The 3rd International Symposium on Radiation Emergency Medicine at Hirosaki University

Organized by
Hirosaki University Graduate School of Health Sciences

Sponsored by
Hirosaki University Institute of Radiation Emergency Medicine
Hirosaki University Education Program for Professionals in Radiation Emergency Medicine

Supported by
National Institute of Radiological Sciences (NIRS)

PREFACE

Since 2008, Hirosaki University Graduate School of Health Sciences has worked to create an advanced professional education program in radiation emergency medicine, including such subject areas as nursing of exposed patients, measures for contamination control and decontamination, radiation dosimetry, and specific clinical assays. The mission of this project is to improve the quality of education and to promote research based on the development of human resources among health professionals, particularly in the field of radiation emergency medicine. Consequently, curriculums have been developed at the School of Health Sciences for both entry-level undergraduate education and the postgraduate course, and the new education program began in April last year.

In line with these initiatives, the 1st International Symposium on Radiation Emergency Medicine at Hirosaki University was held on 1 August 2009, under the theme "Basic Research on Radiation Sciences and Radiation Emergency Medicine." The 2nd International Symposium was held on 10 October 2010, under the theme "Missions and Challenges of Health Professionals in Radiation Emergency Medicine." Recently, the executive committee was making preparations to host the 3rd International Symposium on Radiation Emergency Medicine on 17 September 2011. During this preparation, we faced the unprecedented accident caused at the Fukushima nuclear energy plant by the Great East Japan Earthquake on 11 March 2011. Therefore, the focus of the 3rd International Symposium was "Dose Estimation and Protection in Radiation Exposure." Major topics included current support activities in the wake of the Fukushima nuclear power station accident as related to radiation emergency medicine.

The Symposium consisted of talks by invited speakers, regular presentations, and poster presentations. It was our privilege to have three distinguished invited speakers: Pat Kenny from the International Atomic Energy Agency (IAEA) in Austria and Natalia I. Ossetrova and Mark H. Whitnall from the Armed Forces Radiobiology Research Institute (AFRRI) in the USA. Presentations comprised four sessions, and 26 speakers lectured on topics related to their current research. Participants were deeply impressed by the special guest speakers, and the Symposium was highly successful in promoting the exchange of recent, new developments in this field. This report summarizes the presentations and discussions held at the Symposium. We would like to acknowledge all of the people who have contributed to this Symposium, including the members of the governing board and, above all, the authors and participants. Finally, we hope that the situation in Fukushima will return to normal as soon as possible.

Hitoshi Tsushima
Chief Chair, The 3rd International Symposium on Radiation Emergency Medicine at Hirosaki University
Dean of Graduate School of Health Sciences, Hirosaki University

Symposium

Opening by Professor Hitoshi Tsushima, Dean of Hirosaki University Graduate School of Health Sciences

Guest Professor Mikinori Kuwabara, Hirosaki University Chair of Symposium I

Professor Shinji Tokonami, Hirosaki University The 1st presenter

Assistant professor Masahiro Hosoda, Hirosaki University The 2nd presenter

Associate professor Yuka Noto, Hirosaki University The 3rd presenter

Professor Mitsuaki Yoshida, Hirosaki University Chair of Symposium II

Dr. Takako Tominaga, National Institute of Radiological Sciences The 4th presenter

Dr. Pat Kenny, International Atomic Energy Agency The 5th presenter

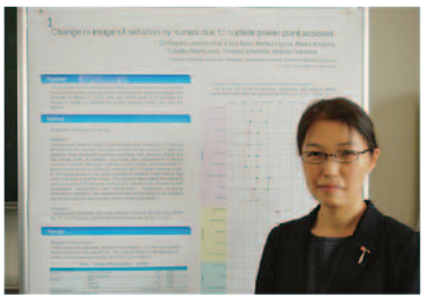
Professor Takashi Kondo, Toyama University
Professor Ikuo Kashiwakura, Hirosaki University
Chair of Symposium IV

Lecture Tomisato Miura, Hirosaki University
The 6th presenter

Professor. Kazunori Anzai, Nihon Pharmaceutical University The 7th presenter

Dr. Natalia I. Ossetrova, Armed Forces Radiobiology Research Institute The 8th presenter

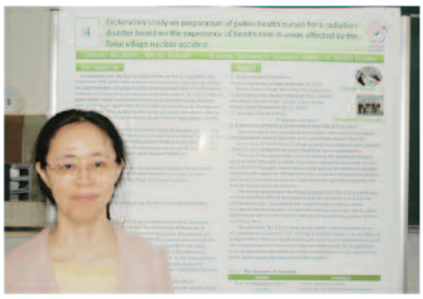
Dr. Mark H. Whitnall, Armed Forces Radiobiology Research Institute The final presenter



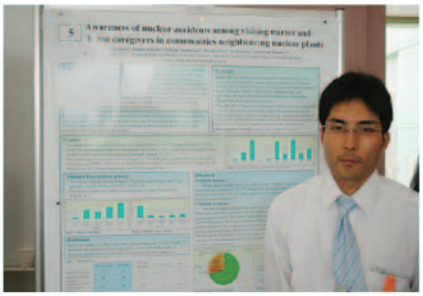
Lecture Mayumi Urushizaka, Hirosaki University
Poster presentation 1

Lecture Noriko Ogura, Hirosaki University
Poster presentation 2

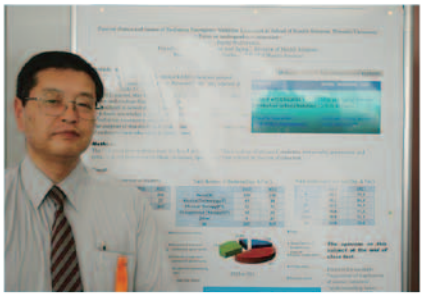
Assistant Keiko Aizu, Hirosaki University Poster presentation 3



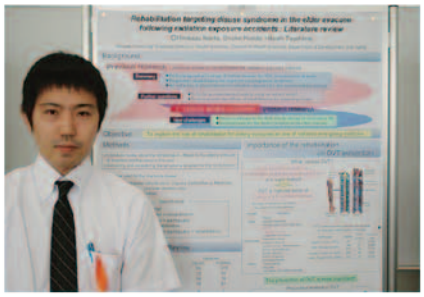
Associate professor Chiaki Kitamiya, Hirosaki University Poster presentation 4



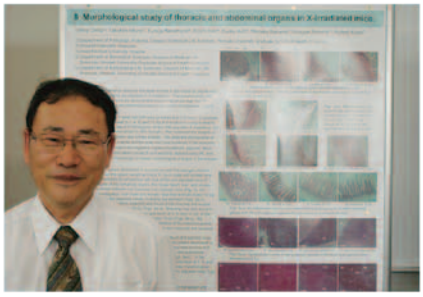
Assistant Daisuke Murakami, Hirosaki University of Health and Welfare Poster presentation 5



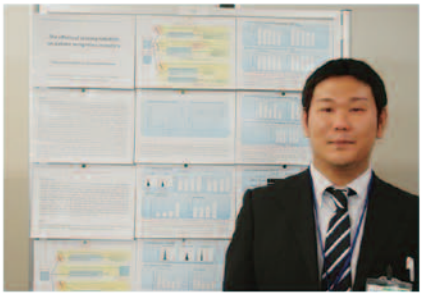
Professor Saichi Wakayama, Hirosaki University Poster presentation 6



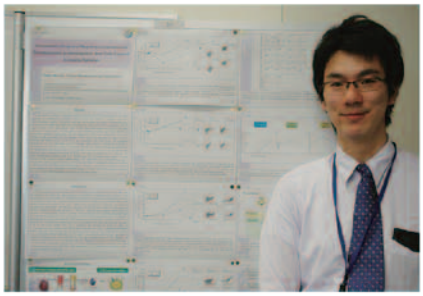
Assistant professor Hirokazu Narita, Hirosaki University Poster presentation 7



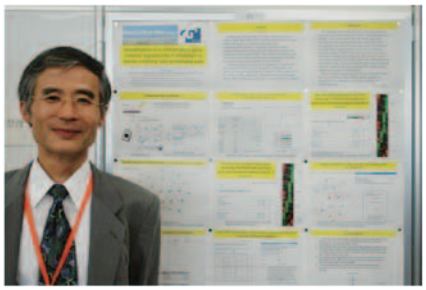
Professor Shoji Chiba, Hirosaki University Poster presentation 8



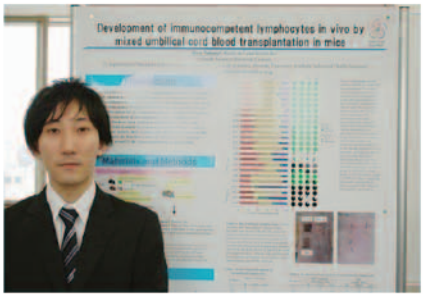
Assistant professor Hironori Yoshino, Hirosaki University Poster presentation 9



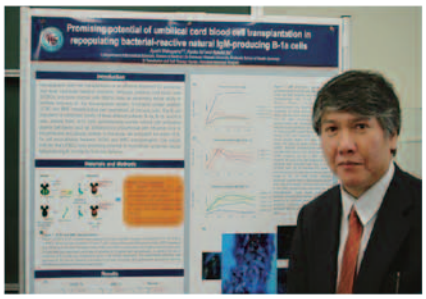
Assistant professor Satoru Monzen, Hirosaki University Poster presentation 10



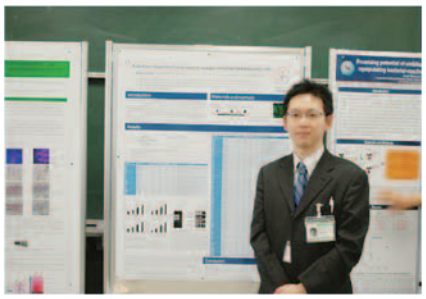
Professor Takashi Kondo, Toyama University Poster presentation 11&12



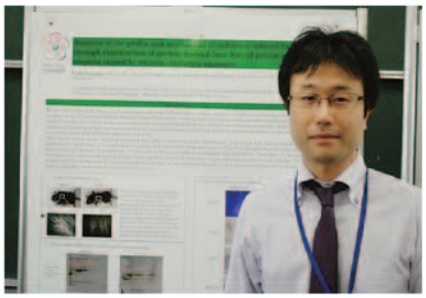
Doctoral Course Akira Nakano, Hirosaki University Poster presentation 13



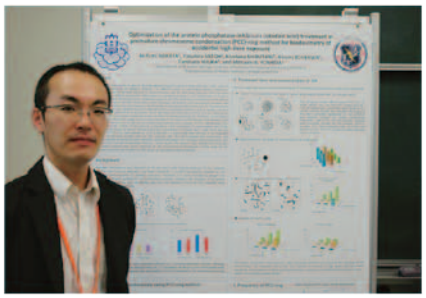
Associate professor Koichi Ito, Hirosaki University Poster presentation 14



Assistant professor Mitsuru Chiba, Hirosaki University Poster presentation 15



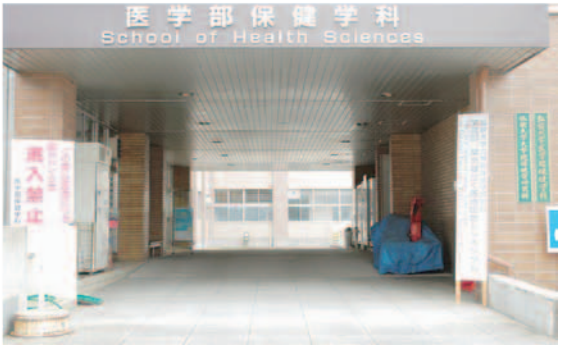
Lecture Naoki Nanashima, Hirosaki University Poster presentation 16



Assistant professor Akifumi Nakata, Hirosaki University Poster presentation 17

Closing by Professor Toshiya Nakamura, Hirosaki University


Registration
Registration


医学部保健学科
School of Health Sciences

Banquet


Reception
Sep.17.2011
The 3rd International Symposium on Radiation Emergency Medicine
in Hirosaki Universi aduate School of Health Sciences


iation Emergency Medicine
ool of Health Sciences


Reception
Sep.17.2011
The 3rd Interna ymposium on Radiation E ergency Medicin
in Hirosaki ity Graduate School of Health Sciences


Reception
Sep.17.2011
The 3rd International Symposium on Radiation Emergency Medicine
in Hirosaki University Graduate School of Health Sciences


Recept
Sep.17.2011
3rd International Symposium ation Emergency Medicine
in Hirosaki University Gradu ool of Health Sciences


The 3rd International Sy on Radiation Emergency Medicine
in Hirosaki University uate School of Health Sciences


Reception
Sep.17.2011
tional Symposium on Radiation Emer Medicine
University Graduate School of Heal nces

Contents

Symposium Ⅲ
Poster Presentation

SymposiumⅣ

Measures against Nuclear Accident at TEPCO Fukushima Daiichi Nuclear Power Plant: Activities carried out by Hirosaki University

Shinji Tokonami[1*]

[1] *Department of Radiation Physics, Institute of Radiation Emergency Medicine, Hirosaki University, Hirosaki, Japan*

Abstract. On March 11, 2011, an earthquake and tsunami of unprecedented magnitude led to major problems in the stabilization of nuclear power reactors in northeast Japan. Operating reactors were automatically shut down, with control rods inserting into the reactor cores. However, a 14-meter tsunami triggered by the earthquake disabled all AC power to Unit 1, 2, and 3. Fuel tanks for emergency diesel generators were carried off by the tsunami. Water injection failed in the emergency core cooling system of Units 1, 2, and 3.Since the normal cooling system was not available, a pressure valve was opened to reduce pressure. In spite of such efforts, hydrogen explosions damaged these facilities. Eventually a large amount of radioactive material was released to the environment. On March 13, members of Radiation Safety Council at Hirosaki University were convened and discussed a policy for this accident. Upon request of the Japanese government, the council concluded that university staff members would be dispatched to Fukushima so as to support their lives. Main actions we took were as follows: 1. Screening tests for radioactive contamination for the general public in Fukushima Prefecture; 2. Field work of radiation measurement and sampling for restructuring their doses. In this paper, an overview of our activities in Fukushima is introduced.

Key Words: earthquake, tsunami, nuclear accident, radioactive contamination, dose assessment, screening test, field survey

Introduction

On Friday, March 11, 2011, an earthquake struck off the coast of Japan, churning up a devastating tsunami that swept over cities and farmland in the northern part of the country. The magnitude of the earthquake was 9.0 on the Richter scale and was the most powerful quake ever hit to our country. This disaster led to major problems in the stabilization of nuclear power reactors in northeast Japan. Operating reactors were automatically shut down, with control rods inserting into the reactor cores. However, a 14-meter tsunami triggered by the earthquake disabled all AC power to Unit 1, 2, and 3. Fuel tanks for emergency diesel generators were carried off by the tsunami. Water injection failed in the emergency core cooling system of Units 1, 2, and 3.Since the normal cooling system was not available, a pressure valve was opened to reduce pressure. In spite of such efforts, hydrogen explosions damaged these facilities. Eventually a large amount of radioactive material was released to the environment. In this paper, Fukushima Daiichi event sequence and an overview of our activities in Fukushima are introduced.

Fukushima Daiichi Event Sequence

Table 1 demonstrates the Fukushima Daiichi event sequence from March 11 to 15[1],. It was derived from information collected by Japan's

* Corresponding to: Shinji Tokonami, Professor, Hirosaki University, 66-1, Hon-cho, Hirosaki, 036-8564, Japan. E-mail: tokonami@cc.hirosaki-u.ac.jp

national nuclear r egulator, the Nuclear and Industrial Safety Agency.

public in Fukushima Prefecture. The second was to assist a temporary visit to homes in a 20-km zone by evacuees. Besides these projects, we planned a

Table 1. Fukushima Daiichi event sequence. (March 11 thru 15)

Date Ti	me	Events
March 11	2:46 p.m.	The 9.0 m agnitude earthquake strikes. Ground acceleration trigge rs automatic shutdown of all three reactors in operation.
	3:42 p.m.	A 14-meter tsunam i trigge red by t he earthquake disables all AC power to Unit 1, 2 and 3.
	3:45 p.m.	Fuel tanks for e mergency diesel genera tors are c arried off by the tsunami.
	4:46 p.m.	Water injection fails in the emergency core cooling systems of Unit 1 and 2.
March 12	9:07 p.m.	A pressure relief valve is opened on the Unit 1 pressure vessel.
	3:36 p.m.	A hydrogen explosion damages the structure of the Unit 1 reactor building.
	8:20 p.m.	Seawater injection to the Unit 1 pressure vessel begins.
	5:58 a.m.	Water injection fails in the emergency core cooling system of Unit 3.
March 13	9:20 a.m.	A pressure relief valve is opened on the Unit 3 pressure vessel.
	4:46 p.m.	Water injection fails in the emergency core cooling systems of Unit 1 and 2.
	11:01 a.m.	A hydrogen explosion damages the external structure of the Unit 3 reactor building.
	1:25 p.m.	The water level in the Unit 2 pressure vessel is found to be low,
March 14		leading operators to conclude that reactor cooling system is no longer functional.
	4:34 p.m.	Seawater injection into the Unit 2 pressure vessel begins.
	6:20 a.m.	An explosion sound is heard at Unit 2 and it concluded to indicate an abnormality in the pressure suppression pool. At the same time, part of a wall in the operation area of Unit 4 is damaged.
	9:38 a.m.	A fire breaks out in the Unit 4 reactor building.
March 15	12:29 p.m.	The unit 4 fire is extinguished.

field work of radiation measurements and sampling for restructuring their doses.

Activities by Hirosaki University

On March 13, members of Radiation Safety Council at Hirosaki University were convened and discussed a policy against this accident. Upon request of the Japanese government, the council concluded that university staff members would be dispatched to Fukushima so as to support people living there. The first team at Hirosaki University was dispatched to Fukushima on March 15.

The dispatched team was involved in two important projects. The first was to do screening tests for radioactive contamination for thegeneral

Screening test for radioactive contamination

For this pr oject, the team consists of a radiation expert, a nurse and a cle rk as their supporting staff. In total 20 team s were dispatched to Fukushima until the end of Jul y. 82 people were involved in t he university. More than 5 ,000 people were examined in this project. In this screening test, a GM survey m eter (e.g. TGS-146B, Aloka, Co., Japan) wa s u sed. **Figure 1** illustrates a screening test carried out by Hirosaki University staff. In this nuclear crisis, a special criterion was set up in terms

of contamination. If the count rate exceeds 100,000 cpm, it is regarded as radioactive contamination. Therefore, decontamination will be needed. As shown in **Figure 2**, a decontamination room was prepared by Self-Defence Force. If the count rate is between 13,000 and 100,000 cpm, the decontamination will be suggested. If the count rate is less than 13,000 cpm, no decontamination will be needed.

Temporary visit to homes in a 20 km zone by evacuees

In order to support their temporary visit, a team was organized, consisting of a medical doctor, a nurse, radiation expert and a clerk. 11 teams were dispatched to Fukushima from late may through early August. 51 people were involved in the university. **Figure 3** illustrates guidance of the temporary visit for evacuees living in a 20 km zone.

Field work of radiation measurement and sampling

Radiation measurements were additionally carried out as well as taking environmental samples. The dose rate in air was measured in several cities in Fukushima Prefecture. Environmental samples were collected from the middle of March. They were soil, water, plants, air, milk and so on. For internal dose assessment, biological sample such as urine was collected from evacuees. Regarding measurement of the dose rate in air, our article has just come out in Scientific Reports. The other analyses are still in progress.

Future Activities

Hirosaki University will make an agreement for comprehensive activities with Namie town where heavily contaminated areas were found. The following activities will be ready:

- Radiation monitoring and environmental/biological assessment
- Decontamination
- Medical support
- Any other supports for remediation of the town

References

[1] Cox M. Special Report: Fukushima Daiichi after the Earthquake and Tsunami. NUCLEAR NEWS: 17-18 (2011).

[2] Hosoda M, Tokonami S, Sorimachi A, Monzen S, Osanai M, Yamada M, Kashiwakura I, Akiba S. Time variation of dose rate artificially increased by the Fukushima nuclear crisis. *Scientific Reports*, DOI: 10.1038/srep00087 (2011)

Acknowledgments

On behalf of all the dispatched teams, the author deeply appreciate President Masahiko Endo and all the staff at Hirosaki University for their kind understanding and cooperation. The author also would like to express his respect to all the dispatched members for their bravery and devotion to people in Fukushima.

Figure 1 Screening test carried out by Hirosaki University staff.

Figure 2 Decontamination room set up by Self-Defence Force

Figure 3 Guidance of the temporary visit for evacuees living in a 20 km zone

Environmental radiation in Fukushima after the nuclear power station accident

Masahiro Hosoda[1*], Shinji Tokonami[2], Atsuyuki Sorimachi[2], Satoru Monzen[1], Minoru Osanai[1], Masatoshi Yamada[2], Ikuo Kashiwakura[1], and Suminori Akiba[3]

[1] Hirosaki University, Graduate School of Health Sciences, Hirosaki, Japan
[2] Hirosaki University, Institute of Radiation Emergency Medicine, Hirosaki, Japan
[3] Kagoshima University, Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Abstract. A car-borne survey of dose rate was carried out in March and April 2011 along an expressway passing northwest of the Fukushima Daiichi Nuclear Power Station (Fukushima NPS) that released radionuclides after the Great East Japan Earthquake on March 11, 2011, and in an area closer to the Fukushima NPS which was known to have been strongly affected. Dose rates along the expressway relatively far from the power station were higher after March 11, in some places by several orders of magnitude, implying that there were additional releases of radionuclides from the Fukushima NPS. The maximum dose rate of the residential area was 32 μGy h^{-1}, and the maximum cumulative external dose of the residential area was estimated to be 177 mSv. On the other hand, the estimated cumulative external dose for evacuees who came from Namie Town to evacuation sites (e.g. in Fukushima, Koriyama, and Nihonmatsu Cities) was from 57 to 68 mSv. As this dose rate was one-third of that in the residential area, the evacuation was justified from the standpoint of radiation protection.

Key Words: car-borne survey, expressway, Fukushima, evacuee, cumulative external dose

Introduction

At 2:46 pm (JST) on March 11, 2011, Japan experienced one of the most powerful earthquakes (M = 9.0) in recorded history, now known as the Great East Japan Earthquake[1],[2]. The power supply for the cooling system in the Fukushima Daiichi Nuclear Power Station (Fukushima NPS) was stopped by the effects of the tsunami generated by the earthquake[3]. Hydrogen explosions at Units 1, 3, and 2 occurred at 3:36 pm on March 12, at 11:01 am on March 14, and at 6:14 am on March 15, respectively. Artificial radionuclides such as ^{133}Xe, ^{131}I, ^{134}Cs, and ^{137}Cs were released from the reactor buildings into the environment[4]. These radionuclides were detected not only in Japan[5], but also in the United States[6], Canada[7], Russia[8], and all over Europe[9]-[11]. The nuclear crisis has not yet ended, thus dose estimations for workers and residents and environmental assessments can only be considered as preliminary. In this study, the effects on the living environment by artificial radionuclides released during the Fukushima NPS crisis were estimated, and the temporal variation of dose rate in air was discussed. Dose rates in the air along an expressway passing northwest of the Fukushima NPS were measured in a car-borne survey. A similar survey was also conducted in a high level contamination area (HLCA) to estimate the external dose for the persons who lived in the area but were evacuated as the crisis developed.

Materials and Methods

Car-borne survey along an expressway

[*] Corresponding to: Masahiro Hosoda, Assistant Professor, Hirosaki University, 66-1, Hon-cho, Hirosaki, 036-8564, Japan.
E-mail: m_hosoda@cc.hirosaki-u.ac.jp

The car-borne survey technique is a common method for the fast assessment of dose rate in emergency situations. The dose rate measurements were conducted 3 times: on March 16, April 11, and April 25, 2011. The distance between Fukushima NPS and each measurement point was from 60 to 355 km, and the typical distance between measurements points were approximately 3 km. Total distance on the expressway was about 1256 km. A 1" × 1" NaI(Tl) scintillation survey meter (TCS-171, ALOKA Co., Japan) was used for each measurement, and measurements were carried out every minute. The survey meter was calibrated with a 2" × 2" NaI(Tl) scintillation spectrometer (SPA-3, Eberline Co., U.S.A). Latitude and longitude at each measurement point were measured using a global positioning system (WPL-2000, Wintec Co., Ltd., Taiwan). More than 100 measurements were obtained in each survey. The shielding effect of the car body was estimated by making measurements inside and outside of the car at 56 points. A shielding factor of 1.9 ± 0.04 was found. The dose rates on pavement and on unsealed surfaces were also compared.

In-situ gamma spectrometry

The *in-situ* gamma spectra was measured using a 3" × 3" NaI(Tl) scintillation spectrometer (JSM-112, ALOKA Co., Japan). Measurements were carried out 1 m above the ground at each measurement site.

Car-borne survey within the HLCA

The car-borne survey within the HLCA was carried out from April 11 to April 15. The survey route was selected after reviewing the MEXT database. Total distance for the car-borne survey in this area was 730 km, and 1623 measurements were performed. The measured values of longitude, latitude, and dose rate were entered into contour mapping software.

Results and Discussion

Car-borne survey along an expressway

Figure 1 shows temporal variation of dose rates in the air before and after the start of the Fukushima NPS crisis. In the 1st survey, the dose rates along the expressway between Fukushima City and Osaki City were found to have drastically increased, from about 0.08 to up to 11 μGy h^{-1}. On the other hand, in the remaining part of the survey area, the dose rates did not increase by much, only by a factor of about 1.5 to 2.5. In the 2nd and 3rd survey, the dose rate profile of the survey area looked quite different. The effect was particularly striking around Oshu City, where the dose rate increased by a factor of up to 10 between the 1st and the 2nd surveys.

Figure 2 shows the temporal variation of *in-situ* gamma spectra measured at Kunimi City, Fukushima Prefecture and Oshu City, Iwate Prefecture. Photon peaks generated by ^{132}Te, ^{131}I, ^{132}I, ^{134}Cs, ^{136}Cs, and ^{137}Cs were observed in the spectra on March 19 at both measurement points. No photon peaks generated by ^{132}Te (3.2 d), ^{131}I, (8.0 d), and ^{132}I (2.3 h) –all short half life nuclides– were observed in the spectra on April 25. The counts of ^{134}Cs and ^{137}Cs at Oshu City on April 25 were much higher than on March 19. This implies that there were additional releases of radionuclides from Fukushima NPS.

The dose rates on pavement and unsealed surfaces

Figure 3 shows the comparison of the measured dose rates on pavement and unsealed surfaces. The dose rates were from 1.0 to 2.1 times (average: 1.3 times) higher on unsealed surfaces than on pavement. Therefore, contamination on sealed surfaces can be removed by wind (re-entrainment) and rain (washout) [12]-[15].

External dose estimation within the HLCA

The dose rates did not depend on the distance from the Fukushima NPS, and the most contaminated area was to the northwest of the facility (**Figure 4**). The highest observed dose rate was 36 μGy h^{-1} (5:08 pm, April 12, 2011) at Hirusone, Namie Town. The maximum value in a residential area was 32 μGy h^{-1} (1:12 pm on April 15, 2011) at Koakuto, Namie Town.

The monitoring data measured by Fukushima Prefecture were used for the calculation of the cumulative external dose from March 12, 2011, to the author's measurement date. Some assumptions were considered for the estimation of the cumulative external dose from March 12, 2011, to March 11, 2012.

- The temporal variation of dose rate between Hirusone and the MEXT monitoring station at Iitate Village was the same.

- The dose rates at Hirusone were proportional to the values at Iitate Village**.**
- The missing monitoring data from March 12 to 14 were of the same level as the dose rate before rainfall at 2:00 pm on March 14.
- The contribution rate of ^{134}Cs and ^{137}Cs were set as 0.75 and 0.25, respectively. These values were estimated using a 3" × 3" NaI(Tl) scintillation spectrometer (JSM-112, ALOKA Co., Japan) in Namie Town.
- The decrease of dose rate due to physical decay of ^{134}Cs and ^{137}Cs was considered.
- The conversion factor from pavement to soil was set at 1.3.
- The indoor and outdoor dwelling times were set as 16 hours and 8 hours, respectively, and the shielding coefficient of walls was set as 0.4, the same values as used by MEXT.

The cumulative external dose at Hirusone was estimated to be 194 mSv, and was close to the value presented by MEXT. This value at Koakuto was estimated to be 177 mSv, and this was estimated to be the external dose for evacuees who were living in Koakuto until May 10. This result included an assumption that the evacuees lived in Fukushima City, Koriyama City, or Nihonmatsu City from May 11, 2011, to March 11, 2012. The estimation was based on the measurement data from Fukushima City (0.27 – 3.2 μGy h^{-1}), Koriyama City (0.45 – 0.91 μGy h^{-1}), and Nihonmatsu City (0.77 – 2.2 μGy h^{-1}) in April. The cumulative external dose for evacuees to Fukushima, Koriyama, and Nohonmatsu Cities was estimated to be from 57 to 68 mSv.

Conclusions

The dose rates along the expressway were higher after March 11. Specifically, the counts of ^{134}Cs and ^{137}Cs around northern Miyagi Prefecture on April 25 were much higher than those on March 19. This implies that there were additional releases of radionuclides from the Fukushima NPS. The maximum dose rate and the cumulative external dose of the residential area in the HLCA were 32 μGy h^{-1} and 177 mSv, respectively. The estimated maximum cumulative external dose for evacuees who came from the HLCA to the evacuation sites was 68 mSv. As this value was one third of the 177 mSv found in the HLCA, the evacuation is justified from the standpoint of radiation protection.

References

[1] Monateersky R. Giant shock rattles ideas about quake behavior. *Nature*. 471: 274 (2011).

[2] Ozawa S, Nishimura T, Suito H, Kobayashi T, Tobita M, Imakiire T. Coseismic and postseismic slip of the 2011 magnitude-9 Tohoku-Oki earthquake. *Nature*. 475: 373-376 (2011).

[3] Tanimoto T, Uchida N, Kodama Y, Teshima T, Taniguchi S. Safety of workers at the Fukushima Daiichi nuclear power plant. *Lancet*. 377: 1489-1490 (2011).

[4] Butler C. Radioactivity spreads in Japan. *Nature*. 471: 555-556 (2011).

[5] Matsumura H, Saito K, Ishioka J, Uwamino Y. Behavior of radioactive materials from Fukushima Daiichi nuclear power station obtained by radiation san on the expressways. *J. At. Energy Soc. Jpn*. 10: 152-162 (2011).

[6] Bowyer TW, Biegalski SR, Cooper M, Eslinger PW, Haas D, Hayes JC, Miley HS, Strom DJ, Woods V. Elevated radioxenon detected remotely following the Fukushima nuclear accident. *J. Environ. Radioact*. 102: 681-687 (2011).

[7] Sinclair LE, Seywerd HCJ, Fortin R, Carson JM, Saull PRB, Coyle MJ, Van Brabant RA, Buckle JL, Desjardins SM, Hall RM. Aerial measurement of radioxenon concentration off the west coast of Vancouver Island following the Fukushima reactor accident. *J. Environ. Radioact*. 102: 1018-1023 (2011).

[8] Bolsunovsky A, Dementyev D. Evidence of the radioactive fallout in the center of Asia (Russia) following the Fukushima Nuclear Accident. *J. Environ. Radioact*. **1**02: 1062-1064 (2011).

[9] Manolopoulou M, Vagena E, Stoulos S, Ioannidou A, Papastefanou C. Radioiodine and radiocesium in Thessaloniki, Northern Greece due to the Fukushima nuclear accident. *J. Environ. Radioact*. 102: 796-797 (2011).

[10] Lozano RL, Hernández-Ceballos MA, Adame JA, Casas-Ruíz M, Sorribas M, San Miguel EG, Bolívar JP. Radioactive impact

of Fukushima accident on the Iberian Peninsula: Evolution and plume previous pathway. *Environ. Int*. 37: 1259-1264 (2011).
[11] Pittauerová D, Hettwig B, Fischer HW. Fukushima fallout in Northwest German environmental media. *J. Environ. Radioact.* 102: 877-880 (2011).
[12] Halldin S, Rodhe A, Bjurman B. Urban storm water transport and wash-off of cesium-137 after the Chernobyl accident. *Water Air Soil Pollut*. 49: 139-158 (1990).
[13] Mueck K, Steger F. Wash-off effects in urban areas. *Radiat. Prot. Dosim*. 37: 189-194 (1991).
[14] Garger EK, Paretzke HG, Tschiersch J. Measurement of resuspended aerosol in the Chernobyl area I. Discussion of instrumentation and estimation of measurement uncertainty. *Radiat. Environ. Biophys*. 36: 139-148 (1997).
[15] Garger EK, Kashpur V, Paretzke HG, Tschiersch J. Measurement of resuspended aerosol in the Chernobyl area Part II. Size distribution of radioactive particles. *Radiat. Environ. Biophys*. 36: 275-283 (1998).
[16] Minato S. Distribution of terrestrial γ ray dose rates in Japan. *J. Geography* (*Chigaku Zasshi*) 115: 87-95 (2006).

Acknowledgments

This work was supported in part by a Grant for the Co-medical Education Program in Radiation Emergency Medicine by the Ministry of Education, Culture, Sports, Science and Technology, Japan (2011). The authors would like to thank Prof. Yasushi Asari, Hirosaki University, Prof. Michikuni Shimo, Fujita Health University and Dr. Susumu Minato, Radiation Earth Science Laboratory for their kind assistance in conducting this study. This study was already published as an original research in *Scientific Reports* (http://www.nature.com/srep/2011/110907/srep00087/full/srep00087.html).

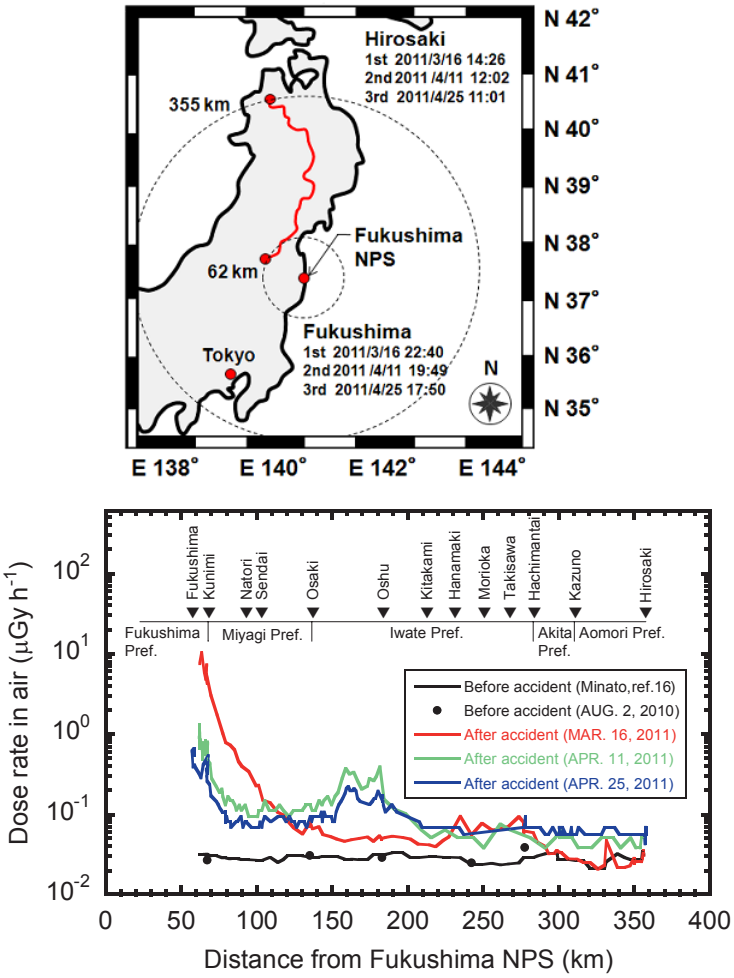


Figure 1 Expressway survey route for measuring dose rates in the air from Hirosaki City to Fukushima City. Temporal variation of dose rates in the air before and after the start of the Fukushima NPS crisis.

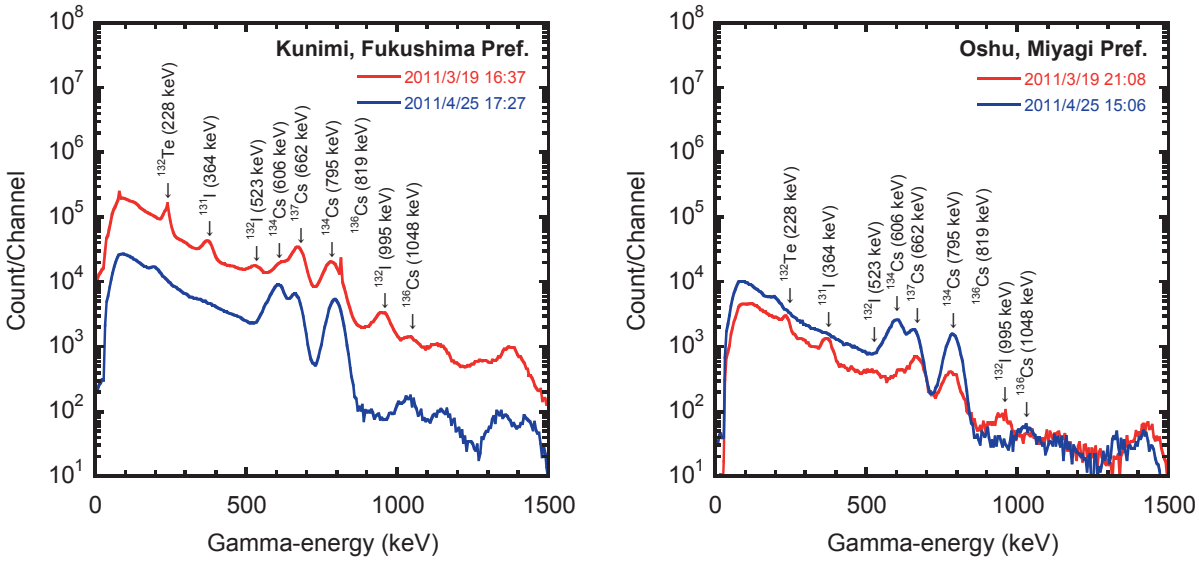


Figure 2 Temporal variation of in-situ gamma spectra measured at Kunimi, Fukushima Prefecture and Oshu, Iwate Prefecture.

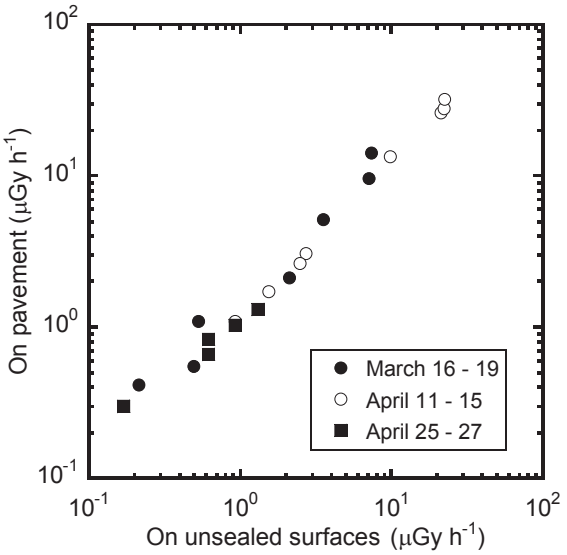


Figure 3 Comparison of measured dose rates in the air over pavement and uncovered surfaces.

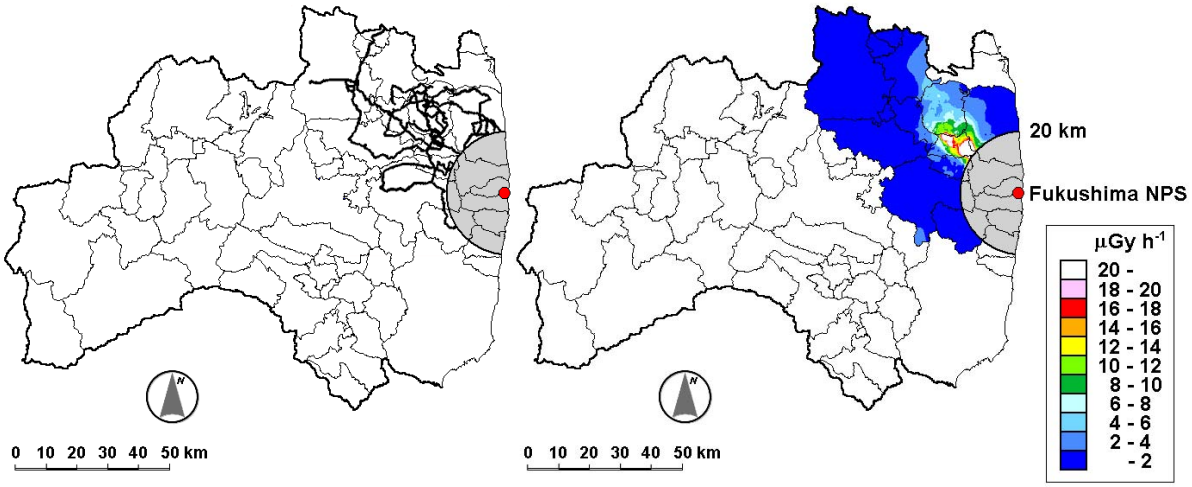


Figure 4 Survey route for measuring the dose rates in the air within the high level contamination area. Distribution map of the dose rates in the air within the high level contamination area in Fukushima Prefecture.

Role of nurses in a nuclear disaster

Yuka Noto

Department of Disability and Health, Division of Health Sciences,
Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan

Abstract. A severe earthquake occurred in Japan on March 11, 2011. The giant tsunami unleashed by the magnitude 9.0 earthquake devastated many coastal communities across a wide area of eastern Japan and crippled the Fukushima Daiichi nuclear power plant. The battle to bring the situation under control continues. The Japanese government decided to conduct a large-scale survey of residents affected by the nuclear and natural disasters in Fukushima prefecture and requested the dispatch of contamination survey personnel from Hirosaki University. In response to the government's request, Hirosaki University decided to dispatch a Radiation Exposure Research Team to Fukushima prefecture. In addition, the Fukushima prefecture Temporary Home Visit Project was initiated. This presentation describes the activities of the Radiation Exposure Research Team and the Temporary Home Visit Project. Next, based on my experience of the 2 projects described above, I would like to talk about the characteristics of the Fukushima nuclear accident and what I feel is important in terms of the care of residents affected by this nuclear disaster. This unprecedented nuclear disaster will leave a large scar within Fukushima and throughout Japan. Our role, as medical staff in the field of radiation medicine, is to protect the health of those affected and try to reduce their anxiety. In addition, as the persons responsible for implementing the Radiation Emergency Medicine Project, it is also necessary that we educate and foster the development of medical care personnel with the appropriate knowledge and skills to carry out the measures required.

Key Words: nuclear disaster, role of nurses, radiation emergency medicine

Introduction

Even if careful attention is paid to the safe use of radiation and appropriate radiation protection measures are taken, the probability of a radiation exposure accident is not zero. Whether a nuclear disaster or a radiation accident, when such an event occurs, it has a great impact on the people in and near the local community, as well as on the facility workers. To be specific, the most serious problem is the health damage to the human body. Therefore, as a precaution against such an unlikely accident, it is necessary to maintain a system of radiation emergency medicine. However, in actuality, this system was not ready.

A severe earthquake occurred on March 11, 2011. The giant tsunami unleashed by the magnitude 9.0 earthquake devastated many coastal communities across a wide area of eastern Japan and crippled the Fukushima Daiichi nuclear power plant. The battle to bring the situation under control continues. With radiation continuing to be released, the evacuees from around the facility continue to live in shelters, unable to begin restoring their community.

1. Outline of the Hirosaki University Radiation Exposure Research Team and Temporary Home Visit Project for evacuated residents

The Japanese government decided to conduct a large-scale survey of Fukushima prefecture residents affected by these nuclear and natural disasters and requested Hirosaki University to send contamination survey personnel to the area.. In response to the government's request, Hirosaki University decided to dispatch the Radiation Exposure Research Team to Fukushima prefecture.

Approximately 80 staff members were sent from March 15 until July. In addition, the Fukushima prefecture Temporary Home Visit Project was initiated in May. In this project, the participants' safety was ensured by providing radiation protection and by conducting contamination surveys. Hirosaki University also took part in this project and dispatched approximately 60 staff members. Having spent several days working with each of these projects, I will now describe their activities.

1) The activities of the Radiation Exposure Research Team

My participation lasted from March 16 to March 18. The team was comprised of radiologists, lecturers from the School of Health Sciences who were also qualified nurses or radiological technologists, and clerical officers. We set up the survey site and examined local residents for any signs of contamination. If the contamination dose was found to be high, we then conducted decontamination procedures. However, only a few of the people who took part in the survey actually required decontamination. Decontamination was achieved by simply removing the outer layer of their clothes.

At the same time, the university researchers began an investigation of the environment and its influence on the human body.

2) The activities of the Temporary Home Visit Project for evacuated residents

The Temporary Home Visit Project allowed those residents who lived within a 20-km radius of the nuclear plants but were currently living in evacuation shelters to return to their homes for several hours. The residents were assembled at a relay point and, after putting on protective clothing, were allowed to return to their homes. They spent several hours there and then returned to the relay point where they were checked for contamination before going back to the evacuation shelters or other accommodation. I participated in this project as part of the medical team.

2. Characteristics of the Fukushima nuclear accident and the role of nurses

Based on my experience with the two aforementioned projects, I would like to describe the characteristics of the Fukushima nuclear disaster and discuss what I feel important in terms of the care of residents affected by this disaster is.

1) Characteristics of this nuclear accident

The situation and circumstances experienced exceeded the assumptions of the radiation emergency medicine system

(i) The disaster was on a larger scale than anticipated

Until now, it was not possible to predict that such a large-scale nuclear accident could be caused by an earthquake or tsunami. Japanese nuclear plants were believed to be equipped with the world's best safety features to protect them against natural disasters. If nuclear accidents were to occur, the more probable causes were considered to be human error or terrorism. As we can see from the widespread use of the adjective "unexpected", the strength and scale of the Fukushima disaster far exceeded our expectations. It was this "unexpected" situation that triggered the subsequent accidents at nuclear plants that had previously been considered to be perfectly safe.

Since the disaster was on such a large scale, areas other than Fukushima were also affected and the difficulty of coordinating and concentrating medical aid was a problem. It was not possible to dispatch a sufficient number of nurses in the early stages of the disaster. Also, as reported in the press, female nurses were particularly hesitant to travel to field sites due to their concerns about the high levels of radiation.

(ii) Compounded by damage from the earthquake and tsunami

A unique characteristic of this nuclear disaster was that the damage was caused by the combination of an earthquake and a tsunami. Therefore, the residents who lived near the nuclear power plants suffered multiple instances of damage as they were forced to evacuate in addition to losing families and homes from the earthquake and tsunami. Compared with other disasters, the enormous amount of emotional, physical, economic, and social distress can be imagined.

(iii) The situation is still not under control

Considering that the worst-case scenario involving a meltdown has actually occurred, it is necessary to re-examine the process schedule chart presented earlier. That is to say, the forecast for the future is still unclear at this point. Therefore, the evacuees remain in an uncertain situation where there is no clear indication as to how long their evacuation status will continue or when they will be able to return home.

(iv) Prediction of health hazards is proving difficult

Until now, it was considered that, when a nuclear disaster or accident occurred, radiation could be measured and health damage could be predicted, at least to some degree, by examining past cases. However, there is a critical shortage of basic data regarding the risk arising from long-term exposure to low doses of radiation in adults, pregnant women, and children.

In the future, it will be necessary to examine how nursing staffs handle these difficult situations, what kind of effect their efforts will have, and how their performances can be evaluated.

2) Role of nurses in a nuclear disaster

From my experience with these two projects, I was convinced that the role of nurses in a nuclear disaster is very important.

(1) Experience of the Hirosaki University Radiation Exposure Research Team

(i) Flexible support depending on individual circumstances

When conducting a contamination survey, it was found that only a limited number of residents actually required decontamination such as removing the outer layer of their clothes. However, in cases where residents are evacuated with only the clothes on their back and are not allowed to return home to get their belongings, they may not be able to change their clothes. In addition, if their hands and hair are contaminated, washing cannot be carried out in a satisfactory manner if the water system has not been restored. Therefore, it is essential not only to conduct contamination surveys and decontamination procedures, but also to provide support by checking conditions in the area and the circumstances of the individuals affected. We all need to think about the best ways to deal with these problems and to offer possible choices and solutions so that each person's individual needs can be satisfied. Therefore, I consider that an important role of nursing in such circumstances is to assess, and carefully consider the individual situations of the residents concerned.

(ii) Setting up a site for contamination surveys and supporting activities

In any disaster, not just a nuclear accident, it is very important to provide thoughtful consideration towards the most vulnerable people in the affected area. Some people will need special consideration, such as the elderly who may have difficulty walking and those people with a vision, hearing or language disability or dementia. In general, the site used for a contamination survey should be set up so that the lines of people participating in the survey do not overlap. However, if set up in this way, the line from the entrance to the exit can become rather long and, even within the site, this distance may pose a problem for some of the people participating in the survey. People who have difficulty walking and use a walking stick outside the site may be forced to walk and to leave their walking sticks at the entrance. The procedure used for making necessary announcements to the group can make it difficult to deal with individual disabilities. If present at the survey site, nurses can quickly identify people with disabilities, approach them individually, and provide them with appropriate support. In this project, the nurses either stayed with these persons during the survey or requested that the survey staff come to the entrance so that people with a disability did not have to move as far.

When setting up a site, consideration must be given not only to the facilities and human and material resources required, but also to the following issues: the number of people to be surveyed, whether or not they will arrive in groups, how many are likely to have a high dose of radiation and require decontamination, and how many will be elderly or disabled. Depending on the particular circumstances encountered, it is necessary to take the location, resources, and local situation into account when setting up a site.

(iii) Conducting a contamination survey involving children

Special consideration is necessary when carrying out a survey involving children. The sight of many adults wearing masks and protective clothing in a strange place will be something they've never experienced. Strange monitoring machines they have never seen will be held over their bodies and their parents will be watching with tense expressions on their faces. In order to reduce their feelings of anxiety and fear as much as possible and to prevent bad memories, it was considered important to provide as much care and consideration as possible. When children were with their parents, we asked them, "would you like to take the survey with your mother?" and we asked their parents to stay nearby. When the children underwent the survey alone, nurses stayed nearby and said things like, "it won't hurt", "it will be over soon", and "you did well" and when the survey was complete we gave the children candy as a present,

saying, “you did a great job”. By the time the survey was completed, many children were looking rather glum but they smiled when they received the candy. Therefore, the “candy strategy” was considered to be an effective means of easing anxiety in children.

(iv) Providing an opportunity to answer questions and express anxiety in words

As part of the survey team, a surveyor and a clerical officer worked together as a pair to explain the contamination survey certificate. Clerical officers were also put in charge of guiding residents through the survey process in order to allow the nurses and healthcare professionals to concentrate on providing care. We were able to identify the persons who needed to be supported and were able to respond promptly. In the case of people in tears telling us, “our home has been washed away by the tsunami and nothing is left”, we listened to them for as long as time allowed. When explaining the contamination survey certificate, many people asked questions and we listened and observed them carefully. If it seemed that they wanted to talk longer, we provided a more detailed explanation and discussed it further.

Our survey team was in operation from the 7th to the 9th day after the disaster. Most of the residents participating in the survey already understood the meaning of radiation and the purpose of the survey, at least to some degree, due to the government’s explanations, press reporting, etc. Some residents had taken part in the survey before or had come at their own initiative. Therefore, it was not considered necessary to explain the meaning or the methodology of the survey to most of the residents. The results of the survey generally showed no significant effect on health, except for several people who could be decontaminated simply by taking off the outer layer of their clothing. By telling these persons that “it is almost the same level as natural background radiation”, the survey team found that they understood and were relieved. Most of the people who went through the decontamination process of taking off the outer layer of their clothes did not seem to be upset and appeared to accept and understand the reason: because “I was wearing these clothes all through the disaster”. However, there were a small number of people who seemed to be irritated and resentful, and we tried to communicate with these people as politely and carefully as possible. Some people also asked questions or expressed concerns such as “it is alright now, but we don’t know about the future”, “I work outside and is it alright to take part in the survey again tomorrow?”, and “I would like my children to take potassium iodine as a precaution and I would like to know what to do”. In these cases we provided explanations and helped as much as we could. It can be very difficult for people in these situations, and it was considered desirable to set up an individual consultation service staffed by specialists to assist the residents visiting the survey sites.

(2) Experience of the Temporary Home Visit Project for evacuated residents

(i) Health check for the participants

Notable aspects of the Temporary Home Visit Project were the long hours required from the participants and their exposure to high levels of risk. It was important that any health check took these characteristics into consideration. To take part in the Temporary Home Visit Project, participants had to leave their evacuation shelters early in the morning in order to arrive at the relay point on time. They were then forced to follow a tight schedule as it took one hour to arrive at their homes where they could stay for only two hours before returning to the relay point, where they underwent a screening before returning to the evacuation shelters, which took another two hours. Some residents who had been evacuated to the homes of their children or relatives, located even further away than the evacuation shelters, had to travel a long way by car to participate. After spending three months as evacuees, some participants were suffering a decline in physical strength, etc. In addition, there were many elderly people and many people with medical conditions such as hypertension, cardiac disorders, asthma or diabetes mellitus and who were receiving medication and treatment. These participants ran a high risk of falling sick during their visit because, in addition to the high temperatures, the requirement to wear protective clothing meant that some became nervous and some refrained from taking fluids while on the bus because they were worried about using the bathroom. Others hid their ill-health because they did not want to miss this chance to visit their homes as they did not know when the next chance would be. The nurses needed to keep these things in mind when conducting the health checks. Cross-checking all the names on the list with medical or drug administration records allowed us to obtain detailed

information. We also checked things such as the frequency of drug intake throughout the day, whether or not the right drug was taken in the morning, and whether or not there were any disease symptoms present. In the case of diabetes mellitus, the administration of the relevant drugs and insulin injections and breakfast and lunch plans were all checked. Necessary instructions were written on the margins of the list of participants and all information was concentrated in one place so that details regarding any residents who needed to be carefully observed could be shared by all the medical staff. We tried to ensure that the same staff supported each resident after their return and, so that a change in staff would not lead to any further problems, we re-checked the above information with all the staff when the bus arrived. Also, by putting Post-it notes on the questionnaire forms, we made the information accessible and understandable to everyone. As an additional precaution, we checked any residents using antihypertensive diuretics for fluid intake and use of the bathroom. In the case of residents who had forgotten to take their antihypertensive medicine, we checked if they had their medicine on them and reminded them to take their pills.

We observed how each resident got down from the bus after the ride back and provided any necessary support, such as asking them about their condition, assisting them while walking, recommending that they use a wheelchair, helping them to sit down or stand up, and providing escorts during the contamination survey. We escorted anyone feeling unwell to a cool, shaded area, notified the doctors, and requested help, as required. We checked the condition of those participants who had come to our attention through the results of a prior health check and passed this information on to the standby physicians. We paid particular attention to the risk of fatigue and dehydration in the elderly, and checked their intake of water and, as needed, arranged for some to undergo a contamination survey or change of clothes on a priority basis.

(ii) Consideration of psychological aspects

The participants included some people who had lost family members or whose home had been washed away. As a result of joining the project, some people met old acquaintances again for the first time after the disaster and, while talking, became overwrought and started crying. One person who returned to their home to console the spirits of the dead said, “I saw the place where the house was located” and “I walked around the surroundings”. Generally, people may appear emotionally calm, but since they have encountered a nuclear accident in addition to other natural disasters, they may be experiencing a wide range of serious anxieties. Therefore, while we need to consider efficiency when coordinating a line of the people so that time is not wasted, it is also desirable to have an extra room for staff so that nurses can stay and care for peoples’ anxieties.

Conclusion

The nuclear disaster that has happened here will leave a large scar within Fukushima and throughout Japan. Our role as medical staff in the field of radiation medicine is to protect the health of those affected and to reduce their anxiety. In addition, as the people responsible for implementing the Radiation Emergency Medicine project, we must also educate medical care personnel and foster the development of the appropriate knowledge and skills.

Acknowledgements

I am deeply grateful to Prof. Yamabe and Associate Prof. Kitamiya of Hirosaki University for their valuable advice.

Role of NIRS in response to the accident at TEPCO Fukushima Daiichi NPS - from viewpoint of radiation emergency medical preparedness -

Takako Tominaga, Takaya Gotoh, Hideo Tatsuzaki, Misao Hachiya, Nobuyuki Sugiura, Makoto Akashi

National Institution of radiological Sciences, 4-9-1 Anagawa Inage-ku, Chiba, Japan
Email: tominaga@nirs.go.jp

In January 2010, National Institute of Radiological Science (NIRS) has established the Radiation Emergency Medical Assistance Team (REMAT) to support primary medical care when an accident of radiation exposure or contamination with radioactive materials has occurred overseas. The team consists of physicians, nurses, radiation protection experts, and health physicists ready to respond to radiation emergencies, and the team is equipped with advanced portable radiation measuring instruments and medicines. NIRS is ready for providing international medical assistance for radiation emergencies, based on human resources with experiences in this field. However, the first REMAT activity was made for the TEPCO Fukushima Daiichi Nuclear Power Station (NPS) accident. Before NIRS established REMAT, we had a system for dispatching staffs to an accident site in Japan based on the Act on Special Measures Concerning Nuclear Emergency Preparedness. However, this system was for providing advice to local staff and the activities were limited.

The Great East Japan Earthquake attacked the Pacific coast area of eastern Japan on 11 March 2011 and this earthquake and tsunami caused enormous damage to the NPS of Tokyo Electric Power Co. (TEPCO). NIRS dispatched REMAT to the local headquarters located 5 km from the NPS almost 17 hours after the earthquake. Since then, many experts at NIRS have been sent to Fukushima. REMAT is still being involved in responses to this nuclear accident including the public issue in Fukushima. Thus, this nuclear accident requires response at an unprecedented scale and over a lengthy period.

In this accident, the response system for radiation emergency medicine did not work effectively since community lifelines such as water supply and electricity were severely damaged. Thus, hospitals lost their function including radiation emergency medicine. In our efforts for recovery from the damages, reconstruction of the medical system in the affected areas has to be hurried. Moreover, reestablishment of the system for radiation emergency medical response is also a key point since reactors have not been stabilized. From our response to this combined disaster of earthquake, tsunami, and radiation, we have learned that there is an urgent need for all-hazard approaches.

IAEA system for responding to radiological and nuclear emergencies and its response to the accident at TEPCO's Fukushima Daiichi

Pat Kenny

Incident and Emergency Centre (IEC)
International Atomic Energy Agency (IAEA), Vienna Austria
Email: p.kenny@iaea.org

The Incident and Emergency Centre (IEC) of the International Atomic Energy Agency (IAEA) is the global focal point for international preparedness and response to nuclear and radiological safety or security related incidents, emergencies, threats or events of media interest. The Convention on Assistance in Case of a Nuclear Accident of Radiological Emergency and the Convention on Early Notification of a Nuclear Accident (Emergency Conventions) are the prime legal instruments that establish an international framework to facilitate the exchange of information and the prompt provision of assistance in the event of a nuclear accident or radiological emergency. They place specific obligations on the Parties and the IAEA, with the aim of minimizing consequences for health, property and the environment. The IEC was established to implement the IAEA's obligations.

The Incident and Emergency System (IES) consists of an on-call system with 24/7 coverage and a response system. The on-call system ensures that the IEC initial response to received message concerning an actual or potential nuclear or radiological incident or emergency or requests for assistance or media reports is prompt and efficient. It also allows for rapid activation of the IEC if required while the response system under activation ensures smooth and efficient implementation of the Agency's REPLIE.

Parities to the Assistance Convention have agreed to cooperate with each other and with the IAEA to facilitate prompt provision of assistance in case of a nuclear or radiological emergency, in order to mitigate its consequences. As part of the IAEA's strategy for supporting practical implementation of the Assistance Convention and in order to coordinate a global response, the IEC manages the global Response Assistance Network (RANET). RANET aims to facilitate assistance in case of a nuclear or radiological incident or emergency in a timely and effective manner and in principal on a regional basis.

The IEC was activated into Full Response Mode shortly after the 11 March earthquake and tsunami and the subsequent accident at TEPCO's Fukushima Daiichi. Since that time the IEC has remained activated as it fulfills the obligations of the IAEA in accordance with the Emergency Conventions.

Change in image of radiation by nurses due to nuclear power plant accident

Mayumi Urushizaka[1*], Yuka Noto[2], Noriko Ogura[3], Maiko Kitajima[1], Yoshiko Nishizawa[1], Tomoko Ichinohe[1], Hideki Yamabe[1]

[1] Department of Health Promotion
[2] Department of Disability and Health
[3] Department of Development and Aging,
Division of Health Sciences, Hirosaki University Graduate School of Health Sciences

Abstract. Many people are concerned about exposure to radiation due to the nuclear power plant accident caused by the Great East Japan earthquake that occurred on March 11th, 2011. The aim of this study is to elucidate the change in image of radiation by nurses between before and after the disaster. The subjects for the this study were 307 nurses. A placement method employing a questionnaire was conducted in February 2010 (before the disaster) and again in May through June 2011 (after the disaster). They answered questions regarding their personal details and a scale of their image of radiation. The scale was composed of 3 factors including 17 items: affectivity, usefulness and certainty, and each of the 17 items use the semantic differential methods with a 7-stage response scale. The conducted analyses were a two-sample t-test and ANOVA, with the significance level set at $p < 0.05$. There were 262 responses (88 before the disaster, 174 after the disaster) with a collection rate of 85.3%. The scores for all items were significantly lower after the disaster than before it. The group who had attended seminars on radiation before the disaster showed a significantly higher score than the group who had not in regards to the "dangerous – safe" indication before the disaster, the difference between both groups diminished after the disaster. In spite of attending seminars on radiation, the "dangerous-safe" indication tended towards having a more dangerous image after the disaster and this may be due to people thinking the impact of the nuclear power plant accident was so serious.

Key Words: radiation image, nuclear power plant accident, scale, nurse

[*] Corresponding to: Mayumi Urushizaka, Lecture, Hirosaki University, 1, Hon-cho, Hirosaki, 036-8564, Japan.
E-mail: urushima@cc.hirosaki-u.ac.jp

Introduction

Many people are concerned about exposure to radiation due to the nuclear power plant accident caused by the Great East Japan earthquake that occurred on March 11th, 2011. In addition, the importance of enhancing radiation emergency medicine and improving the related roles of medical care professionals are widely recognized. Nurses have to care for radiation accident victims as a professional over a long period from the occurrence time, but their training has almost not covered radiation emergency medicine. Therefore, it is thought that a lack of their knowledge about radiation and radiation emergency medicine, or fears to themselves radiation exposure could interfere with nursing practices. The aim of this study is to elucidate the changes in image of radiation held by nurses from before and after the disaster.

Methods

Subjects and Method

The subjects for this study were 307 nurses and a placement method employing a questionnaire was conducted in February 2010 (before the disaster) and again in May through June 2011 (after the disaster). They answered questions regarding their personal details and a scale of their image of radiation. The scale was composed of 3 factors (affectivity, usefulness and certainty) covering 17 items. Each of the 17 items use semantic differential methods with a 7-stage response scale. In this questionnaire a low score indicates a negative perception and a high score indicates a positive perception. The subjects were asked questions regarding their "experience working in a radiation unit which performs a radiographic examination and radiotherapy ", "experience attending workshops or lectures or seminars about radiation", and "familiarity with radiographic examinations and radiotherapy". We explained the aim of this study to the subjects and the anonymous and voluntary nature of their participation

Statistics Analysis

The conducted analyses were a two-sample t-test and ANOVA, with the significance level set at $p < 0.05$.

Results

Subjects characteristics

There were 262 responses (88 before the disaster, 174 after the disaster) with a collection rate of 85.3%. There were 251 female subjects and 9 males, and their average age was 36.08 ±10.91 years (table 1).

Table 1 Subject characteristics (n=262)

Nurses(n=262)			
Number	Before the disaster	88	(33.6)
	After the disaster	174	(66.4)
Gender	Male	9	(3.4)
	Female	251	(95.8)
	Unknown	2	(0.8)
Average Age(years)		36.08±10.91	
Experience working in a radion unit			
	Yes	81	(30.9)
	No	181	(69.1)
Experience attending seminars about radiation			
	Yes	85	(32.4)
	No	177	(67.6)
Familiarity with radiographic examination and radiotherapy			
	Familiar	197	(75.2)
	neither	42	(16.0)
	not familiar	23	(8.8)

n(%) or mean±SD

Change in image of radiation from before and after the disaster

The scores for all items (affectivity, usefulness and certainty) were significantly lower after the disaster than before it ($p < 0.01$; figure 1).

Change in image of radiation from before and after the disaster by subject characteristics

Having "experience working in a radiation unit which performs a radiographic examination and radiotherapy " or a "familiarity with radiographic examinations and radiotherapy" did not change the image of radiation before and after the disaster. "Experience attending workshops or lectures or seminars about radiation" has significant difference in regards to the "dangerous – safe". Although the group who had attended seminars on radiation before the disaster showed a significantly higher score than the group who had not in regards to the "dangerous – safe" indication before the disaster ($p < 0.01$), the difference between both groups diminished after the disaster (figure 2).

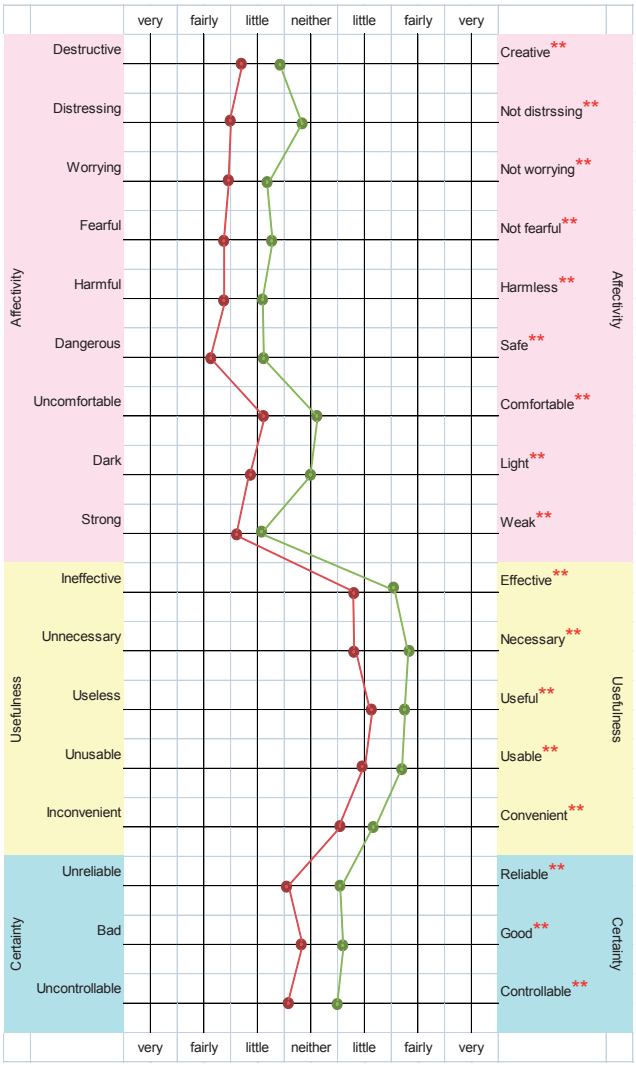


Figure 1 Change in image of radiation from before and after the disaster (n=262)

Before the disaster After the disaster two sample t-test **p<0.01

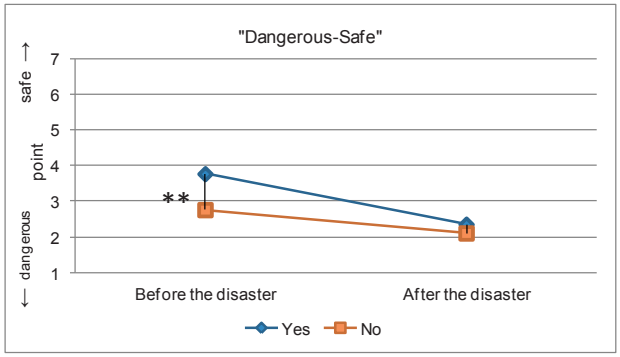


Figure 2 Change in image of radiation from before and after the disaster "Experience attending seminars about radiation" (n=262)

ANOVA **p<0.01

- Low score indicates a negative image(dangerous)
- High score indicates a positive image(safe)

Discussion

In Japan, while small nuclear power plant accidents have occurred several times in the past, the Fukushima Daiichi Nuclear Power Station accident caused by the Great East Japan earthquake was so serious the Japanese government declared a nuclear state of emergency. This accident had a huge impact on nurses as well as the public. Even though nurses often have far more opportunities to be involved with radiographic examinations and radiotherapy and better know the usefulness of radiation than the general public, the results of this study showed the scores for all items (affectivity, usefulness and certainty) were significantly lower after the disaster than before it and that the image of radiation had become more negative. As to the reasons, we suppose that previously they thought the possibility of a nuclear accident was extremely low, and then when the serious accident actually occurred, the nuclear accident affected not only people's bodies but their living environment and food as well. Therefore, as nurses personally felt the fear and risk of radiation, we think that their image of radiation has changed to be more negative after the disaster.

The group who had attended workshops or lectures or seminars about radiation before the disaster showed a significantly more positive image than the group who had not in regards to the "dangerous – safe" indication before the disaster, but after the disaster both groups shifted to a negative image and there were no significant difference between their scores. Nevertheless, why did the nurses who attended workshops or lectures or seminars about radiation and gain some knowledge on radiation, change to having a image of radiation being so dangerous? As to the reasons, in addition to the serious accident, we think there are several factors. Every day there were reports on radiation by numerous television channels, newspapers, and magazines and the information they contained inflamed a fear of radiation. It is difficult to determine the authenticity of such information unless you have specialist knowledge and it was still difficult to evaluate the quality and accuracy of this information with knowledge obtained from workshops. Though they had a positive image of radiation when they attended workshops or lectures or seminars about radiation, the nurses were misled by so much information and

were shifted to having a more negative image of radiation.

Ohta[1)] said that in addition to providing knowledge, knowledge leading to a negative image can be modified by experience interspersed with practice and then correct knowledge can be acquired. To gain knowledge of radiation through experience, measuring shapeless radiation with a survey meter or practicing protection of radiation, a possibility of being connected not to a vague understanding but to a dependable understanding can be considered, as Ohta has stated. In this study we did not investigate the contents of the workshops and lectures attended or the teaching methods used. However, one of reasons why the group who had attended workshops changed to having a negative image in regards to the "dangerous – safe" indication was assumed to be caused by only providing knowledge and that none of the participants could actually practice anything to gain experience. To reduce the negative image of radiation and radiation emergency medicine and to obtain dependable knowledge, we need to discuss how to teach and the curriculum content. We need to investigate not only about the change of image of radiation by nurses over a period after an accident but also the contents of the workshops and the teaching methods used in future.

Conclusion

Nurses had a more negative image of radiation after the disaster than before it. In spite of attending seminars on radiation, the "dangerous – safe" indication tended towards having a more dangerous image after the disaster and this may be due to people thinking the impact of the nuclear power plant accident was so serious. As to how to teach radiation emergency medicine to reduce the negative image of radiation and to provide more correct knowledge, it was suggested that we need to consider not only the provision of knowledge but also experiential education incorporated with experience and practice.

References

1) Ohta, K. Hosyasen ya hibaku toiu kotoba kara kangogakusei ha nani wo renso suruka, Quality Nursing, 6(7), 585-590, 2000. (In Japanese)

Acknowledgments

This study was supported by a Grant for Co-medical Education Program in Radiation Emergency Medicine by The Ministry Education, Culture, Sports, Science and Technology, Japan.

Quality of life and problems in daily living among breast cancer patients undergoing radiotherapy

Noriko Ogura [1*], Mayumi Yamauchi[2], Yuka Noto[1], Yoshiko Nishizawa[1], Hideaki Yamabe[1], Yoichiro Hosokawa[1], Masahiko Aoki[2], Eriko Kudo[2], Yumiko Sato[2], Harumi Shikanai[2], Makoto Akashi[3], Yoshiko Ando[3], Noriko Kudo[3], Yayoi Tsutsumi[3], Akiko Nezato[3], and Yoshiko Fukushima[3]

[1] Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan
[2] Hirosaki University School of Medicine and Hospital, Hirosaki, Japan
[3] Hospital at Research Center for Charged Particle Therapy, National Institute of Radiological Sciences (NIRS), Chiba, Japan

Abstract. The purpose of this study was to clarify the quality of life (QOL) and symptoms of acute radiation damage in breast-cancer patients undergoing radiotherapy. Subjects were 47 breast-cancer patients. The patients were asked to record symptoms using a symptom diary from the day before the start of radiotherapy until its completion. QOL and clinical data were collected from before irradiation until 4-6 weeks after irradiation. The symptoms tended to appear from around the third week of irradiation. Fatigue and pain tended to appear at an early stage. After completion of irradiation, the QOL scores increased in all 8 domains, but the scores 4–6 weeks later were not significantly different from the scores at the completion of irradiation. Symptom scores during irradiation were negatively correlated with QOL scores. Lymphocyte and platelet counts decreased in the third through fifth weeks, but other clinical measures remained stable. Our results indicated that QOL scores did not decrease with radiotherapy. However, there was a negative correlation between symptoms during irradiation and QOL. Therefore, to improve QOL of patients during radiotherapy, nursing intervention is necessary to alleviate these symptoms.

Key Words: radiotherapy, breast cancer, quality of life, acute radiation damage, nursing care

Introduction

Radiotherapy as a form of cancer treatment causes less physical damage and has a lower impact on patients' ability to function compared to conventional surgical therapies. The outcome for breast-cancer patients using radiotherapy is particularly favourable; radiotherapy is used after breast-conserving surgery and mastectomy, as preoperative therapy for advanced breast cancer, and as treatment for recurrent and metastatic breast cancers. The Japanese Breast Cancer Society published the "Breast Conservation Therapy Guideline" in 1999, which recommends that irradiation after breast-conserving surgery should be started promptly. Thus, in Japan, breast-conserving surgery and postoperative irradiation are widely used in the treatment of early-stage breast cancer.

Radiotherapy is effective, but symptoms of acute radiation syndrome such as fatigue and dermatitis are accompanied by physical and mental suffering, contributing to decreased QOL in these patients. Thus, the purpose of this study was to clarify QOL and symptoms of acute radiation damage in breast-cancer patients undergoing radiotherapy.

[*] Corresponding to: Noriko Ogura, Lecturer, Hirosaki University Graduate School of Health Sciences, 66-1, Hon-cho, Hirosaki, 036-8564, Japan. E-mail: ogu1224@cc.hirosaki-u.ac.jp

Methods

Patients

Subjects were 47 breast-cancer patients treated with radiotherapy at Hirosaki University School of Medicine and Hospital.

Methods

1. Basic information

Data was obtained from medical charts regarding types, dose, and method of irradiation.

2. Symptoms during irradiation

We generated a "symptom diary"(Figure 1), and the patients were requested to record their symptoms every day from the day before irradiation until completion of irradiation. Irradiation was conducted 5 times a week. The recorded symptoms were classified into Grade 1 to Grade 5 by the physicians according to the Common Terminology Criteria for Adverse Events v3.0, Japanese translation JCOG/JSCO.

3. QOL

The SF-8^{TM} standard version was used to determine patient QOL. The survey covered 8 areas: physical functioning (PF), role physical (RP), body pain (BP), general health perceptions (GH), vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH). The survey was conducted 3 times: before irradiation, after completion of irradiation, and 4-6 weeks after irradiation.

Patient's name

Date of hospitalization

Date of irradiation starting

Site;Breast

		Sun.	Mon.	Tues.	Wed.	Thurs.	Fri.	Sat.
Date		/	/	/	/	/	/	/
Irradiation days		Before	1	2	3	4	5	
Symptoms and strength	Fatigue							
	Loss of appetite							
	Nausea							
	Vomiting							
	Loss of weight							
	Dermatitis							
	Itching							
	Pain(Site:)							
	Pain(Site:)							
If you have troubled, please write symptoms.								

Figure 1*: Sample of symptom diary*

4. Clinical data

Clinical data were collected for leukocytes, lymphocytes, granulocytes, erythrocytes, platelets, hemoglobin, total protein, albumin, and CRP. Data were obtained from medical charts when the patients had a test.

Ethical considerations

This study was approved by the Ethics Committee of Hirosaki University Graduate School of Medicine. The intent of the study was explained to the patients, and the study was conducted after obtaining informed consent.

Data collection period

Data was collected from July 2010 until May 2011.

Results

Basic information

The average age of the patients was 56.2 ± 10.2 years. The type of radiation was X-ray, the number of irradiations was 25 to 30, and the total irradiation dose was 50 to 60 Gy.

Symptoms occurring during irradiation

Figure 2 shows the appearance of symptoms. The average of the symptom scores was calculated and 1 point was designated Grade 1; 2 points, Grade 2, and 3 points, Grade 3.

Fatigue appeared in 25 of 47 patients; 20 patients were classified as Grade 1 and 5 patients as Grade 2. Six patients already had fatigue before irradiation. Pain appeared in 37 of 47 patients; 33 patients were classified as Grade 1, 3 patients as Grade 2, and 1 patient as Grade 3. Six patients already had pain before irradiation. Many patients complained of pain in multiple areas, and by the 15th irradiation, pain had appeared in approximately 50% of all patients. Dermatitis appeared in 30 of 47 patients; 23 patients were classified as Grade 1, 6 patients as Grade 2, and 1

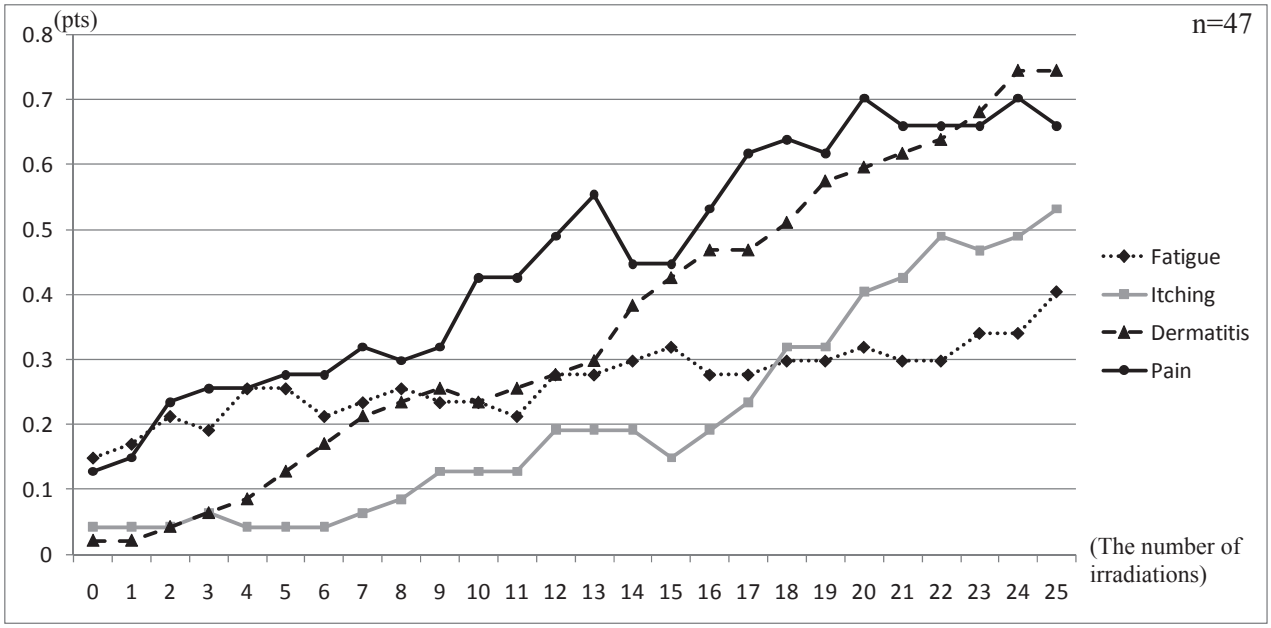


***Figure 2**: Symptoms occurring during irradiation*

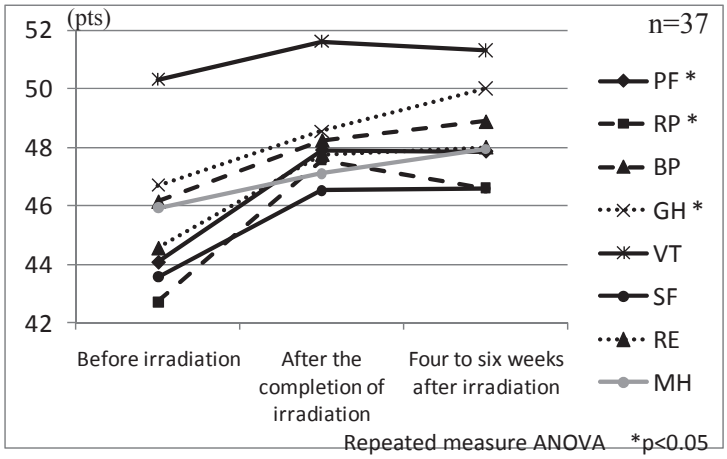


***Figure 3**: Changes in QOL scores*

***Table 1**: Symptom scores during irradiation and relation with QOL*

n=36

	After the completion of irradiation		Four to six weeks after irradiation	
PF	−0.280		−0.045	
RP	−0.290		−0.139	
BP	−0.685	***	−0.652	***
GH	−0.632	***	−0.480	**
VT	−0.457	**	−0.341	*
SF	−0.259		−0.288	
RE	−0.345	*	−0.240	
MH	−0.385	*	−0.370	*

Pearson's product-moment correlation coefficient
*** p<0.001 ** p<0.01 * p<0.05

patient as Grade 3. By the 15th irradiation, dermatitis had appeared in approximately 50% of all patients. Itching appeared in 31 of 47 patients; 29 patients were classified as Grade 1, 1 patient as Grade 2, and 1 patient as Grade 3. By the 20th irradiation, itching had appeared in approximately 50% of all patients.

Overall, the symptoms tended to appear from around the third week of irradiation. It was clear that fatigue and pain tended to appear at an early stage; dermatitis and pain were seen in at least half the patients when the irradiation dose reached approximately 30 Gy. Itching appeared in half the patients when the irradiation dose reached approximately 40 Gy and tended to appear somewhat later than dermatitis.

Changes in QOL scores

Changes in the QOL scores in 8 areas are shown in Figure 3. The scores before irradiation were slightly lower than the Japanese national average (women). After completion of irradiation, the scores increased in all 8 areas, but the scores 4–6 weeks later were not significantly different from the scores at the completion of irradiation.

Significant differences were seen in PF, RP, and GH. PF and RP scores were higher after completion of irradiation and 4-6 weeks after irradiation than before irradiation. The GH score was higher 4–6 weeks after irradiation than before irradiation.

Symptom scores during irradiation and relation with QOL

Because most symptoms tended to appear from around the third week of irradiation, the grades for symptoms that appeared 3–5 weeks after the start of irradiation (11 to 25 irradiations) were incorporated into the scoring system. The correlation coefficient with QOL scores was calculated (Table 1). The symptom scores were negatively correlated with QOL scores.

Clinical data

Subjects consisted of 6 patients. Significant differences were seen in leukocytes, lymphocytes, and platelets. These counts decreased in the third through the fifth week of irradiation. Other clinical measures remained stable (Figure 4-7).

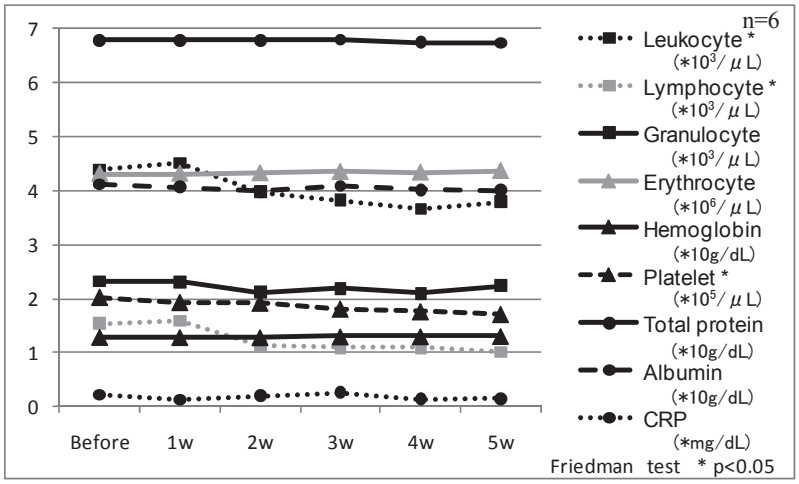


***Figure 4**: Clinical data*

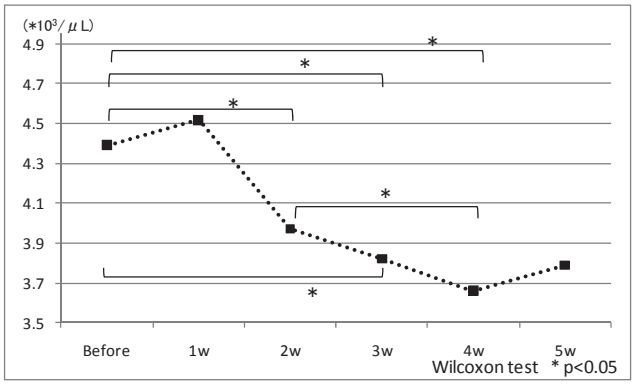


***Figure 5**: Leukocyte*

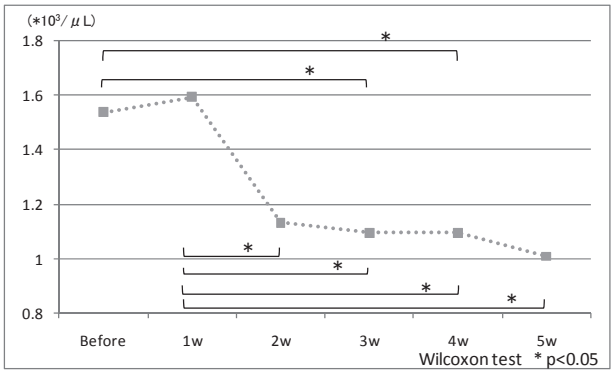


***Figure 6**: Lymphocyte*

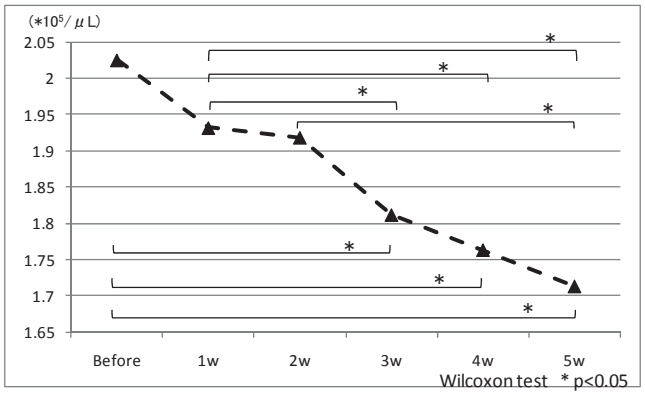


***Figure 7**: Platelet*

Conclusion

The results from our analysis indicate that QOL scores did not decrease with radiotherapy.

In some cases, fatigue and pain appeared at an early stage and it was presumed that this was caused by surgery or chemotherapy rather than from radiotherapy. However, in the latter half of the irradiation regimen, dermatitis and itching appeared in approximately half of the patients, and there was a negative correlation between the symptoms during irradiation and QOL. Therefore, to improve QOL of patients during radiotherapy, nursing intervention is necessary to alleviate these symptoms.

Acknowledgment

We are grateful to the patients who supplied us the symptom and QOL data. This study was supported by a Gant for Co-medical Education Program in Radiation Emergency Medicine by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Perception of radiation risk among first-year students of a health sciences school -Differences before and after the March 2011 Fukushima nuclear power plant disaster-

Keiko Aizu[1], Chieko Itaki[2], Toshiko Tomisawa[1], and Ayako Ohgino[2]

Departments of [1]Health Promotion and [2]Disability and Health, Division of Health Sciences, Hirosaki University Graduate School of Health Sciences

Abstract. The aim of this study was to clarify the differences in radiation risk perception between first-year students who entered a school of health sciences before and after the March 2011 Fukushima nuclear power plant disaster. A questionnaire survey regarding radiation was conducted in 196 students who entered the school in 2010, and 199 students who entered in 2011. The items of "exposure" and "nuclear power generation" scored higher as radiation-associated terms in the 2011 group than in the 2010 group. The items of “difficulty in understanding radiation” and "fearfulness" had a positive correlation, while “difficulty in understanding radiation” and "understanding of radiation" had a negative correlation in the 2011 group. It seems that educating accurate knowledge can ease the excessive fear about radiation. It is necessary to examine and tailor educational curriculums according to the current student perception of radiation.

Key words: perception of radiation risk, education, students of a health sciences school.

Introduction

It is important in risk communication that those who are engaged in medical treatment have an appropriate perception about radiation. Itaki et al.[1)] have stated that appropriate perception of risk and interest in radiation are influenced by educational content. Moreover, in order to educate students effectively, it is important to grasp the students’ interest in radiation and knowledge about radiation.

On the other hand, various reports about radiation have been relayed by mass media since the March 2011 Fukushima nuclear power disaster. Students entering a school of health sciences are assumed to have had many opportunities to be exposed to various types of information about radiation and its risks before they entered the school and could start any special study about the use of radiation in medical treatment. It is possible that such information from mass media has affected the formation of the students’ risk perception. The aim of this study was to clarify the differences in radiation risk perception of first-year students enrolled in the Hirosaki University School of Health Sciences before and after the March 2011 Fukushima nuclear power plant disaster, and to consider the results as fundamental data on risk communication about medical radiation.

Methods

The subjects were first-year students enrolled in the Hirosaki University School of Health Sciences. A questionnaire survey was conducted in 196 students who entered the school in 2010 (2010 group), and 199 students who entered in 2011 (2011 group). The survey consisted of eight

Corresponding to: Keiko Aizu, Assistant Professor, Hirosaki University Graduate School of Health Sciences, 66-1, Hon-cho, Hirosaki, 036-8564, Japan. E-mail: aizu@cc.hirosaki-u.ac.jp

questions about the risk perception of radiation, one question about the health safety risks in daily life, and one question about the health risk of radiation. Data analyses were done using the chi-square test, the Mann-Whitney test, and Spearman's rank correlation coefficient.

Results

In regard to the image of radiation, the items of "exposure" and "nuclear power generation" scored significantly higher, and "Mr. and Madame Curie" and "cancer treatment" scored significantly lower, as radiation-associated terms in the 2011 group than in the 2010 group (Figure.1). In regard to radiation-related items in which they have interest in knowing more about, "correspondence when an accident involving radiation exposure happens" scored significantly higher in the 2011 group than in the 2010 group (Figure.2). In regard to perceived health safety risks in daily life, "X-rays and CT applications"

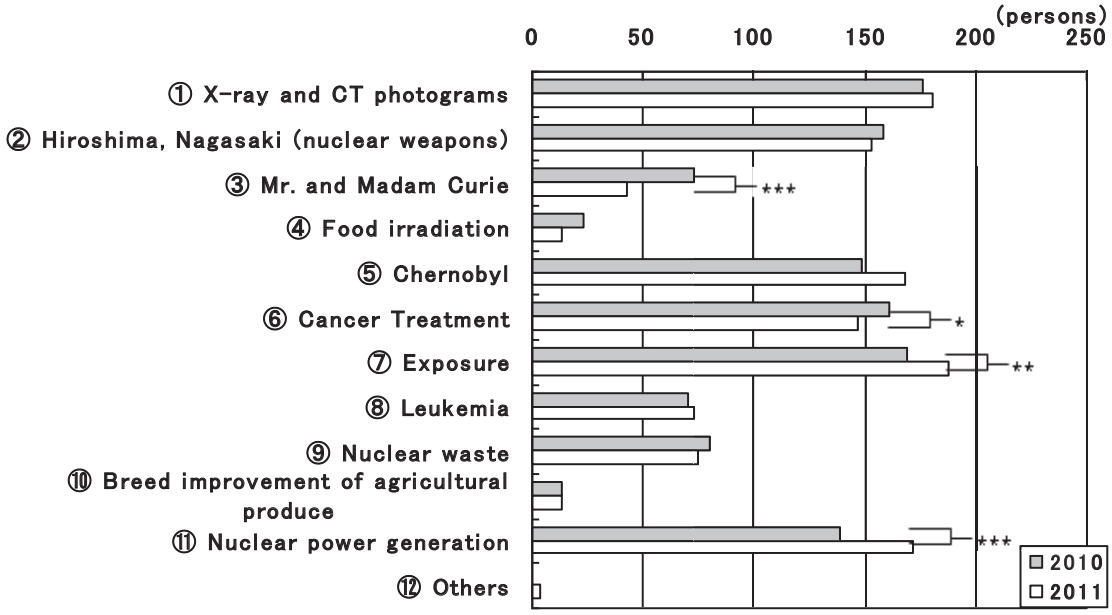


Figure.1 Terms associated with “radiation” (Unlimited answers)
(2010 group n=196, 2011 group n=199) Data analyses were done using the chi-square test. *=p<0.05, **=p<0.01, ***=p<0.00

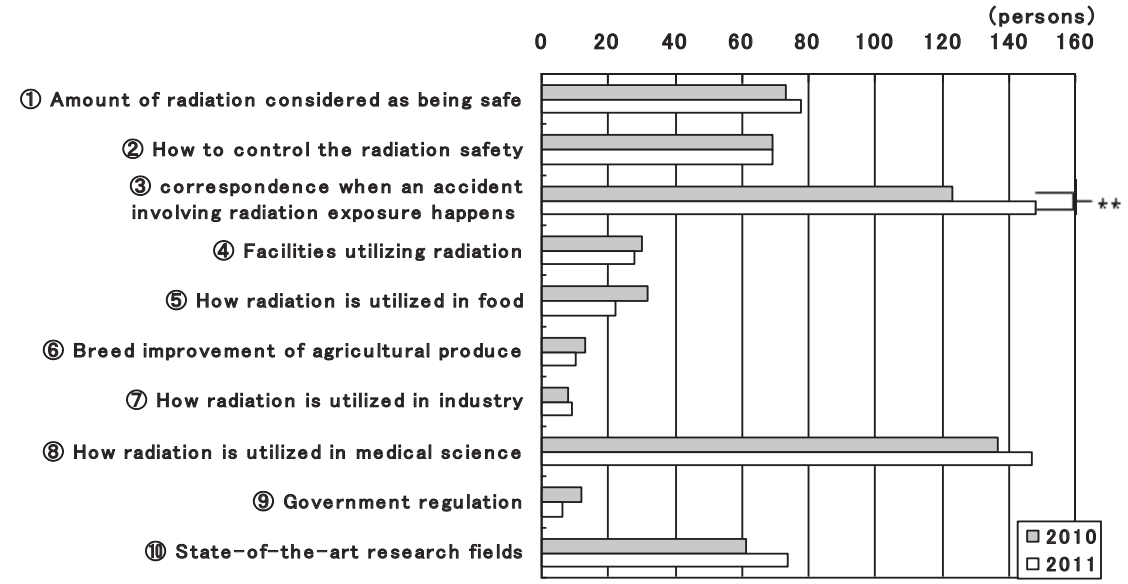


Figure.2 Items of interest to the students regarding”radiation” (Selection of up to three items)
(2010 group n=196, 2011 group n=199) Data analyses were done using the chi-square test. *=p<0.01

scored significantly lower, and "infection with *Escherichia coli* 0157" scored significantly higher in the 2011 group than in the 2010 group (Table.1). In regard to perceived risks of radiation on health, "Radiological treatment", "Air travel", "Rocks and soil(naturally radioactive material)", "CT scan", "Chest X-ray photograms" and "Airport baggage inspection" scored significantly lower in the 2011 group than in the 2010 group (Table.2).

In regard to the perceived fearfulness, difficulty in understanding, interest in, and understanding of the effects of radiation, the subjects were asked to answer questions by choosing from four options: (1) yes, (2) a little, (3) not much, (4) not at all. In regard to the fearfulness of radiation, there was a significant difference between the 2010 and 2011 groups: "(2) a little" (85 persons, 43.81%) and "(3) not much" (52 persons, 26.80%) scored highly in the 2010 group, while "(1) yes" (72 persons, 37.69%) and "(2) a little" (100 persons, 50.25%) scored highly in the 2011 group. There was also a significant difference between the groups in regard to the difficulty in understanding radiation: "(2) a little" (88 persons, 45.36%) and "(3) not much" (54 persons, 27.84%) scored highly in the 2010 group, while "(1) yes" (76 persons, 38.19%) and "(2) a little" (112 persons, 56.28%) scored highly in the 2011 group. In regard to interest in radiation, there was a significant difference between the groups: "(2) a little" (72 persons, 37.11%) and "(3) not much" (62 persons, 31.96%) scored highly in the 2010 group, while "(1) yes" (83 persons, 41.71%) and "(2) a little" (102 persons, 51.26%) scored highly in the 2011 group. In response to "Do you understand the effects of radiation on the human body?", "(2) a little" (131 persons, 67.53%) and "(3) not much" (114 persons, 26.80%) scored highly in the

Table 1. Ranking of perceived health safety risks

Rank	2010 group	Score	2011 group	Score	P-value
1	HIV (AIDS)	1.97	HIV (AIDS)	1.93	
2	Hepatic fever	3.05	O157 (bacteria)	2.74	**
3	O157 (bacteria)	3.76	Hepatic fever	2.85	
4	Smoking (cigarettes)	4.91	Smoking (cigarettes)	4.50	
5	Surgery	5.56	Surgery	5.82	
6	Antibiotics	6.42	Antibiotics	6.55	
7	Riding a motorcycle	6.53	Obesity (overweight)	6.91	
8	Obesity (overweight)	7.05	Riding a motorcycle	7.12	
9	X-rays and CT applications	7.64	Drinking (alcoholic beverage)	7.92	
10	Drinking (alcoholic beverage)	8.10	X-rays and CT applications	8.65	**

Note. The subjects were asked to rank the items, with the most risky item as "1" and the least risky as "10". (2010 group n=187, 2011 group n=194) Data analyses were done using the Mann-Whitney test. **p<0.01
HIV=human immunodeficiency virus; 0157=*Escherichia coli* 0157; CT=computed tomography

Table 2. Ranking of perceived risks of radiation on health

Rank	2010 group	Score	2011 group	Score	P-value
1	Living near a nuclear power plant	7.04	Living near a nuclear power plant	7.20	
2	Nuclear testing in other countries	6.87	Nuclear testing in other countries	6.74	
3	Cosmic rays	5.60	Cosmic rays	5.08	
4	Radiological treatment	4.66	Radiological treatment	3.80	**
5	Radium/radon hot springs	3.02	Radium/radon hot springs	2.58	
6	Air travel	2.83	Air travel	2.47	**
7	Rocks and soil	2.80	Rocks and soil	2.03	**
8	CT scan	2.77	CT scan	1.88	**
9	Chest X-ray photograms	2.61	Chest X-ray photograms	1.85	**
10	Airport baggage inspection	1.58	Airport baggage inspection	1.11	**

Note. The subjects were asked to rank the items, with the most risky item as "10" and the least risky as "1". (2010 group n=193, 2011 group n=195) Data analyses were done using the Mann-Whitney test. **p<0.01
CT: computed tomography

2010 group, and was similar to the 2011 group, in which "(2) a little" (80 persons, 40.20%) and "(3) not much" (114 persons, 57.29%) scored highly as well (Figure.3).

The items of "fearfulness" and "difficulty"/"interest", and "difficulty" and "interest" had significantly positive correlations in the 2010 group. "Difficulty" and "fearfulness", "interest" and "understanding the effects of radiation" had significantly positive correlations, and "difficulty" and "understanding the effects of radiation" had a significantly negative correlation in the 2011 group.

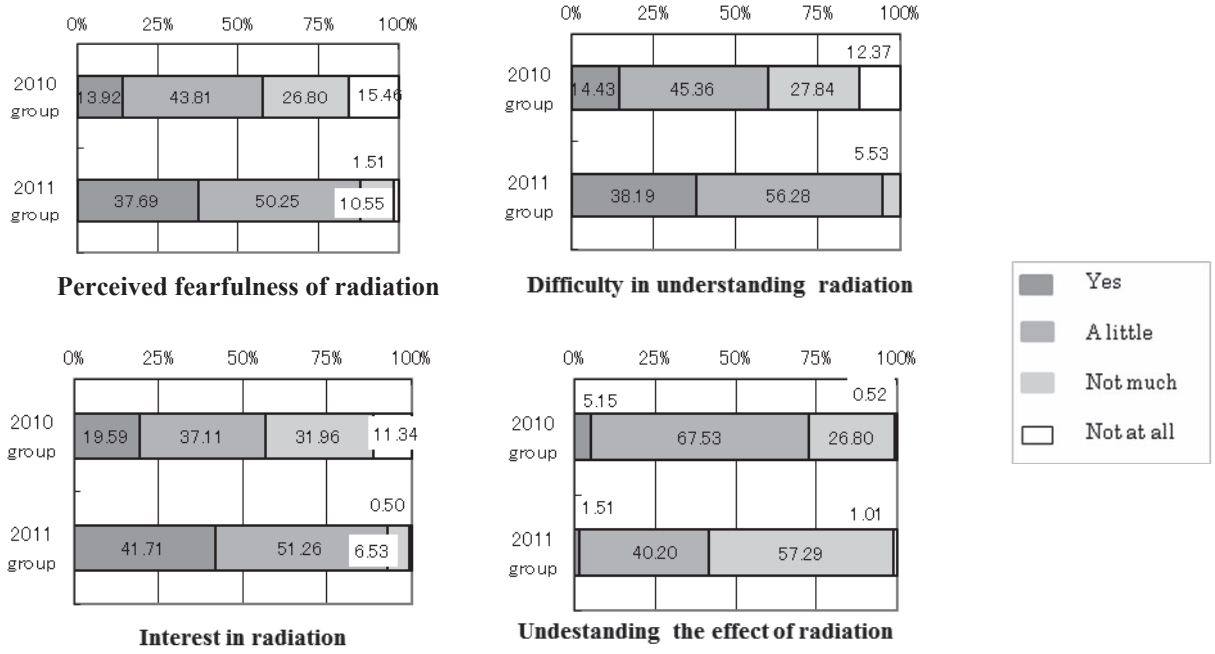


Figure.3 Fearfulness, Difficulty of knowing, Understanding, Interest about radiation.
(2010 group n=194, 2011 group n=199)

Discussion

Saito & Kusama[2] investigated the perception of radiation and reported that 89% of high school students and 94% of college students reported "television" as their main source of information about radiation. Moreover, Combs & Slovic[3] have said that the influence of media is great in forming the general public's perception of risk. We consider that the reports by mass media on the nuclear accident in March 2011 have affected the risk perception of the first-year students, as the items of "exposure" and "nuclear power generation" scored significantly higher as radiation-associated terms, and "correspondence when an accident involving radiation exposure happens" scored significantly higher as an item of interest regarding radiation in the 2011 group than in the 2010 group. Moreover, during the same period, a mass food poisoning incident in a restaurant chain broke out, and many reports about food poisoning were being relayed by media in Japan. That is also considered a reason for why the item of "infection with *E. coli* O157" scored higher as a risk in daily life in the 2011 group.

While the 2011 group had a higher recognition of the fearfulness of radiation, they had a lower recognition of radiation risk in a medical sense when compared to the 2010 group. Okamoto[4] pointed out that mass media often tend to depict bereaved families' feelings about risk, and pointed out that such a tendency creates fear that leads to the loss of balance in the general public's perception of risk. From the results of this study, it seems that the first-year students had lost balance in their perception of radiation risk, and that radiation related to medicine and radiation of nuclear power plants are recognized as different things. From the

above point, it appears that the perception of radiation risk is influenced by social problems and media more than by scientific knowledge at the time of school entrance. Moreover, the more highly students recognized the risk of radiation, the more they thought that radiation was not difficult to understand, and these students were more interested in radiation. Since perceived difficulty increases fear, it seems that teaching accurate radiation knowledge can ease excessive fear of radiation, and will help students to form a correct perception about radiation. It is necessary to examine and tailor educational curriculums according to the current student perception and interest in radiation.

Conclusion

While the first-year students who entered school after the March 2011 Fukushima nuclear power plant disaster had a high perception about the risk of radiation, they had a low perception about radiation used in medical treatments. Moreover, more students answered that they are reminded of the terms "exposure" and "nuclear power generation" as associative items about radiation, and showed interest in "correspondence when an accident involving radiation exposure happens". It is necessary to examine and tailor educational curriculums according to the current student perception and interest in radiation.

References

[1] Itaki, C., Tomisawa, T., Ohgino, A. & Aizu, K. Study on risk communication in education of radiation protection: Risk perception in health sciences students. The Proceedings of the 2nd International Symposium on Radiation Emergency Medicine at Hirosaki University. 65-69 (2010).

[2] Saitoh,S., & Kusama, T. Ippan Kousyuu no housyasen /housyanou ni kansuru ninsiki no teido to ninsikikatei. Hokenbutsuri. 27,23-26 (1992).

[3] Combs,B., & Slovic, P. Newspaper coverage of causes of death. Journalism Quarterly. 56,837-843 (1979).

[4] Okamoto, K. Risuku to Masukomi. Risuku sinrigaku nyuumon. Tokyo. 113-136 (1992).

Preparing a paper on the proceedings of an exploratory study on the preparation of public health nurses for a radiation disaster based on the experience of health care in areas affected by the Tokai Village nuclear accident

Chiaki Kitamiya[1*] and Ruriko Kidachi [2]

[1] Department of Health Promotion, Division of Health Sciences, Graduate School of Health Sciences, Hirosaki, Japan
[2] Department of Development and Aging, Division of Health Sciences, Graduate School of Health Sciences, Hirosaki, Japan

Abstract. We attempted to reconstruct the experience of public health nurses who respond to a radiation disaster based on an analysis of narrative accounts of the Tokai Village JCO nuclear accident. This study was done through half-structured interviews. It was approved by the Committee of Medical Ethics of Hirosaki University Graduate School of Medicine, Hirosaki, Japan. Interviews were conducted at Higasidori Village in Aomori on November 8, 2010 and at Tokai Village, Naka City, Hitachinaka City, Hitachi-Omiya City, Ibaraki prefectural office, Ibaraki Hitachinaka Health Center, and Ibaraki Hitachi-Omiya Health Center in Ibaraki on November 24 and 25, 2010. The average interview lasted 30 minutes to 1.5 hours. In this analysis, data from interviews in three cities surrounding Tokai Village were compiled. As a result of a qualitative analysis of this data, three categories were extracted from nine subcategories: (1) activity for mitigating residents' anxiety; (2) mental attitude toward a radiation disaster; and (3) training of public health nurses as members of the local government. By continuing training based on the experience gained in the JCO accident, we can prepare inexperienced persons who may need to respond to future emergencies. The data we gathered on the JCO disaster established the need to prioritize mitigation of residents' anxieties as a critical role for public health nurses.

Key Words: radiation disaster, public health nurse, health crisis management, preparation

* Corresponding to: Chiaki Kitamiya, Associate Professor, Hirosaki University, 1-66, Hontyou,036-8564, Japan
E-mail:chiaki@cc.hirosaki-u.ac.jp

Introduction

Immediately after the fatal accident known as the JCO accident occurred in 1999 at the Tokai uranium conversion plant operated by JCO Co. Ltd., approximately 220 public health nurses provided mental health care to residents at shelters, first-aid stations, and health centers. While a small number of the public health nurses had attended a lecture on special training, many did not have training experience for emergencies such as this one. Many of the public health nurses received at least some part of a lecture about radiation and mental health care immediately after the accident.

Purpose

We attempted to reconstruct the experience of public health nurses who respond to a radiation disaster based on an analysis of narrative accounts of the Tokai Village JCO nuclear accident.

Method

Method of research

The study was conducted using the half-structured interviewing method.

Procedure

The study requests were mailed to each local government and health-care center. The main points of the study, its purpose, and content were included in the document. Consent was obtained by mail. Recommendations for candidates were requested from the organizations that expressed their intention to cooperate in the study.

A cooperation request document was mailed to the candidates before the interviews began. The main points of the study, purpose, details of the study, and ethical considerations were specified in the document. After obtaining consent to participate, we coordinated the time and place of the interview with the candidate.

We provided a verbal and written explanation of the ethical considerations to the candidate at the time of the interview. Interviews began after obtaining written informed consent.

This study was approved by the Committee of Medical Ethics of Hirosaki University Graduate School of Medicine, Hirosaki, Japan.

Interview survey content

・Is the role of a public health nurse required for an emergency drill?

・What is the role of a public health nurse at the time of a disaster such as a radiation disaster?

・How is a public health nurse's experience with previous disasters used in future disasters?

Candidates for the study

Public health nurses from health-care centers and various cities, towns, and villages

Aomori: Higashidoori Village

Ibaraki: Toukai Village, Naka City, Hitachinaka City, Hitachi-Oomiya City, Ibaraki Prefectural Office, Ibaraki Hitachinaka Health Center, Ibaraki Hitachi-Oomiya Health Center

Interviewees

- Public health nurses from cities, towns, and villages: 8 people
- Village mayor: 1 person
- Health center public health nurses: 3 people
- Prefectural office public health nurses: 3 people
- The section head of prefectural health-crisis management: 1 person

Survey data

The candidate's consent was obtained and recorded at the interview. The data in the letter were analyzed as qualitative data. The data provided at the time of the interviews were also used for analysis.

Results

1. Instiutions consenting to the study

Aomori: Higashidoori Village (November 8, 2010)

Ibaraki: Toukai Village, Naka City, Hitachinaka City, Hitachi-Oomiya City, Ibaraki Prefectural Office, Ibaraki Hitachinaka Health Center, Ibaraki Hitachi-Oomiya Health Center (November 24–25, 2010)

2. Average interview time

30 minutes–1.5 hours

3. Experience of the local governments near the JCO accident

Table 1. The structure of the narrative

Category	Subcategory
Activity for mitigating residents' anxiety	・Health support in a shelter ・Survey and consultation have top priority ・Consultation by public health service
Mental attitude toward a radiation disaster	・To have a willingness to support residents ・To maintain a channel of information through consultation activities ・To learn from an uncertain situation
Training of public health nurses as members of the local government	・It shares that a radiation disaster may occur ・A role as a member of an organization is created ・The public health nurse was undergoing continual training in radiation disasters

Data from the 3 cities around the JCO accident were used in this report. Five participants were in their 50s and 2 participants were in their 40s.

As a result of our analysis, 3 categories and 9 subcategories were determined (Table 1).

The 3 categories were extracted from the 9 subcategories. The role of the public health nurse in relieving the anxiety of residents was a priority in the JCO accident. Their role in general public health services was halted so they could work in the shelters to provide mental health support to affected residents, survey the effects of the radiation, and provide consultation services. This support role continued after the disaster at the request of residents.

The mental attitude that the nurses acquired from the JCO accident was to understand the difficult decisions faced by the residents and to provide mental health care. In order to provide better support to the residents, it was considered important to have as much information as possible. Many public health nurses did not have disaster training or procedural guidelines prior to the JCO accident.

Ten years after the JCO accident, public health nurses have been given the opportunity to continue their training in radiation disasters as members of

an organization as radiation disasters could occur in the future. As such, future generations of nurses who have not experienced the accident first-hand are given the opportunity to receive training based on the expertise of public health nurses who experienced the accident.

Discussion

By continuing training based on the experience gained in the JCO accident, we can prepare inexperienced persons who may need to respond to future emergencies. The data we gathered on the JCO disaster established the need to prioritize mitigation of residents' anxieties as a critical role for public health nurses.

This study was conducted before the nuclear power plant accident in Fukushima. Therefore, it is thought that the recognition of a public health nurse's role has now changed. A future research goal is to investigate the changing role and recognition of public health nurses.

Conclusions

The next experience was drawn from the health care activity.

1) Role in mitigating residents' anxiety.
2) Mental attitude toward a radiation disaster.
3) Training of public health nurses as members of the local government.

Acknowledgments

The cooperation of Mr. Hitoshi Araki of the Ibaraki Hitachinaka health center and Ms. Shizuka Kurauchi of the Hirosaki University Graduate School of Health Science in this study is deeply appreciated.

Awareness of nuclear accidents among visiting nurses and home caregivers in communities neighbouring nuclear plants

Daisuke Murakami[1*],Ruriko Kidachi[2], Chikako Yonaiyama[2], Haruka Otsu[2], Yu Kitajima[2], and Chiaki Kitamiya[2]

[1]Hirosaki University of Health and Welfare, Hirosaki, Japan
[2] Division of Health Sciences, Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan

Abstract. This study was conducted to clarify if visiting nurses and home caregivers working in prefectures with nuclear plants are aware of their responsibilities and the measures they would need to take in identifying those who might need assistance in the event of a nuclear accident.Subjects were employees at visiting nurse stations and home-care helper stations located near nuclear plants in 10 prefectures. Information was collected by a self-administered questionnaire. Consent was obtained from 15facilities (7.85%). Currently, 60 questionnaires have been returned(55.56%). The surveys indicate that interest in radiation is high, but that knowledge of and opportunities to learn about radiation are insufficient. Most respondents imaged “earthquake” from the word “disaster” and"nuclear power plant accident" from the phrase “nuclear accident”. In the free writing section, many comments were in regards to “correct knowledge” and “accurate information”. Other opinions included “I noticed an indifference to nuclear power plants in this accident”. A correlation analysis (Spearman) was performed. As a principal result, “interest in radiation” showed a strong correlation with “interest in nuclear accidents”. “Interest in nuclear accidents” also showed a strong correlation with “interest in disaster drills for nuclear accidents.”

Key Words: radiation, nuclear accident, visiting nurse, home caregiver, natural disaster, nuclear plants

Background

In Japan, the guidelines for responding to a nuclear accident were established by the Fundamental Disaster Relief Act. At the prefectural and municipal levels, there are established regional disaster plans, and disaster drills are conducted. However, guidelines for dealing with individuals requiring assistance during disasters have not been included in any of these plans. Therefore, visiting nurses and home caregivers should play an active role in the establishment of such guidelines. Studies on natural disasters have stated the importance of collaboration among various institutions and individuals.[2][3] Visiting nurses and home caregivers are key players in disaster response because they know the location of people who require assistance after a disaster and the level of assistance required during an evacuation.

Purpose

This study was conducted to clarify if visiting nurses and home caregivers working in prefectures with atomic facilities are aware of their responsibilities and the measures they would need to take in identifying those who might need assistance in the event of a nuclear accident.

[*]Corresponding to: DaisukeMurakami, Research Assistant,Hirosaki University of Health and Welfare, 3-18-1, Sanpinai, Hirosaki, Aomori 036-8102, Japan.E-mail: h10gg108@stu.hirosaki-u.ac.jp

Table1 About interest

	Very interested	interested	Not really interested	Not interested	total
You are interested in radiation.	22	28	6	0	56
You are interested in nuclear accidents	25	26	3	1	55
You are interested in disaster drill for nuclear accidents	16	29	8	3	56

Table2 Knowledge of radiation

	Very knowledgeable	knowledgeable	little	non	total
knowledge of radiation	1	6	48	1	56

Methods

Subjects were employees at visiting nurse stations and home-care helper stations located near nuclear plants in 10 prefectures: Hokkaido, Aomori, Niigata, Ishikawa, Ibaraki, Fukui, Ehime, Shimane, Saga, and Kagoshima. The prefectures of Miyagi, Fukushima, and Shizuoka were not included. Information was collected by a self-administered questionnaire. Requests to participate in the survey were sent to office directors, and consent was obtained along with an agreement on the number of questionnaires that would be completed. The requisite number of copies of the questionnaire was then sent to each director, and employees at the office were asked to complete the questionnaires and return them via mail. The survey began on April 22, 2011 and will continue until March 31, 2012.

Results

Results to date indicate that consent was obtained from 15 of the 191 specialty facilities (7.85%). Of the 108 questionnaires that were sent, 60 (55.56%) of them have been returned, however, 4 were invalid, so there were 56 valid responses.

The rate of response for each prefecture was 100% in Hokkaido, Aomori, and Saga and 92.86%, 0%, 16.67%, 41.38%, 50%, 44.83%, and 58.33% in Niigata, Ishikawa, Ibaraki, Fukui, Ehime, Shimane, and Kagoshima, respectively.

[Distance from nuclear plants]

The responses indicate that the distance of the facilities from nuclear plants was generally more than 21 km (34.55%), and the distance from the respondent's home was most often less than 5 km (55.36%).

[Radiation]

Although interest in radiation was high (very interested and interested: 55.36%)(Table1), there was insufficient knowledge about radiation (little or none: 55.36 %)(Table2). Opportunities to learn about radiation were also insufficient (learned: 32.14%).

[Disasters]

<Natural disasters>

When asked what they associate with the word "disaster", most respondents answered "earthquake" (71.15%). When asked what was necessary to prepare individuals for such a disaster, the most common answer was "to confirm the location of the evacuation site and escape routes" (26.21%, multiple answers).

<Nuclear accidents>

Interest in nuclear accidents was high (very interested and interested: 92.73%)(Table1). And opportunities to learn about radiation were also insufficient (learned: 20.37%).When asked what they associate with the word "nuclear accident", most respondents answered "a nuclear power plant accident" (34.07%, multiple answers). When asked about the differences between natural disasters and nuclear accidents, most respondents answered "prolonged impact" (23.64%, multiple answered), then "confusion in regards to the hazardous area" (21.21%), and then "radiation cannot be felt through the five senses" (20.61%). When asked what was necessary to prepare for a nuclear disaster, respondents most often answered "to confirm the location of the evacuation site and escape routes" (29.41%, multiple answers).

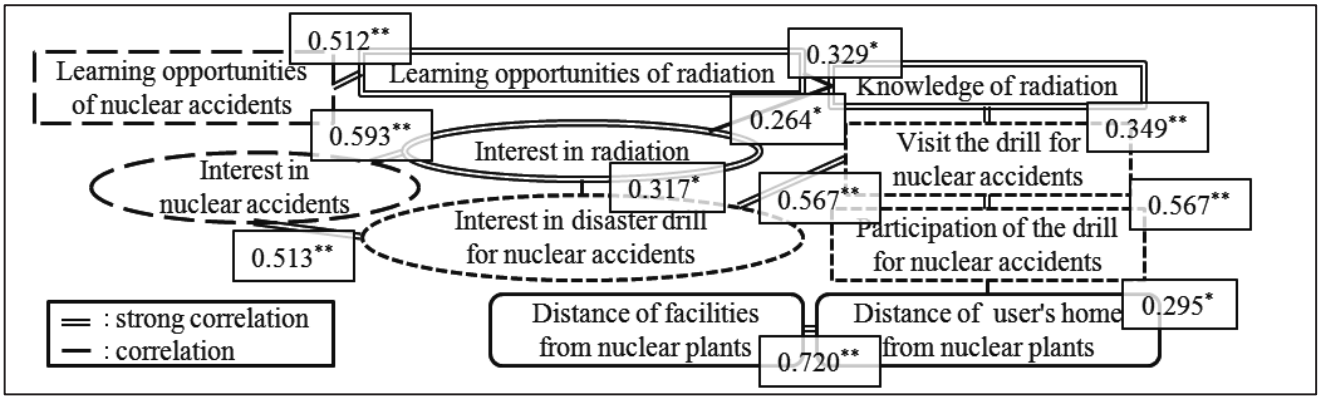


Figure 1 Figure of relationship about correlation

[Free writing responses]

<Necessary from the perspective of the field >

Many comments were received regarding the "correct knowledge" and "accurate information" held by individuals. In addition, a "nuclear accident manual" for each facility was considered necessary.

<Comments>

Some opinions regarding the "awareness of nuclear power plants" were "I noticed that there was indifference towards this accident" and "I did not conscious because the nuclear power plant is too everyday landscape."

[Analysis]

In this study, for analytical methods, a correlation analysis (Spearman) was performed. As a result, "interest in radiation" showed a strong correlation with "interest in nuclear accidents." "Interest in radiation" also showed a correlation with "knowledge of radiation" and "interest in nuclear accident drills." In addition, "interest in nuclear accidents" showed a strong correlation with "interest in disaster drills for nuclear accidents." "Learning opportunities of radiation" showed a strong correlation with "learning opportunities nuclear accidents."“The distance of user's home from nuclear plants" showed a correlation with "participation in drills" (Figure 1).

On the other hand, "whether to participate in disaster activities" was not correlated with the other items. In addition, the distance of the facilitiesfrom a nuclear plant was not correlated with the distance of the respondent's home from a nuclear plant.

Study status

ThisstudywasapprovedbytheResearch Ethics Committee of Hirosaki University. Data collection began in June 2011. Questionnaires are still being collected.

The intention of this report is to communicate the progress of the study. Going forward, we would like to add further discussion and analysis.

Acknowledgments

We are grateful to the directors and employees who participated in the study and to everyone who provided guidance.

References

[1]Mizushima Y, Hayashi K.Study on Disaster Planning at Home-visit Nursing Station -Through Research on Damage and Response Actions at Homevisit Nursing Station in Notohanto Earthquake-, Ishikawa Journal of Nursing vol. 5:39-45(2008)

[2]Ojio Y and others. Study on Disaster Planning for Effort and the Future challenge at Home-visit Nursing Station, Hospics and Home Care, vol. 17(3):263-274(2009)

[3]Mizusima Y, Hayashi K. The current situation and issues of disaster planning at home care nursing agency, Journal of JSDN vol. 9(3):24-30(2008)

[4]Sakai A and others. Disaster NursingTo integrate the expertise of the nursing and associate with the practice, Nanzando (2008)

[5]Kawahara N and others. Disaster preparedness manualVisiting nurse station -Home-care patients and their families-, Journal of JSDN vol. 7(3):28-43(2006)

Current status and issues of radiation emergency medicine education at the School of Health Sciences, Hirosaki University －Focus on undergraduate education－

Saichi Wakayama[1*], Toshiko Tomisawa[2], Mamoru Kubota[3], Shingo Terashima[3], Koichi Ito[4], Takao Osanai[5] and Mitsuaki A. Yoshida[6]

[1] Department of Development and Aging, Division of Health Sciences,
[2] Department of Health Promotion, Division of Health Sciences,
[3] Department of Radiological life Sciences, Division of Medical Life Sciences,
[4] Department of Biomedical Sciences, Division of Medical Life Sciences,
[5] Department of Disability and Health, Division of Health Sciences,
Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan
[6] Department of Radiation Biology, Institute of Radiation Emergency Medicine,
Hirosaki University, Hirosaki, Japan

Abstract. The purpose of this study is to clarify any issues with the current state of undergraduate education in radiation emergency medicine at the School of Health Sciences, Hirosaki University. A course in radiation emergency medicine was established last year as a liberal arts education class for first-year students. The subjects of this study were 245 students in 2010 and 267 students in 2011. TurningPoint 2008 clicker response cards were used to record student responses to several questions about basic radiation during the first day of class. Students were also asked to give their opinions about radiation on the final examination. The results showed that students' knowledge of basic radiation was not the same in 2011 as it was in 2010 because of the March 3, 2011, Great East Japan Earthquake and subsequent disaster at the Fukushima nuclear power plant. In 2010, clicker responses indicated that the students' knowledge of nuclear-related facilities in Aomori Prefecture and their impression of radiation and radioactivity were limited. In regards to the class itself, the students gave many positive opinions such as "it was important that the course contents were duplicated", "I gained a basic understanding of radiation and REM", and "we should be interested in this subject as it is necessary". Some negative opinions were "duplication of course contents", "need to learn more", and "using radiation measuring devices". We are currently analysing the opinions of the 2011 class. The survey results indicated an increasing understanding of the subjects presented in the class, and we obtained positive feedback in regards to the contents of the course. On the other hand, issues of concern with the class were the duplication of education, the course content and study methods, and the ability to create the text. Due to the effects of the nuclear disaster at Fukushima, it is expected that the awareness about health and radiation of the students in the 2011 class will change.

Key Words: radiation emergency medicine, education, undergraduate course school of health sciences

Introduction

The Radiation Emergency Medicine (REM) class "Introduction to Basic Radiation" at Hirosaki University started in April 2010 for first-year students of the School of Health Sciences, except for Radiation Technology course students. In 2011, the class started in May because of the March 3, 2011, Great East Japan Earthquake and nuclear

*Corresponding to: Saichi Wakayama, Professor, Hirosaki University Graduate School of Health Sciences, 66-1 Hon-cho, Hirosaki, 036-8564, Japan, E-mail: swaka@cc.hirosaki-u.ac.jp

disaster at Fukushima. The aim of the class is to provide paramedical personnel with a basic knowledge of radiation protection and an outline of REM as a liberal arts class for first-year students (1). Next year, we will start a faculty education class, “Medical Risk Management”, aimed at providing a further understanding of REM and crisis-management involving cooperation among paramedical personnel (table 1).

The purpose of this study is to clarify any issues with the current state of undergraduate education in the "Introduction to Basic Radiation" class.

Methods

Subjects and procedure

The subjects of this study were students who took the "Introduction to Basic Radiation" class in 2010 and 2011. On the first day of class , we used TurningPoint 2008 clicker response cards to ask the students several basic questions about radiation. We then asked the students for their opinions about radiation on the final examination.

The numbers of students and examination scores were analyzed. The clicker response cards were used to assemble the students’ responses to questions about their knowledge of the nuclear-related facilities in Aomori Prefecture and their impressions of radiation and radioactivity (2). The students’ opinions of radiation solicited on the final examination were also aggregated.

Result

In 2010, 241 students from the School of Health Sciences and 4 other students took the class, and in 2011 the numbers were 240 and 27, respectively (table 2). All the students from the School of Health Sciences passed the class in both years. The average score on the final examination was 81.8 in 2010 and 78.6 in 2011 (table 3).

The clicker response surveys were conducted at the beginning of the class. Responses to question 1, “What nuclear-related facilities are located in Aomori Prefecture?” indicate the students had more accurate knowledge of the situation in 2011 than in 2010 (table 4).

Table 1 REM-related classes for undergraduate students

Subjects	Grade, Semester, Unit	Class Goals
< Liberal Arts Education > Introduction to Basic Radiation	1st Grade, Spring semester 1 Unit, 16 hours	Basic understanding of radiation protection and a general outline of REM
< Faculty Education > Medical Risk Management	3rd Grade, Spring semester 1 Unit, over 30 hours	Further understanding of REM and crisis-management involving cooperation among paramedical personnel

Table 2 Number of Students

	2010	2011
Nurse(N)	160	160
Medical Technology(T)	40	39
Physical Therapy(PT)	21	21
Occupational Therapy(OT)	20	20
Other faculty	4	27
Total	245	267

Table 3 Final Exam. Score

	2010	2011
N	80.2	79.5
T	86.1	82.2
PT	80.5	76.7
OT	79.8	77.3
Other	78.8	73.2
All	81.6	78.6

Table 4 What nuclear-related facilities are located in Aomori Prefecture ?

	2010		2011	
Nuclear power plant	9	5.8%	71	39.9%
Reserves of low-level radioactive waste facility	59	37.8%	9	5.1%
Reserves of high-level radioactive waste facility	55	35.3%	24	13.5%
Nuclear fuel reprocessing plant	15	9.6%	60	33.7%
Nuclear ship 'Mutsu'	18	11.5%	14	7.9%
Total	**156**	**100%**	**178**	**100%**

Responses to question 2, where students were asked for their opinions on radiation and radioactivity, included "scary" 20%, "atomic bomb of Hiroshima and Nagasaki" 42%, "Nuclear power plant" 33%, "Accident of JCO" 2%, and "nothing special" 4% (N=153) in 2010 (table 5-1). In 2011, the items "Nuclear disaster at Chernobyl in Russia" and "Nuclear disaster at Fukushima" were added to the list of choices because of the nuclear disaster at Fukushima on March 3, 2011. These 2 items were frequently chosen (table 5-2). The results for two of the other choices have been omitted.

Results also included the students' opinions on these subjects which they had written on their final examinations for the 2010 class. We received many positive opinions such as "it was important the course contents were duplicated", "I gained a basic understanding of radiation and REM", and "we should be interested in this subject as it is necessary". Negative opinions included "duplication of course contents", "need to learn more", and "using radiation measuring devices".

We are currently analyzing the opinions of the 2011 class.

Discussion

In 2010, the undergraduate Radiation Emergency Medicine Education course "Introduction to Basic Radiation" was started at Hirosaki University. Survey results indicated an increasing understanding of the course, and we obtained positive feedback in regards to the contents of the class. Issues with the class were the duplication of education, course content and study methods, and the ability to create the text.

Students in the 2011 class will be affected by the nuclear disaster at Fukushima, and we expect their awareness of health and radiation exposure to change (2).

References

[1] Radiation Emergency Medicine Committee of Hirosaki University: Radiation Emergency Medicine Human Resource Development Project 2010 Annual Report, 67-75, 2011. (In Japanese)

[2] Japan Atomic Energy Relations Organization Public Opinion Poll on education and dissemination of knowledge and use of nuclear energy (4th), 2010. (In Japanese)

Table 5-1 Impression of radiation and radioactivity

	2010	
Scary	30	19.6%
Atomic bombs of Hiroshima and Nagasaki	64	41.8%
Nuclear power plant	50	32.7%
JCO Accident	3	2.0%
Nothing special	6	3.9%
Total	**153**	**100%**

Table 5-2 Impression of radiation and radioactivity

	2011	
Nuclear disaster at Chernobyl in Russia	58	32.6%
Atomic bombs of Hiroshima and Nagasaki	30	16.9%
Nuclear power plant	13	7.3%
JCO Accident	2	1.1%
Nuclear disaster at Fukushima	75	42.1%
Total	**178**	**100%**

Rehabilitation targeting disuse syndrome in elderly evacuees following radiation exposure accidents: Literature review

Hirokazu Narita[1*], Shuhei Koeda[1], and Hitoshi Tsushima[1]

[1] Department of Development and Aging, Division of Health Sciences, Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan

Abstract. After the nuclear accident at Fukushima nuclear power plant No. 1, many residents living close to the power plant were forced into an unfamiliar life as an evacuee. As this evacuation life continued, health problems were observed in the frail, elderly people. Given this situation, it seemed that rehabilitation for the health problems of elderly evacuees was required as one of the radiation emergency medicines. Therefore, the aim of this study was to consider rehabilitation and its effects by reviewing reports of the rehabilitation offered to evacuees in previous disasters. We found that the conditions targeted for rehabilitation included disuse syndrome and deep vein thrombosis (DVT). The elderly were known to be at a high risk of developing disuse syndrome and DVT, and preventing these was considered to be very important. In order to prevent these conditions, exercising the lower limbs was especially important and had an observable effect on the evacuee. However, as a clot isolated during exercise might lead to pulmonary thromboembolism in DVT-affected persons, we thought that rehabilitation should be undertaken in conjunction with an assessment of DVT. Additionally, in a nuclear-power disaster such as Fukushima, we determined that it was imperative to convey correct information about radiation to evacuees and to reduce their anxiety about the situation.

Key Words: evacuee, deep vein thrombosis, disuse syndrome, radiation emergency medicine

Introduction

We reviewed the literature about rehabilitation for patients with large dose radiation exposure in nuclear accidents such as the Tokai-mura JCO criticality accident, and we considered the role of rehabilitation in radiation emergency medicine [1]. As a result, the positive effects of rehabilitation on severely exposed patients and the importance of rehabilitation focussed on radiodermatitis and respiratory management were specifically determined. However, because there were very few reports about rehabilitation offered to radiation exposed patients, it is uncertain how early rehabilitation should be conducted. The influence of physical stimulation, such as a range of motion exercises, to exposed skin and muscle also remains unclear. Since this point requires further investigation, we are now preparing an experimental system using an animal model to investigate the effects of rehabilitation on large dose radiation exposed patients. Meanwhile, due to the accident at the Fukushima nuclear power plant caused by the Great East Japan Earthquake on March 11th, 2011, residents living close to the power plant were forced into an unfamiliar life as evacuees. As this evacuation life continued, health problems, such as disuse atrophy, were observed in the frail, elderly persons. Given this situation, it seemed that rehabilitation for the health problems of elderly evacuees was required as one of the radiation emergency medicines. Therefore, the aim of this study is to consider the conditions targeted for rehabilitation and the effect of this rehabilitation by reviewing reports of rehabilitation offered to evacuees in prior disasters.

[*] Corresponding to: Hirokazu Narita, Assistant Professor, Hirosaki University Graduate School of Health Sciences, 66-1 Hon-cho, Hirosaki 036-8560, Japan. E-mail: narihiro@cc.hirosaki-u.ac.jp

Methods

Ichushi ver5 (Japana Centra Revuo Medicina) and PubMed were used as the reference data bases of the Japanese and English literature respectively. In Ichushi, the literatures in which only the abstract was registered were excluded. The terms "evacuation center", "evacuation center <and> rehabilitation", "evacuation center <and> earthquake", "earthquake <and> rehabilitation", and "evacuation center <and> earthquake <and> rehabilitation" were used as key words for searches.

Results

In PubMed, 1216 articles were found using the search term "evacuation center". 55, 16, 81, and 1 articles were found using the search terms "evacuation center <and> rehabilitation", "evacuation center <and> earthquake", "earthquake <and> rehabilitation", and "evacuation center <and> earthquake <and> rehabilitation", respectively.

In Ichushi, 164 articles were found using the search term “evacuation center” and 12, 99, 75 and 9 articles were found using the search terms "evacuation center <and> rehabilitation", "evacuation center <and> earthquake", "earthquake <and> rehabilitation", and "evacuation center <and> earthquake <and> rehabilitation", respectively. Further investigation of the retrieved literature confirmed that deep vein thrombosis (DVT) and disuse syndrome were objectives of rehabilitation for evacuated persons in previous disasters.

Discussion

1. Importance of rehabilitation on DVT prevention

DVT is a clinical condition that causes a venous return disorder and congestion, by thrombotic occlusion, in the deep veins of the leg. Life-threatening vascular obstructions due to loose blood clots, such as a pulmonary thromboembolism, are a particularly troublesome problem in DVT. In case of the Niigata Chuetsu earthquake on October 23rd, 2004, the DVT occurred in 17.9 % of the evacuees and there were more than 10 patients with pulmonary thromboembolism especially in the refugees living in cars. Consequently it was reported that at least four of them have died [2].

It is important to consider the prevention of DVT in the case of a natural disaster. According to a guideline for the diagnosis, treatment, and prevention of pulmonary thromboembolism and deep vein thrombosis [3], the usual annual incidence of DVT is 12 per 100 thousand population (0.012 %). However, the incidence of DVT among the evacuees and victims in prior Japanese earthquakes, as shown in Table 1, was reported to be 4.9 to 17.9 % [2, 4-7]. Because these studies are different in several respects, such as investigation period, post-disaster time and targeting age, the results should not be directly compared. Nonetheless, it does appear that the incidence of DVT among the evacuees and victims of previous Japanese earthquakes is much higher than the general incidence of DVT. According to the guideline, elderly and prolonged bed rest are classified as moderate risk factors in strength of additional risk factor for venous thromboembolism [3]. Therefore, a major cause of deep-vein thrombosis in the elderly persons who live in evacuation centers appears to be the prolonged cramped posture and reduced activity due to the unfamiliar environment. The most significant treatment strategy for DVT is prevention. Available methods to prevent DVT are interventions, such as active exercise, walking, massage, and elastic stockings [3], from the preclinical stage. Specifically, ankle dorsiflexion exercise seems to be effective because of the pumping action of the muscles and the improvement of the venous return in the legs [8], and it is possible to easily perform in an evacuation center. However, in some cases affected by DVT, leg exercises and compression with elastic stockings may promote thrombotic isolation and pulmonary thromboembolism. Therefore, rehabilitation for evacuees should be conducted in conjunction with an assessment of DVT and with the cooperation of doctors and other staff.

Table 1. DVT incidence of the evacuees and victims in prior Japanese earthquakes

Earthquake	subject	DVT positive	Incidence rate
Niigata Chuetsu [2]	67	12	17.9%
Noto Peninsula [4,5]	198	21	10.6%
	207	16	7.7%
Niigata Chuetsu-oki [6]	995	49	4.9%
Iwate-Miyagi Nairiku [7]	113	17	15.0%

2. Importance of rehabilitation on disuse syndrome prevention

Disuse syndrome is a generic term for the decline in various mental and physical functions caused by being in a continuous rest state for a long period of time. The principal cause of disuse syndrome in elderly evacuees is thought to be reduced activity and mental stress due to being in an unfamiliar environment such as an evacuation center. The aforementioned DVT is also a disuse syndrome. A decrease in the amount of activity triggers the disuse syndrome, which then leads to the decline of activity of daily living (ADL) and to prolonged bed rest. This decreased ADL and prolonged bed rest leads to a further decline in activity, and a vicious cycle is eventually created. In order to break this negative spiral, it is necessary to promote getting out of bed and actively exercising the lower extremities. Evacuation centers will have to be improved, by installing handrails for example, to prevent the decline of evacuees' activity and to enable the elderly persons to move more easily.

3. Specific considerations in a nuclear power disaster

In the case where an atomic accident is combined with a natural disaster, like the case in Fukushima, evacuees have multiple anxieties, such as the uncertainty about when they will be able to return home and the fear of invisible radioactive material. Therefore, it seems to be necessary that rehabilitation specialists be able to convey correct knowledge and information about radiation to the evacuees and to reduce their anxiety and stress.

References

[1] Narita H, et al.: Considering necessity and feasibility of rehabilitation for radiation-exposed patients from literature review, J Health Sci Res. 2011; 1:49-54.

[2] Hanzawa K, et al.: Acute Pulmonary-venous thromboembolism in Niigata Chuetsu earthquake, Heart. 2007; 39:104-9. (in Japanese)

[3] JCS joint working group: Guidelines for the diagnosis, treatment and prevention of pulmonary thromboembolism and deep vein thrombosis (JCS 2009) -Digest version-, Circ J. 2011; 75:1258-81.

[4] Terakami T, et al.: Deep vein thrombosis in Noto Peninsula earthquake victims, Rinsho Byori. 2009; 57:411-6. (in Japanese)

[5] Sato H, et al.: The meaning of the exercise in the seismic hazard shelter -Decondioning syndrome and pulmonary thoromboembolism (venous thromnoembolism)-, J Jp Soc Resp Care Rehab.2009; 19:83-6. (in Japanese)

[6] Hanzawa K, et al: DVT frequency in Niigataken Chuetsu-oki earthquake, Ther Res. 2008; 29:641-3. (in Japanese)

[7] Shibata M, et al.: Team Kurihara - venous thromboembolism prevention activities, Heart. 2010; 42:473-80. (in Japanese)

[8] Sochart D, et al.: The relationship of foot and ankle movement to venous return in the lower limb, J Bone Joint Surg. 1999; 81:700-4.

Morphological study of thoracic and abdominal organs in X-irradiated mice

Shoji Chiba[1], Takahiro Miura[2], Fumiya Nakamura[3] , Koichi Itoh[4], Kyoko Itoh[4], Manabu Nakano[4], Hiroyuki Nozaka[4], and Kohsei Kudo[5]

1 Department of Pathologic Analysis, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences,
2 Hospital Hakodate Hokkaido
3 Iwate Medical University Hospital
4 Department of Biomedical Sciences, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences,
5 Department of Radiological Life Sciences, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences

Abstract. In order to morphologically confirm acute radiation damage and organ radiosensitivity in mice, we performed whole body X-ray irradiation at single doses of 0 (control), 2, 4, 8, and 10 Gy. The size of the spleen diminished in accordance with the strength of the X-irradiation dose, and its weight decreased significantly in males and females exposed at doses of 8 and 10 Gy. Bleeding was found macroscopically in the stomach and intestine and microscopically in the tissues of the digestive organs, heart, lungs, and urinary bladder. Lymphocyte number in the lymphoid nodules of the ileum and sigmoid colon decreased at 2 Gy. Furthermore, lymphocyte number in the spleen decreased in proportion to the strength of X-irradiation dose, and this decrease was obvious at 8 and 10 Gy.

Key Words: acute X-radiation damage, visceral organs, digestive canal, morphology, mouse

Introduction

Text books on radiation describe the death of cells in the intestinal glands and villi when digestive organs are exposed to X-irradiation [1, 3]. The present study was conducted to confirm previously documented morphological damage from X-irradiation in the thoracic and abdominal organs, especially in the digestive organs.

Materials and Methods

The whole bodies of 7-week-old ICR mice (2 males and 2 females; 2 exposed to each dose) were exposed to 2, 4, 8, and 10 Gy of X-irradiation using a Hitachi MBR-1505R2 at a dose rate of 0.133 Gy/min. On the 10th day after X-irradiation, the mice and their organs were stored in 10% formalin [2]. We measured the weights of the lungs, heart, liver, spleen, and urinary bladder. We obtained photographs of the digestive organs as a whole and the outer and inner surfaces of the stomach.

The above-mentioned organs and digestive organs (duodenum, jejunum, ileum, cecum, and sigmoid colon) were cut into 5-μm sections, stained using HE, and observed under a light microscope to detect morphological changes in the tissues.

Results

The size of the spleen diminished in accordance with the strength of the X-irradiation dose, and the weight of the spleen at 8 and 10 Gy in male and female mice decreased markedly in comparison with that of the non-exposed mice ($P < 0.05$). The weights of the remaining organs (the lungs, heart, liver, and urinary bladder) were similar between non-exposed and exposed mice (Figure 1). Upon macroscopic investigation, both the male and the female mice that received 8 and 10 Gy showed bleeding in the digestive canals, including the stomach (Figure 2a-c).

Upon histological examination, bleeding was found in the mucosa and muscle layer of the stomach at 4, 8, and 10 Gy (Figure 3a-d). Bleeding was also found in the submucosa of the duodenum, jejunum, and ileum at 4, 8, and 10 Gy; of the cecum at 10 Gy; and of the sigmoid colon at 8 and 10 Gy (Figure 4a-e). No differences were found in the morphological appearance of the intestinal glands and villi in the small and large intestines between non-exposed and exposed mice (Figure 5a-d).

Lymphocyte number in the lymphoid nodules of the ileum and sigmoid colon decreased at 2 Gy (Figure 6a-d). Lymphocyte number in the spleen decreased in proportion to the strength of X-irradiation dose; this decrease was obvious at 8 and 10 Gy (Figure 7a-d). In the lungs, bleeding in the alveoli and substantial hypertrophy of the alveoli were found at 4, 8, and 10 Gy (Figure 8a-c). In the urinary bladder, bleeding was found in the mucosa and muscle layer at 2, 8, and 10 Gy (Figure 9a, b). In the tissues of the heart, liver, and pancreas, morphological differences were not observed between non-exposed and exposed mice (Figure 10a-c).

Discussion

As mentioned in the textbook [1], X-irradiation causes a decrease in lymphocyte number in the spleen and lymphoid nodules of the small and large intestines. Jordan et al. (1956) reported that the size and weight of the spleen diminished in inverse proportion to X-irradiation strength [3]. With regards to the spleen, Kohda (1981) pointed out that it was evident the number of cells in the red pulp decreased and the sizes of the white pulp shrank [4]. Because we found no changes in the lengths of the intestinal gland and villi between the exposed and non-exposed mice, it was thought that a 10-Gy dose of radiation is a critical value; exposure at this level influences the growth of the intestinal glands and villi.

References

[1] Masuda K, Sasaki H. Radiobiology (in Japanese). Tokyo: Nanzando. (1996)

[2] Chiba S, Kagamiya M, Itoh K, et al. Acute X-irradiation damages to the appearances and some visceral organs in mice. Proc.of 2nd Intern.Symp.Rad. Emerg.Med. at Hirosaki Univ. 85-92 (2010).

[3] Jordan DL, Clark JW, Vogel HH. The additivity of γ -rays and fission neutrons in producing spleen weight reduction. Radiation Research 4:77-85 (1956).

[4] Kohda S. Effect of quality with various photon radiations (in Japanese with English abstract). J. Tokyo Med. Univ., 39:1093-1103 (1981).

Abbreviations for Figures 1-10

Ac, ascending colon; As, alveolar sac; Br, bronchiole; Ca, central artery; Ce, cecum; Ch, chief cell; Cr, crypt of Lieberkühn; Cv, central vein; Du, duodenum; Es, esophagus; Fu, fundus of stomach; Gc, germinal center; Gp, gastric pit; IL, islet of Langerhans; Il, ileum; Je, jejunum; Ln, lymphatic nodule; Mu, mucous cell; Pa, parietal cell; Rp, red pulp; Te, transitional epithelium; Vi, villi: Wp, white pulp; ☆, bleeding

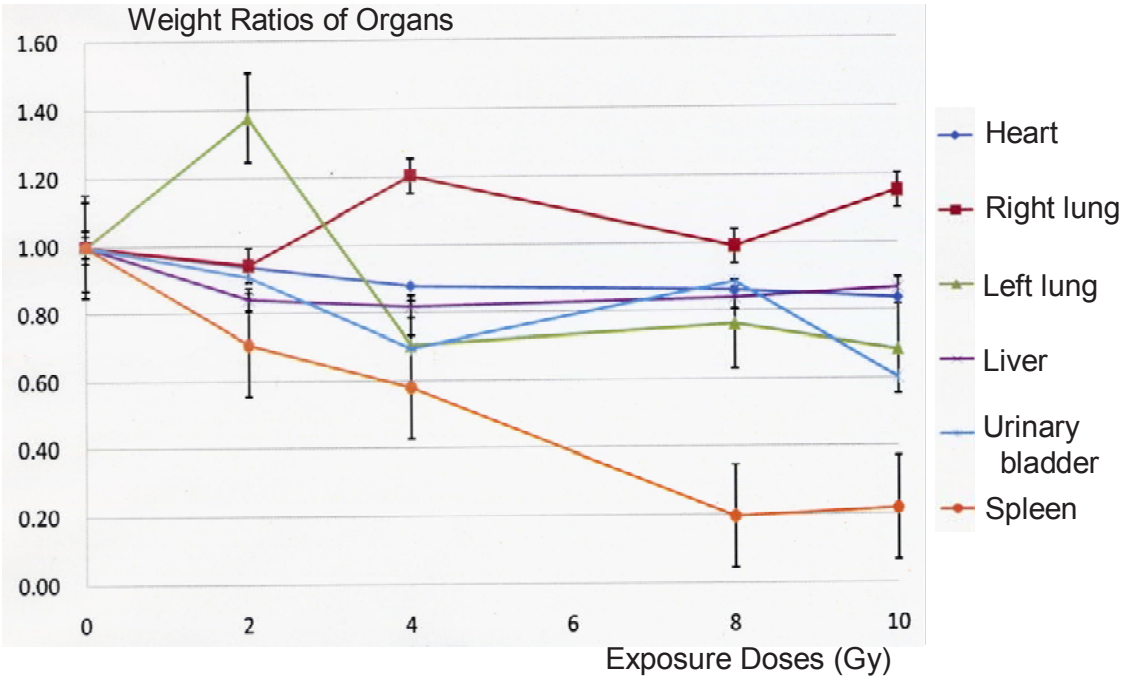
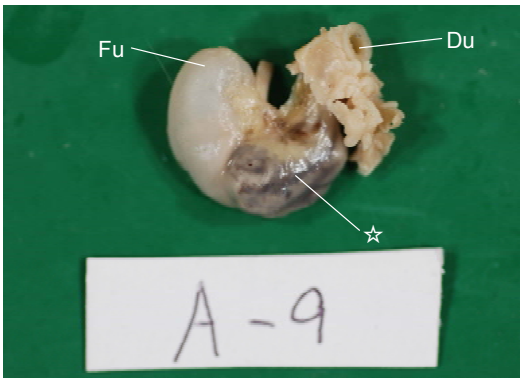
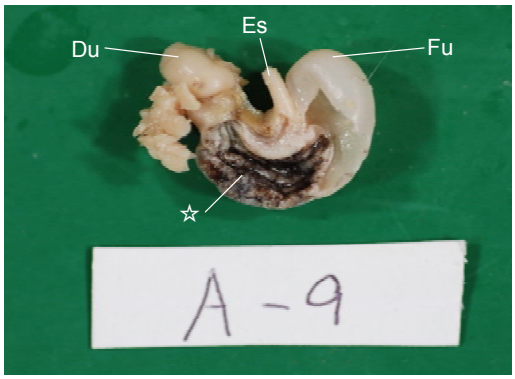


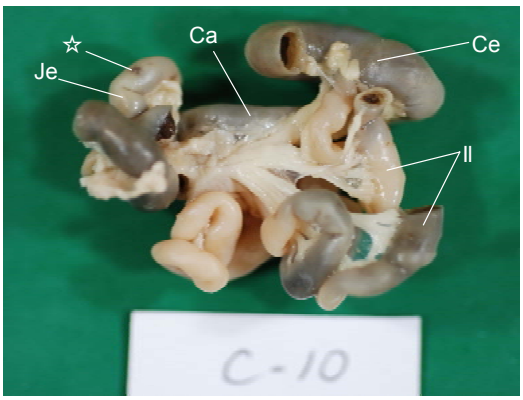
Fig. 1. In comparison with the non-exposed mice, the spleen weight at 8 and 10 Gy was decreased markedly ($P < 0.05$). The weights of the remaining organs (the lungs, heart, liver, and urinary bladder) were similar between the non-exposed and exposed mice.



2a. Outer surface of stomach (Male at 10 Gy)

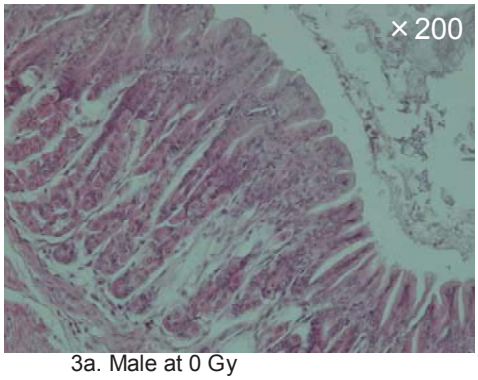


2b. Inner surface of stomach (Male at 10 Gy)

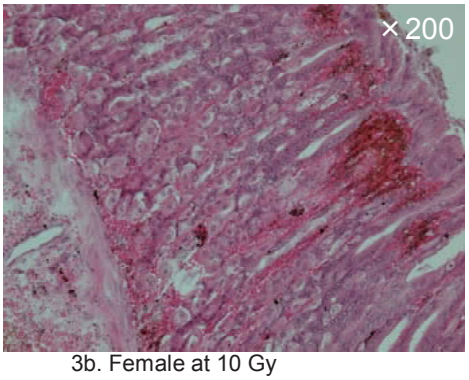


2c. Dorsal view of digestive canal (Female at 10 Gy)

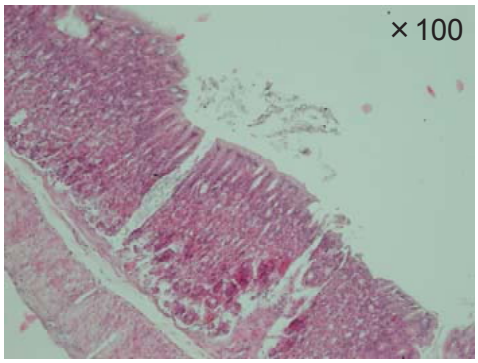
Figs. 2a-c. Bleeding was found macroscopically on the outer and inner surfaces of the stomach in mice that received 8 and 10 Gy, and was shown widely in the digestive canals.



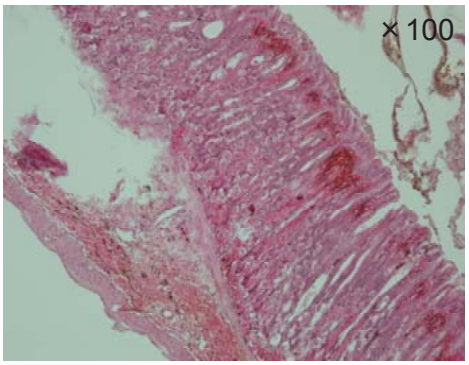
3a. Male at 0 Gy



3b. Female at 10 Gy

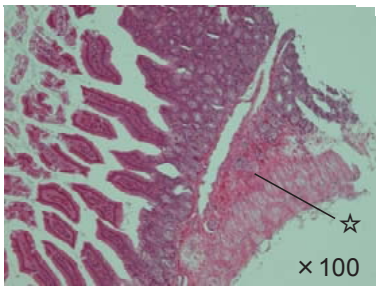


3c. Female at 0 Gy

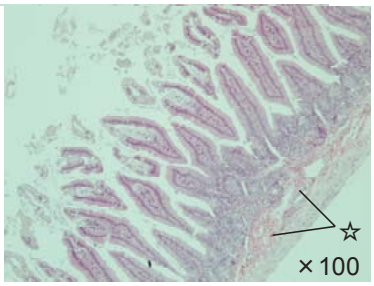


3d. Female at 10 Gy

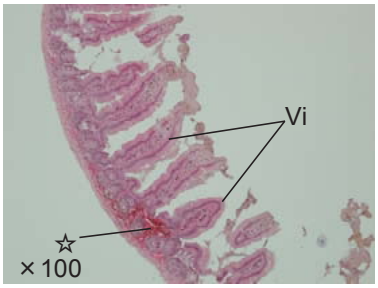
Figs. 3a-d. Histological structures of the gastoric gland were similar between non-exposed and exposed mice. Bleeding was found in the mucosa and muscle layer of the stomach at 4, 8, and 10 Gy. H&E staining. Magnification 100×, 200×.



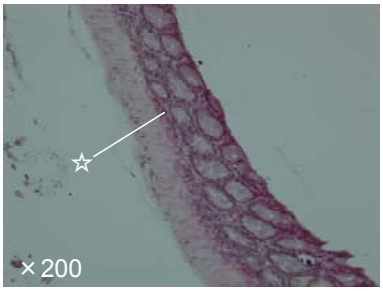
4a. Duodenum (Male at 10 Gy)



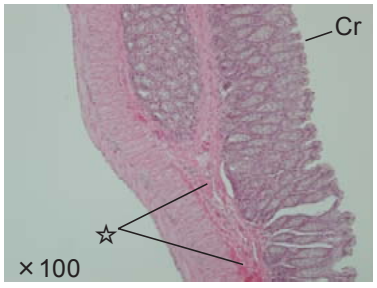
4b. Jejunum (Male at 10 Gy)



4c. Ileum (Female at 10 Gy)

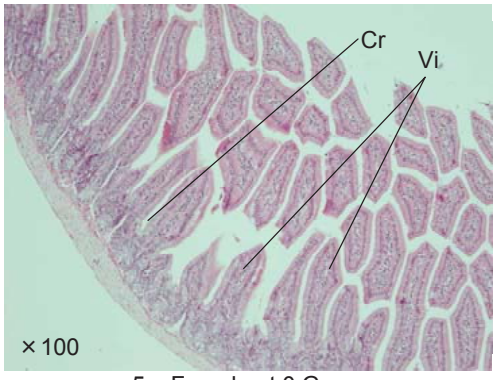


4d. Cecum (Female at 8 Gy)

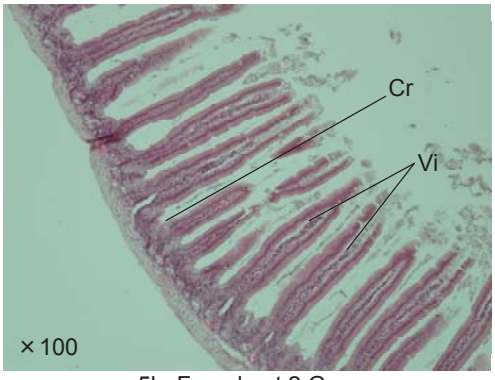


4e. Sigmoid colon (Female at 10 Gy)

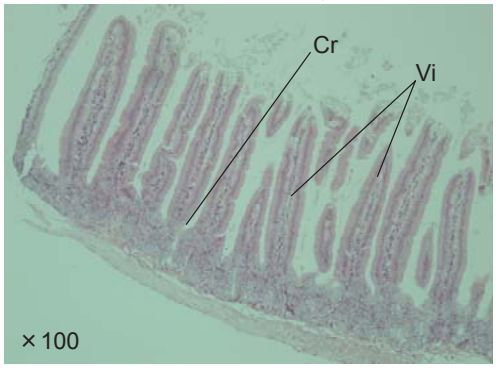
Figs. 4a-e. Bleeding was also found in the submucosa of the duodenum, jejunum, and ileum at 4, 8, and 10 Gy; of the cecum at 10 Gy; and of the sigmoid colon at 8 and 10 Gy. H&E staining. Magnification 100×, 200×.



5a. Female at 0 Gy



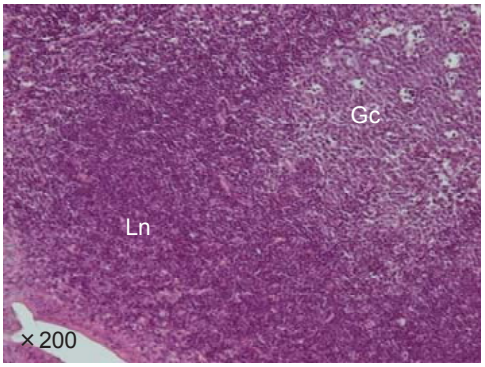
5b. Female at 2 Gy



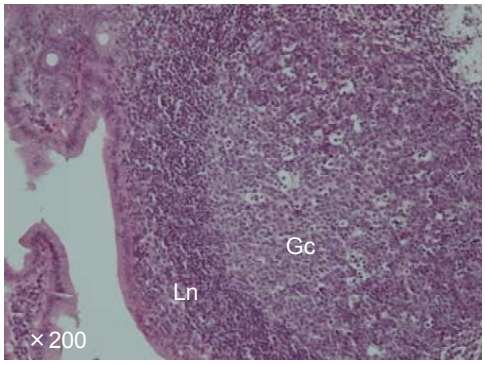
5c. Female at 8 Gy

5d. Female at 10 Gy

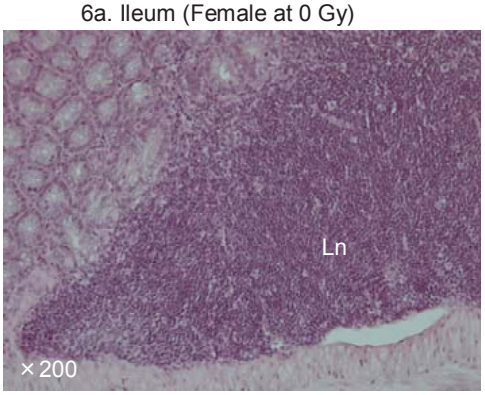
Figs. 5a-d. No differences were found in the morphological appearance of the intestinal glands and villi in the small and large intestines between the non-exposed and exposed mice. H&E staining. Magnification 100 × .



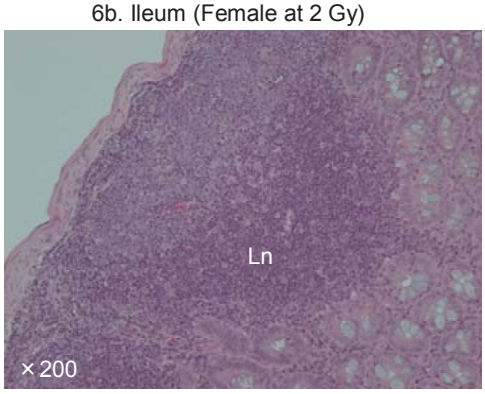
6a. Ileum (Female at 0 Gy)



6b. Ileum (Female at 2 Gy)

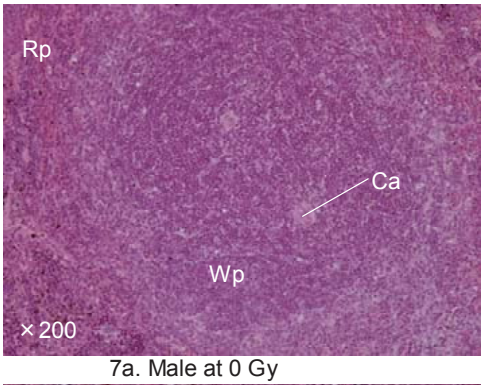


6c. Sigmoid colon (Male at 0 Gy)

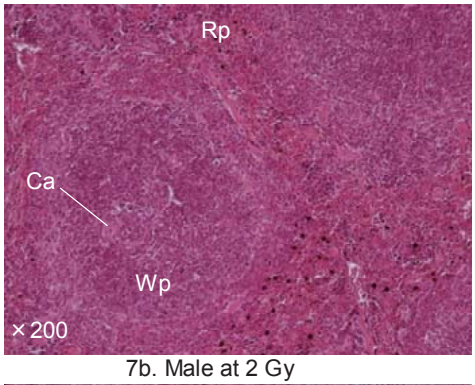


6c. Sigmoid colon (Male at 2 Gy)

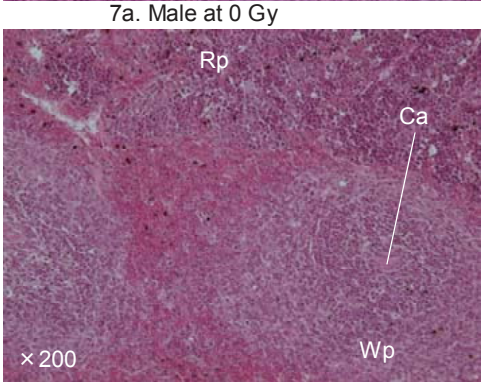
Figs. 6a-d. Lymphocyte number in the lymphoid nodules of the ileum and sigmoid colon decreased at 2 Gy. H&E staining. Magnification 200 × .



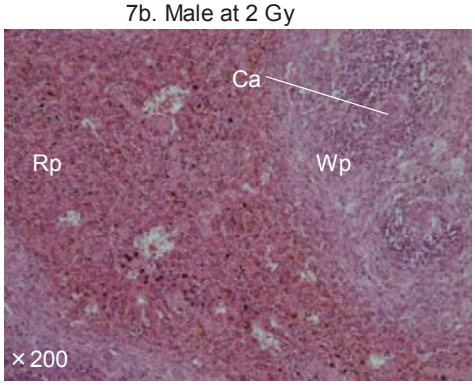
7a. Male at 0 Gy



7b. Male at 2 Gy

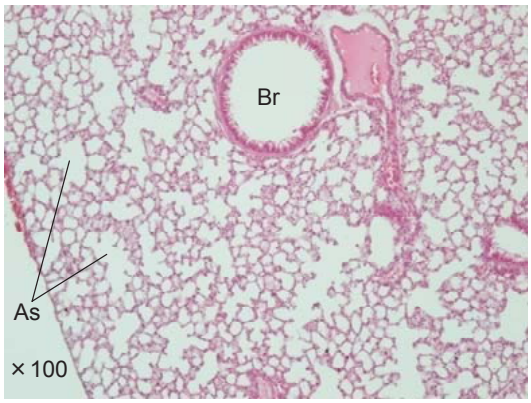


7c. Male at 4 Gy

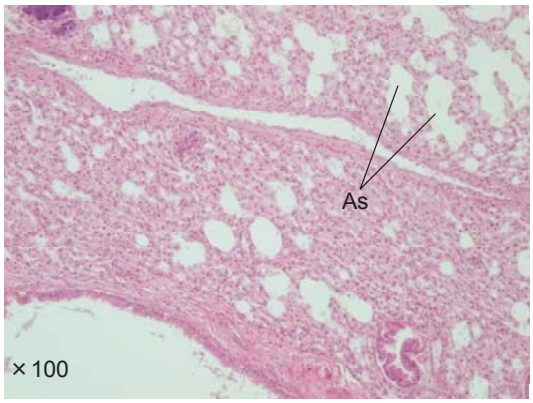


7d. Male at 10 Gy

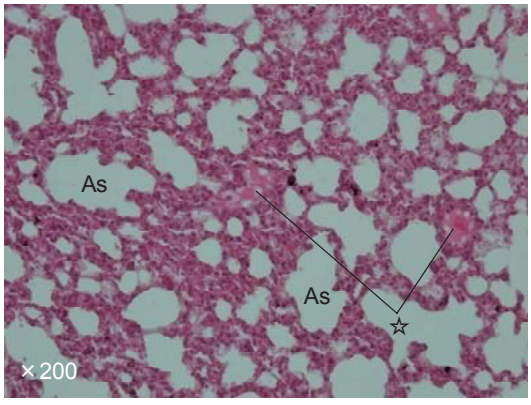
Figs. 7a-d. Lymphocyte number in the spleen decreased in proportion to the strength of X-irradiation dose and this decrease was obvious at 8 and 10 Gy. H&E staining. Magnification 200×.



8a. Male at 0 Gy

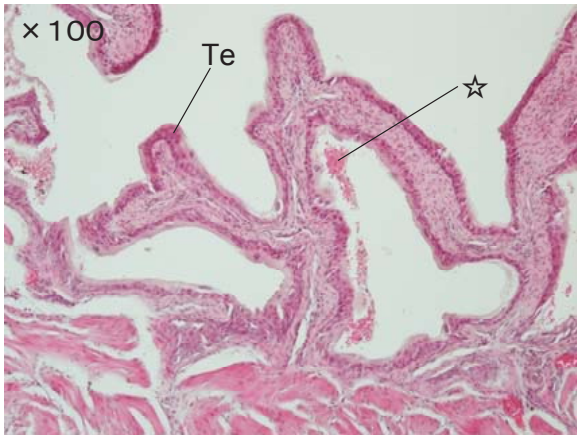


8b. Male at 10 Gy

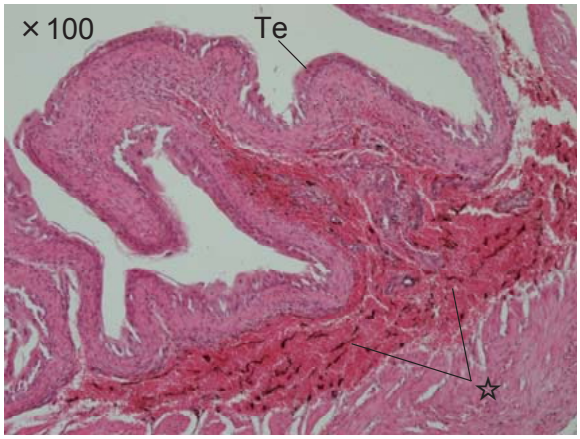


8c. Male at 10 Gy

Figs. 8a-c. In the lungs, bleeding in the alveoli and substantial hypertrophy of the alveoli were found at 4, 8, and 10 Gy. H&E staining. Magnification 100×, 200×.

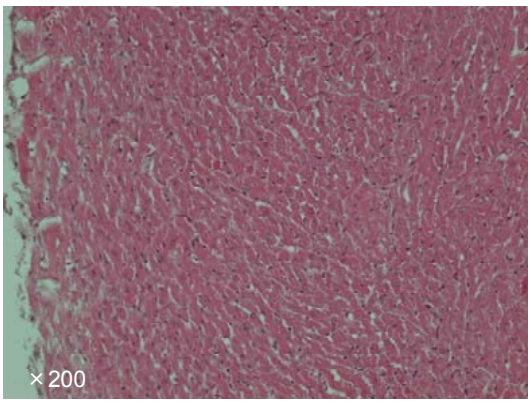


9a. Male at 8 Gy

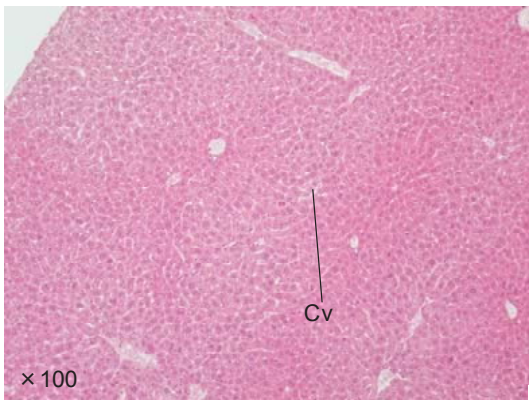


9b. Female at 10 Gy

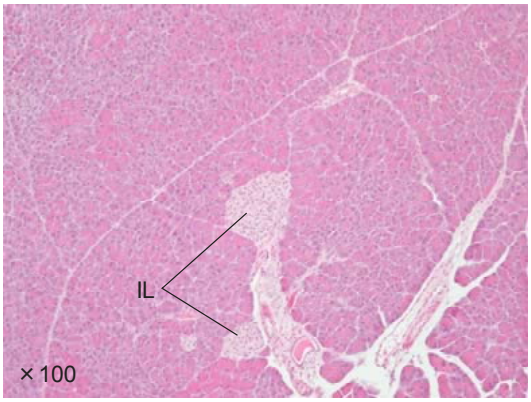
Figs. 9a, b. In the urinary bladder, bleeding was found in the mucosa and muscle layer at 2, 8, and 10 Gy. H&E staining. Magnification 100 × .



10a. Heart (Male at 10 Gy)



10b. Liver (Female at 10 Gy)



10c. Pancreas (Male at 10 Gy)

Figs. 10a-c. In tissues of the heart, liver, and pancreas, morphological differences were not observed between the non-exposed and exposed mice. H&E staining. Magnification 100 × , 200 × .

The effects of ionizing radiation on pattern recognition receptors

Hironori Yoshino and Ikuo Kashiwakura[1]

Department of Radiological Life Sciences,
Division of Medical Life Sciences,
Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan

Abstract. The immune system is composed of innate and adaptive immunity. Antigen presenting cells (APCs), such as dendritic cells and macrophages, serve as a link between innate and adaptive immunity. APCs express pattern recognition receptors (PRRs) which recognize pathogen-associated molecular patterns (PAMPs). Toll-like receptor (TLR) and retinoic-acid inducible gene-I (RIG-I) are well-studied PRRs that play important roles in anti-bacterial or anti-viral immunity. The activation of APCs through PRRs is required for the induction of adaptive immune responses. However, it remains unknown whether ionizing radiation affects PRRs. The effects of ionizing radiation on the expression of PRRs and the response against PAMPs were herein investigated using THP1 cells (human acute monocytic leukemia cells). THP1 expressed TLR2 and TLR4 which are receptors for peptidoglycan and lipoplysacchride (LPS), respectively. Those expressions after X-ray irradiation (1-5 Gy) were higher in irradiated cells than in non-irradiated cells. The response against LPS was estimated by the induction of tumor necrosis factor-α (TNF-α). The TNF-α positive cells were higher in irradiated-cells (2%) than in non-irraidated cells (0.5%). To investigate the effects of ionizing radiation on PRRs in detail, THP1 cells were treated with *phorbol* 12-myristate 13-acetate (PMA) in order to differentiate into macrophage-like cells, and then the similar experiments were performed. In contrast to the results of THP1, the expression of TLR4 of PMA-treated THP1 after irradiation was lower in irradiated cells than in non-irradiated cells. Although TNF-α positive cell after LPS stimulation was higher in PMA-treated cells (40%), no significant difference in the response of LPS was observed between non-irradiated and irradiated conditions. We next investigated the expression of RIG-I which recognizes double-strand RNA. Although non- or PMA-treated cells did not express RIG-I, RIG-I was expressed in PMA-treated cells after LPS stimulation. No significant effects of irradiation on the RIG-I expression in PMA-treated cells after LPS stimulation was observed. In conclusion, this study demonstrated that ionizing radiation affects PRRs expression and the response of PRRs to PAMPs, but these effects depend on the cell types and differentiation state.

Key Words: ionizing radiation, pattern recognition receptors

Introduction

The immune system is composed of innate and adaptive immunity. The adaptive immune system is composed of T cells and B cells, which induces antigen-specific immune responses. On the other hand, antigen presenting cells (APCs), such as dendritic cells and macrophages, play important roles in innate immunity, and serve as a link between innate and adaptive immunity. APCs express pattern recognition receptors (PRRs) which recognize pathogen-associated molecular patterns (PAMPs)[1]. Toll-like receptor (TLR) and retinoic-acid inducible gene-I (RIG-I)-like receptor (RLR)

[1] **Corresponding to:**Ikuo Kashiwakura, Ph.D. Hirosaki University Graduate School of Health Sciences, 66-1 Hon-cho, Hirosaki, Aomori 036-8564, Japan. E-mail: ikashi@cc.hirosaki-u.ac.jp

are well-studied PRRs that are indispensable for anti-bacterial or anti-viral immunity. For example, TLR2 and TLR4 recognize peptidoglycan and lipoplysacchride (LPS), respectively, which results in the host defense against bacteria. The activation of APCs through PRRs is required for the induction of adaptive immune responses. However, it remains unknown whether ionizing radiation affects PRRs. In the present study, to clarify the effects of ionizing radiation on the expression of PRRs and the response against PAMPs using THP1 cells (human acute monocytic leukemia cells).

Experiments

Figure 1 shows the schema of this study.

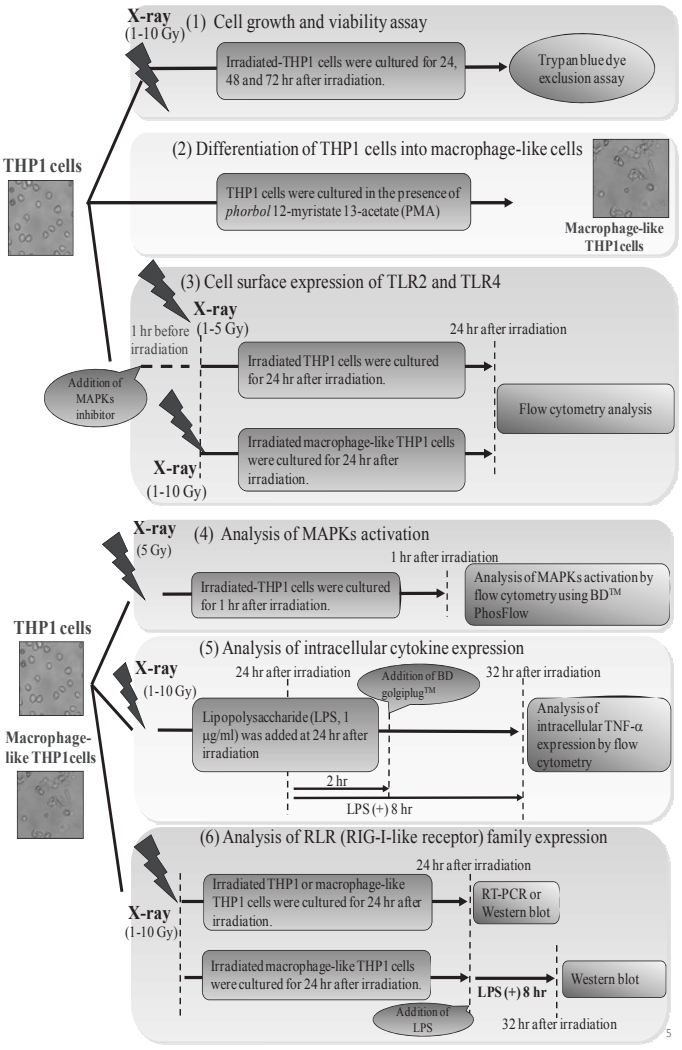


Figure 1. The schema of experiments in the present study.

Results

X-irradiation inhibits cell growth and decreased viability.

We first examined the effects of ionizing radiation on the cell growth and cell viability. Non- and irradiated-THP1 cells (2.0×10^5 cells) were cultured after irradiation. The dose of more than 5 Gy inhibited both cell growth and cell viability (Fig. 2).

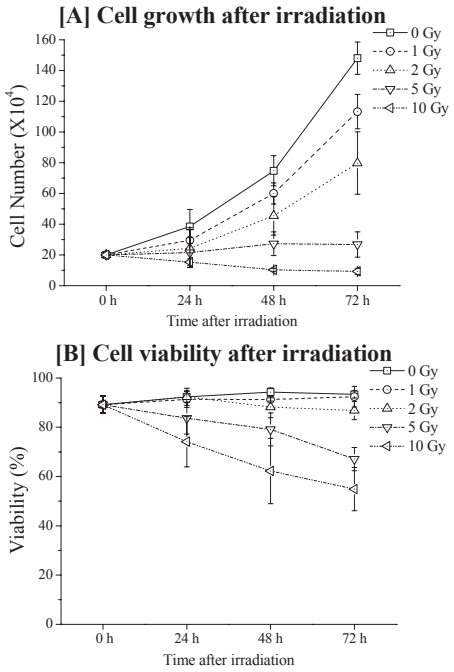


Figure 2. The viable and dead cells were counted by trypan blue dye exclusion assay at 24, 48, 72 hr after irradiation. [A] The number of viable cell was shown. [B] The viability (%) of THP1 cells was calculated as 100×(number of viable cell)/(number of total cell). The data were mean ± SD of four different experiments.

X-irradiation up-regulates cells surface TLR2 and TLR4 expression of THP1 cells, and enhances the response against LPS.

To investigate the effects of ionizing radiation on the expression of TLR2 and TLR4, the cell surface expression of TLR2 and TLR4 on non- and irradiated-THP1 cells were analyzed. Those expressions were higher in irradiated-cells compared with those of non-irradiated control (Fig. 3). Furthermore, we investigated the response of irradiated-THP1 cells against LPS by estimating the induction of tumor necrosis factor-α (TNF-α). In

accordance with the up-regulation of TLR4 expression, the TNF-α positive cells were higher in irradiated-THP1 (2%) than in non-irraidated cells (0.5%) (data not shown).

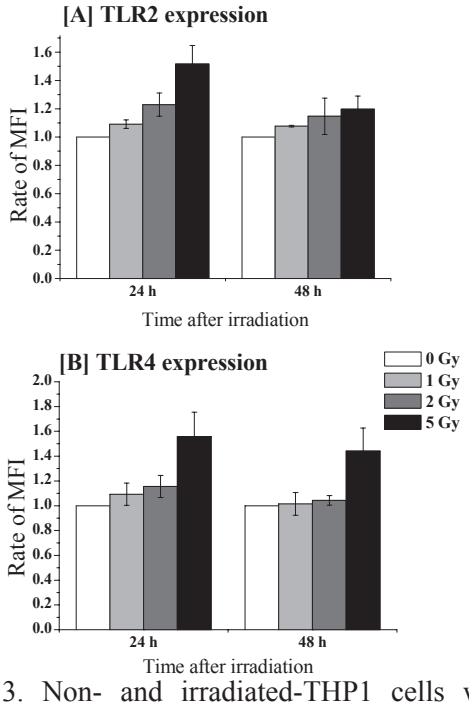


Figure 3. Non- and irradiated-THP1 cells were cultured for 24 or 48 hr, and then the expression of TLR2 [A] and TLR4 [B] were analyzed by a flow cytometer. MFI means mean fluorescence intensity. The data were mean ± SD of three different experiments.

X-irradiation down-regulates cells surface TLR2 and TLR4 expression of macrophage-like THP1 cells, but does not attenuate the response against LPS.

To investigate the effects of ionizing radiation on PRRs in detail, THP1 cells were treated with PMA in order to differentiate into macrophage-like cells, and then the similar experiments were performed. In contrast to the results of THP1, the expression of TLR4 of PMA-treated THP1 after irradiation was lower in irradiated cells than in non-irradiated cells (Figure 4). Although TNF-α positive cells after LPS stimulation was higher in PMA-treated THP1 (40%), no significant difference in the response of LPS was observed between non-irradiated and irradiated conditions (data not shown).

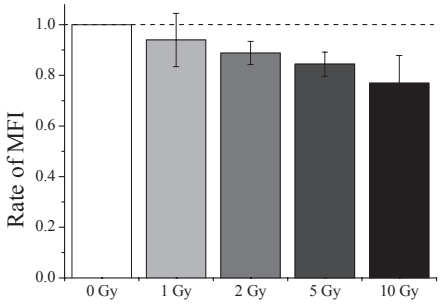


Figure 4. The macrophage-like THP1 cells were irradiated, and then the expression of TLR2 and TLR4 were analyzed at 24 hr after irradiation. The data were mean ± SD of three different experiments.

X-irradiation does not affect RIG-I expression.

We next investigated the expression of RIG-I which recognizes double-strand RNA. Although non- or PMA-treated THP1 did not express RIG-I, RIG-I was expressed in PMA-treated THP1 after LPS stimulation. No significant effects of irradiation on the RIG-I expression in PMA-treated THP1 after LPS stimulation was observed (data not shown).

Conclusions

The present study indicates that X-irradiation enhances TLR expression in THP1 cells. On the other hand, X-irradiation decreased TLR expression in macrophage-like THP1 cells, thus demonstrating that the regulation of TLR expression by ionizing radiation depends on cell differentiation**.** Furthermore, X-irradiation does not significantly affect RIG-I expression.

References

[1] Creagh EM and O'Neill LA. TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. *Trends Immunol.* 27:352-357. (2006).

Acknowledgments

This work was supported by a Grant for the Co-medical Education Program in Radiation Emergency Medicine by the Ministry of Education, Culture, Sports, Science and Technology, Japan (2010). This work also received support from a Hirosaki University Grant for Exploratory Research by Young Scientists (2010).

Characteristic Analysis of Megakaryocytopoiesis and Thrombopoiesis by Human Hematopoietic Stem Cells Exposed to Ionizing Radiation

Satoru Monzen.[1], Toshiya Nakamura[2] and Ikuo Kashiwakura.[1].

1 Department of Radiological Life Sciences, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences

2 Department of Biomedical Sciences, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences

Abstract. Hematopoietic processes, especially megakaryocytopoiesis and thrombopoiesis, are highly sensitive to extracellular oxidative stresses such as ionizing radiation and chemotherapeutic agents. This study examined the terminal maturation of megakaryocytes and platelet production in hematopoietic stem/progenitor cells (HSPCs) irradiated with ionizing radiation. Highly purified $CD34^+$ cells derived from human placental/umbilical cord blood were exposed to X rays (2 Gy, 150 kVp, 20 mA; 0.5-mm aluminum and 0.3-mm copper filters), at a dose rate of approximately 1 Gy/min, and then cultured in a serum-free medium supplemented with thrombopoietin and interleukin-3. The number of cells generated from X-irradiated $CD34^+$ cells decreased with time in the culture. However, the fraction of $CD34^+Tie\text{-}2^+$ and $CD41^+Tie\text{-}2^+$ cells among the total cells generated from X-irradiated cells increased significantly in comparison to non-irradiated controls on day 7. In addition, the $CD42a^+$ particles, which appeared to be platelets, generated from the X-irradiated HSPCs appeared to be normal. Quantitative real-time reverse transcriptase-polymerase chain reaction analysis of the expression of various genes in cells harvested from the cultures showed that the early hematopoiesis-related genes FLI1, HOXB4, and Tie-2, the cytokine receptor genes KIT and IL-3RA, and the oxidative stress-related genes HO1 and NQO1 were up-regulated on day 7. These results suggest that normal terminal maturation of megakaryocytes and platelet production occur in residual HSPCs after ionizing radiation, despite the adverse effect of radiation on proliferation and differentiation of HSPCs. Ionizing radiation may have the potential to promote both megakaryocytopoiesis and thrombopoiesis.

Key Words: hematopoietic stem/progenitor cells, ionizing radiation, terminal maturation of megakaryocytes and platelet production

Introduction

Hematopoietic stem cells can self-renew and differentiate into all hematopoietic lineages throughout the lifetime of an organism, but these cells are sensitive to extracellular oxidative stress (1-3). Damage to the hematopoietic system caused by ionizing radiation remarkably suppresses the production of mature blood cells in a dose-dependent manner (3-6). Previous studies have shown a variation in the recovery of blood cells among individuals exposed to ionizing radiation and a delay in the production of leucocytes and platelets compared to erythrocytes (7, 8). The effective treatment of patients with severe thrombocytopenia consists of platelet concentrate transfusion. Therefore, comprehensive research on the effect of radiation on thrombopoiesis in hematopoietic stem/progenitor cells (HSPCs) is critical (9-11).

Very recently, the thrombopoietin (TPO) mimetics romiplostim and eltrombopag were approved for the treatment of immune thrombocytopenic purpura (12, 13). In addition, Satyamitra *et al.* demonstrated that the TPO antagonist ALXN4100TPO increased bone marrow cellularity and megakaryocytic development and accelerated multilineage hematopoietic recovery in an in vivo study (14). However, the process by which ionizing radiation leads to thrombocytopenia or its effects on the differentiation from residual HSPCs in megakaryocytopoiesis/ thrombopoiesis are not clear, and experimental approaches are therefore necessary. We previously reported that the differentiation pathway from HSPCs to megakaryocytopoiesis/thrombopoiesis is more radiosensitive than that from HSPCs to granulocytopoiesis and erythropoiesis (15, 16). In a prior study, we showed that the combination of TPO and interleukin-3 (IL-3) was effective not only as a promoter of megakaryocytopoiesis and thrombopoiesis but also as a radioprotector in this pathway (17). We also demonstrated that mature megakaryocytes are radiosensitive, but the radiosensitivity decreases at the terminal stages of megakaryocytic maturation, in particular in megakaryocytes entering the proplatelet formation stage (18). In addition, we showed that megakaryocytopoiesis and thrombopoiesis in HSPCs irradiated with heavy-ion beams function in a similar manner as in non-irradiated cells, although heavy-ion beams affect the expression of genes associated with cellular adhesion (19). However, there is currently no information on the process of megakaryocyte differentiation, maturation, and gene expression in X-irradiated HSPCs. To evaluate the effects of ionizing irradiation on the terminal process of megakaryocytopoiesis and thrombopoiesis, the present study investigated the production of mature megakaryocytes and platelets generated from freshly prepared human placental/umbilical cord blood (CB) $CD34^+$ cells exposed to 2 Gy (1/3 of the survival dose of CFU-Meg) X-irradiation in serum-free culture supplemented with TPO and IL-3. This combination is optimal for terminal maturation of megakaryocytes and platelet production (17). The estimation of cell surface antigens and mRNA expression was performed within 24 h (early stage of hematopoiesis) on day 7 (the beginning of megakaryocytic proliferation) and day 14 (the stage with the maximum number of platelets).

Results and Discussion

The fraction of the $CD34^+Tie\text{-}2^+$ and $CD41^+Tie\text{-}2^+$ cell populations generated from irradiated HSPCs was significantly higher than that generated from non-irradiated controls on day 7 (Figure 1). However, the number of surviving cells in the mature stages of megakaryocytopoiesis decreased following irradiation of $CD34^+$ cells. These results suggest that $Tie\text{-}2^+$ cells included in HSPCs are relatively radioresistant cells under conditions of stimulation with TPO and IL-3.

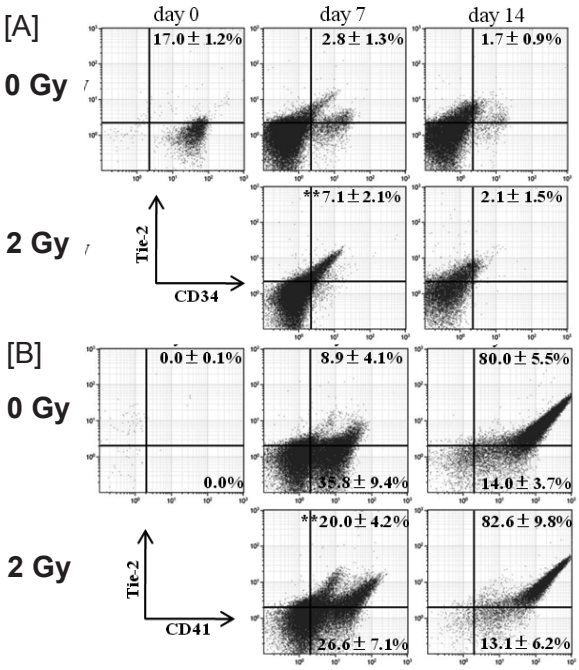


Figure 1. The fraction of $CD34^+Tie\text{-}2^+$ cells [A] and $CD41^+Tie\text{-}2^+$ cells [B] to X-irradiation. $^{**}P < 0.05$ in comparison to 0 Gy - day 7.

Our previous study showed an increase in the Tie-2^+ cell populations derived from CB $CD34^+$ cells exposed to 2 Gy heavy-ion beams of 50 keV/μm on day 7, while the rate of cell survival declined (18, 19). In addition, a statistically significant correlation observed between the percentage of Tie-2^+ cells and the surviving fraction of hematopoietic progenitor cells (16), indicated that the $CD34^+$Tie-2^+ fraction is radioresistant in comparison to the $CD34^+$Tie-2^- fraction. $CD34^+$Tie-2^+ cells in HSPCs are in a more immature state than $CD34^+$Tie-2^- cells and are maintained in G_0 phase through signalling by angiopoietin-1 (Ang-1), a ligand for the Tie-2 receptor (20, 21).

On the other hand, a high expression of adhesion molecules is observed in proplatelets formed in megakaryocytes, which is the process of terminal maturation (22, 23). The present results showed that the aggregation function of platelets generated from X-irradiated HSPCs was enhanced as compared to non-irradiated controls (Figure 2).

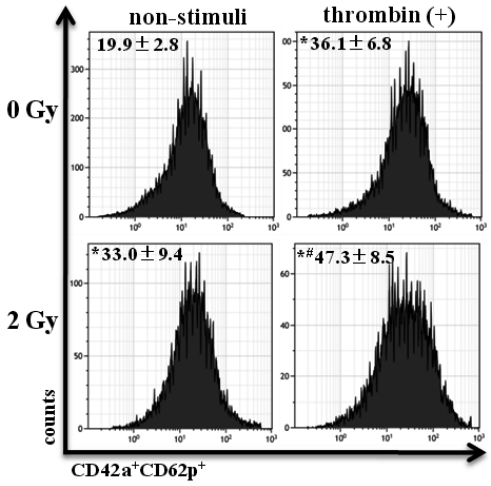


Figure 2. Platelet activation in response to X-irradiation. *P < 0.05 in comparison to non-stimuli 0 Gy control and $^{\#}P$ < 0.05 in comparison to non-stimuli 2 Gy control.

Furthermore, expression of Tie-2 mRNA was significantly up-regulated on day 7 as compared to non-irradiated controls (Figure 3). These results suggest that X-irradiation promotes megakaryocytic differentiation associated with the expression of CD41 and Tie-2 proteins. Schmitz *et al.* reported that IL-3 supports early megakaryocytopoiesis and that IL-6 mainly regulates the late stage of megakaryocyte differentiation (22).

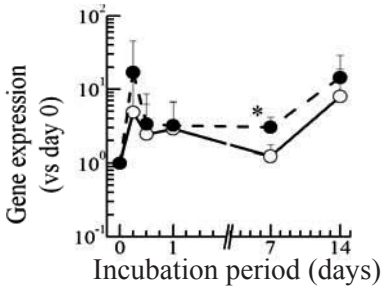


Figure 3. Behaviour of Tie-2 mRNA expression after exposure to X-irradiation. *P < 0.05.

Moreover, IL-3 and IL-6 are reported as cytokines with autocrine capacity in megakaryopoiesis (22). Proplatelets formed in megakaryocytes generally express some adherent molecules (23). These findings suggest that the Ang-1, IL-3, and IL-6 signals maintained by the corresponding receptors in residual HSPCs as a result of X-irradiation promote the terminal maturation stages of megakaryocytopoiesis and thrombopoiesis (24, 25). Ionizing radiation induces reactive oxygen species and causes DNA damage, and insufficient cellular repair mechanisms may contribute to premature aging and apoptosis (26, 27). On the other hand, ionizing radiation enhances cell differentiation in certain normal cells, depending on the specific conditions and radiation dose (28). Thus, one of the biological processes that specifically occur with 2 Gy X-irradiation may be the promotion of megakaryocyte maturation.

The present mRNA expression analysis showed that 7 factors, namely, the early hematopoiesis-related FLI1, HOXB4, and Tie-2 (29-33), Nrf2 oxidative stress regulation system-related HO1 and NQO1, and the cytokine receptor-related KIT and IL3RA genes were up-regulated (Figure 4) (1, 34, 35). The other 3 factors, namely, MPL, CSF2RA,

and GP1BA, which are cytokine receptor- and megakaryocyte-related genes (36), were down-regulated. The 10 factors changed by X-irradiation among the 16 factors analyzed suggest that the up-regulation of specific genes is an important biological characteristic of X-irradiation. This verified that the variation in cell surface antigens and gene expression by ionizing radiation changes with megakaryocytic and thrombopoietic differentiation (Figure 4).

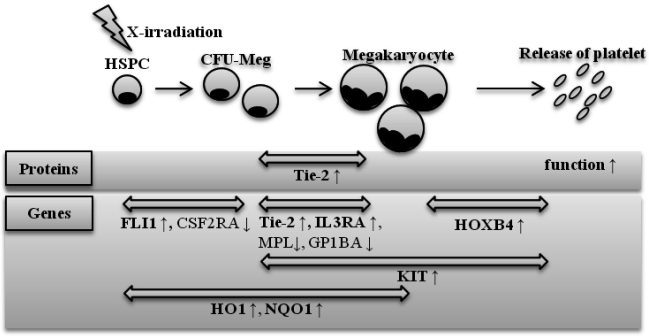


Figure 4. Model illustrating megakaryocytopoiesis and thrombopoiesis in HSPCs irradiated with X-radiation.

Subsequent analyses are underway to determine the mechanism(s) underlying the effect of X-irradiation of HSPCs on the up-regulation of megakaryocytopoiesis- and thrombopoiesis-related molecules.

In conclusion, the present results suggest that normal terminal maturation of megakaryocytes and platelet production occur in residual HSPCs after ionizing radiation, despite the adverse effect of radiation on the proliferation and differentiation of HSPCs. The X-irradiation induced up-regulation of 7 genes associated with the early stages of hematopoiesis, megakaryocytic maturation, and antioxidant systems suggest that ionizing radiation may promote both megakaryocytopoiesis and thrombopoiesis. These results provide useful insights that may help solve problems arising from incidents such as accidental nuclear scenarios, and will help us to understand the risk of lethal- or sub-lethal doses of radiation (18).

References

[1] Nagayama H, Misawa K, Tanaka H, Ooi J, Iseki T, Tojo A, Tani K, Yamada Y, Kodo H, Takahashi TA, Yamashita N, Shimazaki S, Asano S. Transient hematopoietic stem cell rescue using umbilical cord blood for a lethally irradiated nuclear accident victim. Bone Marrow Transplant. 29:197-204 (2002).

[2] Schmidt-Ullrich RK, Dent P, Grant S, Mikkelsen RB, Valerie K. Signal transduction and cellular radiation responses. Radiat Res. 153:245-257 (2000).

[3] Kadhim MA, Wright EG. Radiation-induced transmissable chromosomal instability in haemopoietic stem cells. Adv Space Res. 22:587-596 (1998).

[4] Kato K, Takahashi K, Monzen S, Yamamoto H, Maruyama A, Itoh K, Kashiwakura I. Relationship between radiosensitivity and Nrf2 target gene expression in human hematopoietic stem cells. Radiat Res. 174:177-184 (2010).

[5] Wojcik A, Gregoire E, Hayata I, Roy L, Sommer S, Stephan G, Voisin P. Cytogenetic damage in lymphocytes for the purpose of dose reconstruction: a review of three recent radiation accidents. Cytogenet Genome Res. 104:200-205 (2004).

[6] Haimovitz-Friedman A. Radiation-induced signal transduction and stress response. Radiat Res. 150(5 Suppl):S102-S108 (1998).

[7] Fliedner TM, Graessle D, Meineke V, Dörr H. Pathophysiological principles underlying the blood cell concentration responses used to assess the severity of effect after accidental whole-body radiation exposure: an essential basis for an evidence-based clinical triage. Exp Hematol. 35(4 Suppl 1):8-16 (2007).

[8] UNITED NATIONS. United Nations Scientific Committee on the Effects of Atomic Radiation 1988 Report to the General Assembly, with Annexes. New York, 1998.

[9] Schlenke P, Hagenah W, Irsch J, Sundin D, Corash L, Lin L, Kirchner H, Wagner T. Safety and clinical efficacy of platelet components prepared with pathogen inactivation in routine use for thrombocytopenic patients. Ann Hematol. 2011 Apr 19. [Epub ahead of print].

[10] Greening DW, Sparrow RL, Simpson RJ. Preparation of Platelet Concentrates. Methods Mol Biol. 728:267-278 (2011).

[11] DiCarlo AL, Poncz M, Cassatt DR, Shah JR, Czarniecki CW, Maidment BW. Medical Countermeasures for Platelet Regeneration after Radiation Exposure. Radiat Res. 176:e0001-e0015 (2011).

[12] Miyazaki H, Kato T. Thrombopoietin: biology and clinical potentials. Int J Hematol. 70:216-225 (1999).

[13] Wei P. Thrombopoietin factors. Cancer Treat Res. 157:75-93 (2011).

[14] Satyamitra M, Lombardini E, Graves J 3rd, Mullaney C, Ney P, Hunter J, Johnson K, Tamburini P, Wang Y, Springhorn JP, Srinivasan V. A TPO Receptor Agonist, ALXN4100TPO, Mitigates Radiation-Induced Lethality and Stimulates Hematopoiesis in CD2F1 Mice. Radiat Res. 175:746-758 (2011).

[15] Kashiwakura I, Kuwabara M, Inanami O, Murakami M, Hayase Y, Takahashi TA, Takagi Y. Radiation sensitivity of megakaryocyte colony-forming cells in human placental and umbilical cord blood. Radiat Res.153:144-152 (2000).

[16] Takahashi K, Monzen S, Hayashi N, Kashiwakura I. Correlations of cell surface antigens with individual differences in radiosensitivity in human hematopoietic stem/progenitor cells. Radiat Res.173:184-190 (2010).

[17] Kashiwakura I, Inanami O, Murakami M, Takahashi TA, Kuwabara M, Takagi Y. Effects of the combination of thrombopoietin with cytokines on survival of X-irradiated $CD34^+$ megakaryocytic progenitor cells from normal human peripheral blood. Radiat Res. 158:202-209 (2002).

[18] Takahashi K, Monzen S, Eguchi-Kasai K, Abe Y, Kashiwakura I. Severe damage of human megakaryocytopoiesis and thrombopoiesis by heavy-ion beam radiation. Radiat Res. 168:545-551 (2007).

[19] Monzen S, Takahashi K, Yoshino H, Kasai-Eguchi K, Kashiwakura I. Terminal maturation of megakaryocytes and platelet production by hematopoietic stem cells irradiated with heavy-ion beams. Radiat Res. 176:8-16 (2011).

[20] Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY, Suda T. Tie2/Angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell. 118:149-161 (2004).

[21] Huang YQ, Li JJ, Karpatkin S. Identification of a family of alternatively spliced mRNA species of angiopoietin-1. Blood. 95:1993-1999 (2000).

[22] Schmitz B, Wickenhauser C, Thiele J, Frimpong S, Brockbals C, Selbach B, et al. Megakaryocyte induced fibroblast proliferation is enhanced by costimulation with IL-6/IL-3 and dependent on secretory and adhesion events. Leuk Res. 23:723-729 (1999).

[23] Kojima H, Kanada H, Shimizu S, Kasama E, Shibuya K, Nakauchi H, Nagasawa T, Shibuya A. CD226 mediates platelet and megakaryocytic cell adhesion to vascular endothelial cells. J Biol Chem. 278:36748-36753 (2003).

[24] Dunois-Lardé C, Capron C, Fichelson S, Bauer T, Cramer-Bordé E, Baruch D. Exposure of human megakaryocytes to high shear rates accelerates platelet production. Blood. 114:1875-1883 (2009).

[25] Stasi R, Bosworth J, Rhodes E, Shannon MS, Willis F, Gordon-Smith EC. Thrombopoietic agents. Blood Rev. 24:179-190 (2010).

[26] Motohashi H, Kimura M, Fujita R, Inoue A, Pan X, Takayama M, Katsuoka F, Aburatani H, Bresnick EH, Yamamoto M. NF-E2 domination over Nrf2 promotes ROS accumulation and megakaryocytic

maturation. Blood. 115:677-686 (2010).
[27] Bertram C, Hass R. Cellular responses to reactive oxygen species-induced DNA damage and aging. Biol Chem. 389:211-220 (2008).
[28] von Wangenheim KH, Peterson HP. Control of cell proliferation by progress in differentiation: clues to mechanisms of aging, cancer causation and therapy. J Theor Biol. 193:663-678 (1998).
[29] Pimanda JE, Ottersbach K, Knezevic K, Kinston S, Chan WY, Wilson NK, Landry JR, Wood AD, Kolb-Kokocinski A, Green AR, Tannahill D, Lacaud G, Kouskoff V, Göttgens B. Gata2, Fli1, and Scl form a recursively wired gene-regulatory circuit during early hematopoietic development. Proc Natl Acad Sci U S A.104:17692-17697 (2007).
[30] Donaldson IJ, Chapman M, Kinston S, Landry JR, Knezevic K, Piltz S, Buckley N, Green AR, Göttgens B. Genome-wide identification of cis-regulatory sequences controlling blood and endothelial development. Hum Mol Genet. 14:595-601 (2005).
[31] Göttgens B, Nastos A, Kinston S, Piltz S, Delabesse EC, Stanley M, Sanchez MJ, Ciau-Uitz A, Patient R, Green AR. Establishing the transcriptional programme for blood: the SCL stem cell enhancer is regulated by a multiprotein complex containing Ets and GATA factors. EMBO J. 21:3039-3050 (2002).
[32] Guillon N, Tirode F, Boeva V, Zynovyev A, Barillot E, Delattre O. The oncogenic EWS-FLI1 protein binds in vivo GGAA microsatellite sequences with potential transcriptional activation function. PLoS One. 4:e4932 (2009).
[33] Kyba M, Perlingeiro RC, Daley GQ. HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. Cell. 109:29-37 (2002).
[34] Rothschild G, Sottas CM, Kissel H, Agosti V, Manova K, Hardy MP, Besmer P. A role for kit receptor signaling in Leydig cell steroidogenesis. Biol Reprod. 69:925-932 (2003).
[35] Itoh N, Yonehara S, Schreurs J, Gorman DM, Maruyama K, Ishii A, Yahara I, Arai K, Miyajima A. Cloning of an interleukin-3 receptor gene: a member of a distinct receptor gene family. Science. 247:324-327 (1990).
[36] Suzuki T, Sakagami T, Rubin BK, Nogee LM, Wood RE, Zimmerman SL, Smolarek T, Dishop MK, Wert SE, Whitsett JA, Grabowski G, Carey BC, Stevens C, van der Loo JC, Trapnell BC. Familial pulmonary alveolar proteinosis caused by mutations in CSF2RA. J Exp Med. 205:2703-2710 (2008).

Acknowledgements

This work was supported by a Grant for Co-medical Education Program in Radiation Emergency Medicine by the Ministry of Education, Culture, Sports, Science and Technology, Japan (2010). This work also received support from a Grant for Hirosaki University Institutional Research (2010).

Identification of Radiation-induced Inflammatory Gene Network in Human Umbilical Vein Endothelial Cells

Yukihiro Furusawa[1], Qing-Li Zhao[1], Seiji Yamamoto[2], Yuichi Hattori[2], Takaharu Nomura[3] and Takashi Kondo[1]

1. *Department of Radiological Sciences, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan.*
2. *Department of Molecular and Medical Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan.*

Radiation Safety Research Center, Nuclear Technology Research Laboratory, Central Research Institute of Electric Power Industry, 2-11-1 Iwado Kita, Komae, Tokyo 201-8511, Japan.

Abstract. Cardiovascular disease has been considered a major health-risk factor after radiation exposure such as that experienced by A-bomb survivors. In a trial to understand the molecular mechanisms underlying the inflammatory reaction frequently encountered in the vascular system after exposure to ionizing radiation, we carried out global scale microarray and computational gene expression analyses in human umbilical endothelial cells (HUVEC). Global scale microarray gene expression analysis of irradiated HUVEC (2.5 Gy) identified 1,128 genes that were up- or down-regulated by a factor of 1.8 or greater at 6, 12, or 24 hr after irradiation. Hierarchical cluster analysis of the differentially expressed genes identified four clusters. Bioinformatic analysis using ingenuity pathway analysis tools revealed that the down-regulated genes in cluster I were associated with cell cycle regulation, whereas the up-regulated genes in cluster IV were associated with inflammatory responses. The analysis also identified a gene network containing interferon response factor 7 (IRF7) and its transcriptional target interferon-induced transcripts (IFITs) and Mx1, which have been known to be associated with inflammation in endothelial cells. The up-regulated genes and the gene network identified here may explain the inflammatory response induced by X-irradiation. These findings uncover some of the molecular basis of the mechanism(s) of the inflammatory disorder in response to X-irradiation in human umbilical vein endothelial cells.

Key Words: gene expression, cardiovascular disease

Introduction

Cardiovascular disease has been considered a major health-risk factor after radiation exposure such as that experienced by A-bomb survivors (*1*). Recently, the association of radiation dose with cardiovascular disease mortality in the life span study cohort of 86,000 A-bomb survivors with estimated doses was reported (*2*). Although chronically produced reactive oxygen species and inflammation are thought to be a pathogenic mediator of atherosclerosis, the mechanism has been unclear. In order to better understand the molecular mechanisms underlying the inflammatory reaction frequently encountered in the vascular system after exposure to ionizing radiation, we carried out global scale microarray and computational gene expression analyses in human umbilical endothelial cells.

Materials and Methods

Human umbilical endothelial cells (HUVEC) were irradiated by an X-ray generator at a dose of 2.5 Gy.

Gene expression was analyzed using a GeneChip® system (Affymetrix, Santa Clara, CA; http://www.affymetrix.com/index.affx). Sample preparation for array hybridization was carried out following the manufacturer's instructions. For RNA isolation, total RNA was extracted from cells using

an RNeasy Total RNA Extraction kit (Qiagen, Valencia, CA), and treated with DNase I (RNase-free DNase kit, Qiagen) for 15 min at room temperature to remove residual genomic DNA.

Microarray and computational gene expression analyses were performed using a GeneChip® system with a Human Genome U133-plus 2.0 array spotted with approximately 54,000 probe sets. Samples for array hybridization were prepared as described in the Affymetrix GeneChip® Expression Technical Manual. The scanned arrays were analyzed using GeneChip Analysis Suite Software (Affymetrix). The obtained hybridization intensity data were analyzed using GeneSpring (Silicon Genetics, Redwood City, CA) to extract the significant genes. To examine gene ontology, including biological processes, cellular components, molecular functions, and gene networks, the obtained data were analyzed using Ingenuity Pathways Analysis tools (Ingenuity Systems, Mountain View, CA), a web-delivered application that enables the identification, visualization, and exploration of molecular interaction networks in gene expression data (*3*).

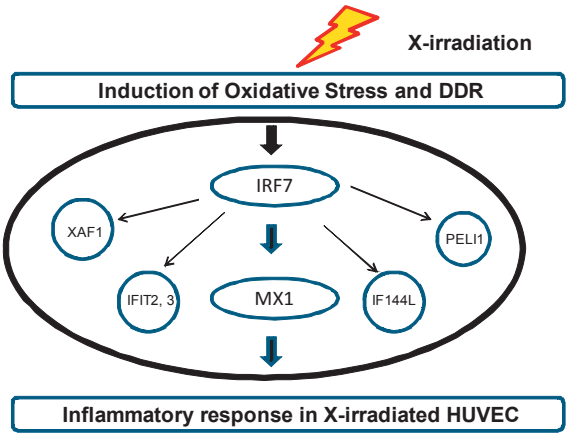
Results

1. Global scale microarray gene expression analysis identified 1,128 genes which were up- or down-regulated in human umbilical endothelial cells (HUVEC) after 2.5 Gy.
2. Bioinformatic analysis using ingenuity pathway analysis tools revealed that the down-regulated genes were associated with cell cycle regulation, whereas the up-regulated genes were associated with inflammatory responses.
3. The analysis also identified a gene network containing interferon response factor 7 (IRF7) and its transcriptional target interferon-induced transcripts (IFITs) and Mx1.

Conclusion

These findings uncover some of the molecular basis of the mechanism(s) of the inflammatory response to X-irradiation in HUVEC.

Figure 1. Scheme of gene expression induced by X-irradiation in HUVEC.



References

1. Shimizu Y, Kato H, Schull WJ, Hoel DG. Studies of the mortality of A-bomb survivors. 9. Mortality, 1950-1985: Part 3. Noncancer mortality based on the revised doses (DS86). Radiat Res 130: 249-266, 1992.
2. Shimizu Y, Kodama K, Nishi N, Kasagi F, Suyama A, Soda M, Grant EJ, et al. Radiation exposure and circulatory disease risk: Hiroshima and Nagasaki atomic bomb survivor data, 1950-2003. Br Med J 340: doi: 10.1136/bmj.b5349, 2010.
3. Tabuchi Y, Takasaki I, Kondo T. Identification of genetic networks involved in the cell injury accompanying endoplasmic reticulum stress induced by bisphenol A in testicular Sertoli cells. Biochem Biophys Res Commun 345:1044-1050, 2006.

Effects of Continuous Low Dose-Rate γ-Irradiation on Diabetic Nephropathy in Type II Diabetes Mellitus Model Mice

Takaharu Nomura[1], Xiao-Han Li[2], Hiromitsu Ogata[3], Kazuo Sakai[1], Takashi Kondo[4], Yasuo Takano[2], and Junji Magae[1]

1. *Radiation Safety Research Center, Nuclear Technology Research Laboratory, Central Research Institute of Electric Power Industry, 2-11-1 Iwado Kita, Komae, Tokyo 201-8511, Japan;*
2. *Department of Diagnostic Pathology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan;*
3. *National Institute of Public Health, 2-3-6 Minami, Wako, Saitama 351-0197, Japan;*
4. *Department of Radiological Sciences, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan.*

Abstract. We previously showed that continuous exposure of mice to low-dose-rate γ-irradiation enhances antioxidant activity. Here we study the ameliorative effect of continuous whole-body irradiation with low-dose-rate γ-rays on diabetic nephropathy. Ten-week-old female *db/db* mice, an experimental model for type II diabetes, were irradiated with low-dose-rate γ-rays from 10 weeks of age throughout their entire lives. Nephropathy was studied by histological observation and biochemical analysis of serum and urine. Antioxidant activities in the kidney were biochemically determined. Continuous low-dose-rate γ-irradiation significantly increases lifespan in *db/db* mice. Three of 24 irradiated mice were free of glucosuria after 80 weeks of irradiation. Histological studies of their kidneys suggest that low-dose irradiation increases the number of normal capillaries in glomeruli. Antioxidant activities of superoxide dismutase, catalase, and glutathione are significantly increased in kidneys of irradiated *db/db* mice. Continuous low-dose rate γ-irradiation ameliorates diabetic nephropathy and increases lifespan in *db/db* mice through the activation of renal antioxidants. These findings have noteworthy implications for radiation risk estimation of non-cancer diseases.

Key Words: low dose rate, kidney damage, antioxidant activity

Introduction

One of the major cellular effects of ionizing radiation is the generation of ROS through interaction with intracellular molecules, which evokes a series of antioxidative reactions within cells called oxidative stress responses. We have shown that whole-body irradiation with low-dose γ-rays enhances antioxidant function in a variety of tissues and protects the tissues from chemically induced oxidative damage (*1-5*). The ameliorative effect of low-dose radiation on type I diabetes has been reported in nonobese diabetic (NOD) mice (*6*) in which autoimmune response causes apoptotic death of β-cells in the pancreas through an ROS-related mechanism (*7-9*). Low-dose radiation increases SOD activity in the pancreas and suppresses β-cell apoptosis and diabetes incidence in this model (*6*). We recently found that continuous whole-body irradiation with low-dose-rate γ-irradiation improves glucose clearance in *db/db* mice (*10*), an experimental model for type II diabetes that causes obesity and diabetes due to a mutation in the leptin receptor gene (*11, 12*). In this model, irradiation does not improve insulin resistance, but does suppress apoptosis and induce SOD expression in β-cells (*10*). A similar result is reported in alloxan-induced type-I diabetic rats, in which a single γ-irradiation at 0.5 Gy prevents elevation of pancreatic lipid peroxidation and blood

glucose (*13*). Because glycation reactions in diabetes are also found in β-cells, resulting in dysfunction and apoptosis of the cells, it can be assumed that continuous irradiation with low-dose-rate γ-rays protects β-cells from the oxidative damage caused by a chronic high glucose environment.

In this study, we exposed *db/db* mice to continuous low-dose-rate radiation for their entire lives and found that the radiation significantly prolongs lifespan and suppresses diabetic nephropathy.

Materials and Methods

Ten-week-old female *db/db* mice (BKS.Cg-+*Lepr*^{db}/+*Lepr*^{db}/Jcl), *db/m* littermates (BKS.Cg-+m/+*Lepr*^{db}/Jcl), and 6-week-old C57BL/6N were used and were treated in accordance with the guidelines for animal experiments of the Central Research Institute of Electric Power Industry.

Continuous whole-body irradiation with low-dose-rate γ-rays was carried out in a clean irradiation room equipped with a 314 GBq 137-Cs γ -ray source.

Glucose, uric acid, creatinine, 8-hydroxy-deoxyguanosine, and insulin were determined biochemically. Activities of SOD, glutathione peroxidase, glutathione reductase, and reduced-type glutathione concentration were assayed by commercially available kits. Measurement of lipid peroxide in the kidney and quantitative pathological examination were also performed.

Results and Discussion

Continuous low-dose-rate γ-irradiation significantly increases lifespan in *db/db* mice. Three of 24 irradiated mice were free of glucosuria after 80 weeks of irradiation. Histological studies of their kidneys suggest that low-dose irradiation increases the number of normal capillaries in glomeruli. Antioxidant activities of superoxide dismutase, catalase, and glutathione are significantly increased in the kidneys of irradiated *db/db* mice. Continuous low-dose-rate γ-irradiation ameliorates diabetic nephropathy and increases lifespan in *db/db* mice through the activation of renal antioxidants.

Our results have important implications for the radiation risk estimation of non-cancer diseases as well as a therapeutic strategy for the amelioration of diabetic complications. These results were recently published (*14*).

References

1. Nomura T, Yamaoka K. Low-dose γ-ray irradiation reduces oxidative damage induced by CCl4 in mouse liver. Free Radic Biol Med. 27:1324-1333, 1999.
2. Kataoka T, Nomura T, Wang DH, Taguchi T, Yamaoka K. Effects of post low-dose X-ray irradiation on carbon tetrachloride-induced acatalasemic mice liver damage. Physiol Chem Phys Med. 37:109-26, 2005.
3. Yamaoka K, Kojima S, Nomura T. Inhibitory effects of post low dose γ -ray irradiation on ferric-nitrilotriacetate-induced mice liver damage. Free Radic Res. 32: 213-21, 2000.
4. Kojima S, Matsuki O, Nomura T, Yamaoka K, Takahashi M, Niki E. Elevation of antioxidant potency in the brain of mice by low-dose γ -ray irradiation and its effect on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced brain damage. Free Radic Biol Med. 26: 388-95, 1999.
5. Yamaoka K, Kataoka T, Nomura T, Taguchi T, Wang DH, Mori S, Hanamoto K, et al. Inhibitory effects of prior low-dose X-ray irradiation on carbon tetrachloride-induced hepatopathy in acatalasemic mice. J Radiat Res. 45:89-95, 2004.
6. Takahashi M, Kojima S, Yamaoka K, Niki E. Prevention of type I diabetes by low-dose gamma irradiation in NOD mice. Radiat Res. 154:680-685, 2000.
7. O'Brien A, Harmon BV, Cameron DP, Allan DJ. Apoptosis is the mode of beta-cell death responsible for the development of IDDM in the nonobese diabetic (NOD) mouse. Diabetes 46:750-757, 1997.
8. Kurrer MO, Pakala SV, Hanson HL, Katz JD. γ-cell apoptosis in T cell-mediated autoimmune diabetes. Proc Natl Acad Sci. 94: 213-218, 1997.
9. Corbett JA, Sweetland MA, Wang JL, Lancaster JR Jr, McDaniel ML. Nitric oxide mediates cytokine-induced inhibition of insulin secretion by human islets of Langerhans. Proc Natl Acad Sci. 90:1731-1735, 1993.

10. Tsuruga M, Taki K, Ishii G, Sasaki Y, Furukawa C, Sugihara T, Nomura T, et al. Amelioration of type II diabetes in *db/db* mice by continuous low-dose-rate γ-irradiation. Radiat Res. 167: 592-599, 2007.
11. Hummel KP, Dickie MM, Coleman DL. Diabetes, a new mutation in the mouse. Sci**.** 153:1127-1128, 1966.
12. Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, et al. Evidence that the diabetes gene encodes the leptin receptor: Identification of a mutation in the leptin receptor gene in db/db mice. Cell 84: 491-495, 1996.
13. Takehara Y, Yamaoka K, Hiraki Y, Yoshioka T, Utsumi K. Protection against alloxan diabetes by low-dose 60Co gamma irradiation before alloxan administration. Physiol Chem Phys Med NMR. 27:149-59, 1995.
14. Nomura T, Li X-H, Ogata H, Sakai K, Kondo T, Takano Y, Magae J. Suppressive effects of continuous low dose-rate γ-irradiation on diabetic nephropathy in type II diabetes mellitus model mice. Radiat Res. 176:356-365, 2011.

Development of immunocompetent lymphocytes in vivo by mixed umbilical cord blood transplantation in mice

Akira Nakano[1], Kyoko Ito[2]and Koichi Ito[2]

[1] Department of Cellular Biochemistry and Molecular Biology,
Hirosaki University Graduate School of Health Sciences, Japan.
[2] Department of Biomedical Sciences,
Hirosaki University Graduate School of Health Sciences, Japan.

Abstract. Umbilical cord blood cell (UCBC) transplantation is an effective treatment for accidental high-dose total-body irradiation exposure. We previously reported that mixed UCBC transplantation has a high success rates and induce development of phenotypically mature lymphocytes in lethal X-ray-irradiated recipient mice. In this study, we evaluated the functional maturity of T and B lymphocytes reconstituted by mixed UCBC transplantation in recipient mice. C57BL/6 female mice X-ray-irradiated with 9-Gray were transplanted with 3 different combinations of mixed UCBCs. At 16 weeks after transplantation, all recipients received skin grafting to assess the functional maturity of the newly developed T and B lymphocytes. Our observations indicate that T and B lymphocytes derived from mixed UCBCs transplants are immunologically fully competent with the ability to distinguish self from non-self using different major histocompatibility complex antigens.

Key words: mixed UCBC transplantation, immune function, major histocompatibility complex, radiation exposure

Introduction

Currently, umbilical cord blood cells (UCBCs) are used as the primary source of hematopoietic stem cells (HSCs) for transplantation instead of bone marrow cells (BMCs). However, one disadvantage of UCBC transplantation is the limited quantity of cord blood that can be obtained from a pregnant woman. To overcome this quantity limitation, the use of mixed cord blood might be necessary. Due to a lack of convenient animal models, analyses of the differentiation capacity of mixed UCBCs in recipients have been limited to *in vivo* xenogeneic experiments [1-3] and clinical observations [4-6]. At the Second International Symposium on Radiation Emergency Medicine held at Hirosaki University last year, we reported that mixed UCBC transplantation has a high rate of success and induces development of phenotypically mature lymphocytes in recipient mice that have been subjected to lethal X-ray irradiation. However, the extent to which the lymphocytes derived from the mixed UCBC transplant recover their immune function is still unclear. In this study, we evaluated the functional maturity of T and B lymphocytes reconstituted by mixed UCBC transplantation in recipient mice.

Mixed UCBC transplantation

C57BL/6 (B6) (H-2^b) female mice subjected to 9-Gray X-ray irradiation received transplants comprising three different combinations of mixed UCBCs: group (1), green fluorescent protein (GFP)-Tg B6 (H-2^b) and C3H (H-2^k); group (2), GFP-Tg B6 (H-2^b) and BALB/c (H-2^d); group (3), C3H (H-2^k) and BALB/c (H-2^d), each combination containing an equal number of cells (Figure 1). At 16 weeks after transplantation, all recipients underwent skin grafting to assess the functional maturity of the newly developed T and B lymphocytes.

* Corresponding to: Koich Ito, Associate professor, Hirosaki University, 66-1 Hon-Cho, Hirosaki, 036-8564, Japan. E-mail:kohito@cc.hirosaki-u.ac.jp

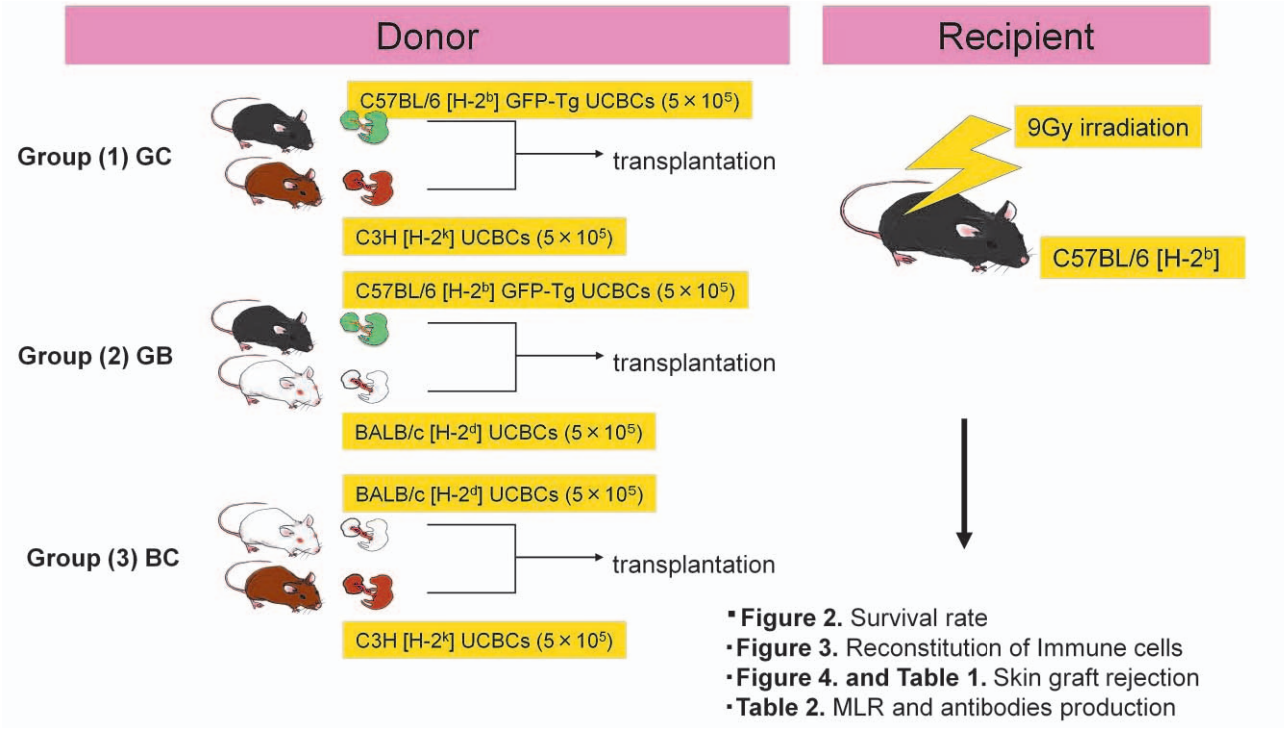


Figure1. Mixed UCBC transplantation

Survival rate of recipient mice after mixed UCBC transplantation

The survival curves up to 16 weeks after transplantation are shown (Figure 2). The survival rate at 16 weeks after transplantation was 92% for group (1) (12/13 mice; indicated by a blue line), 73% for group (2) (8/11 mice; indicated by a red line), and 50% for group (3) (3/6 mice; indicated by a green line). On the other hand, irradiated control mice (indicated by a black line) that did not undergo subsequent

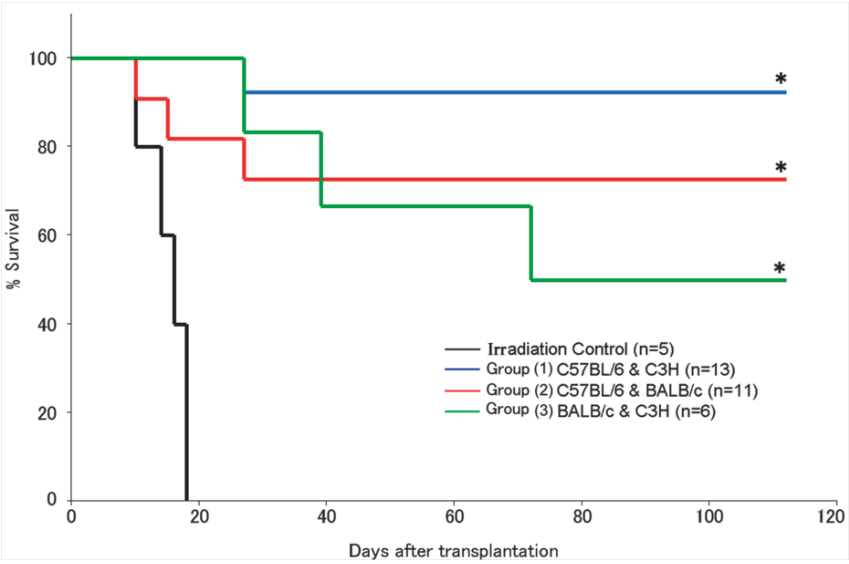


Figure 2. Survival rate of recipient mice after mixed UCBC transplantation

transplantation died within 20 days, indicating that mixed UCBC transplantation can improve hematopoiesis in irradiated recipients.
*P>0.01 by student's *t* test

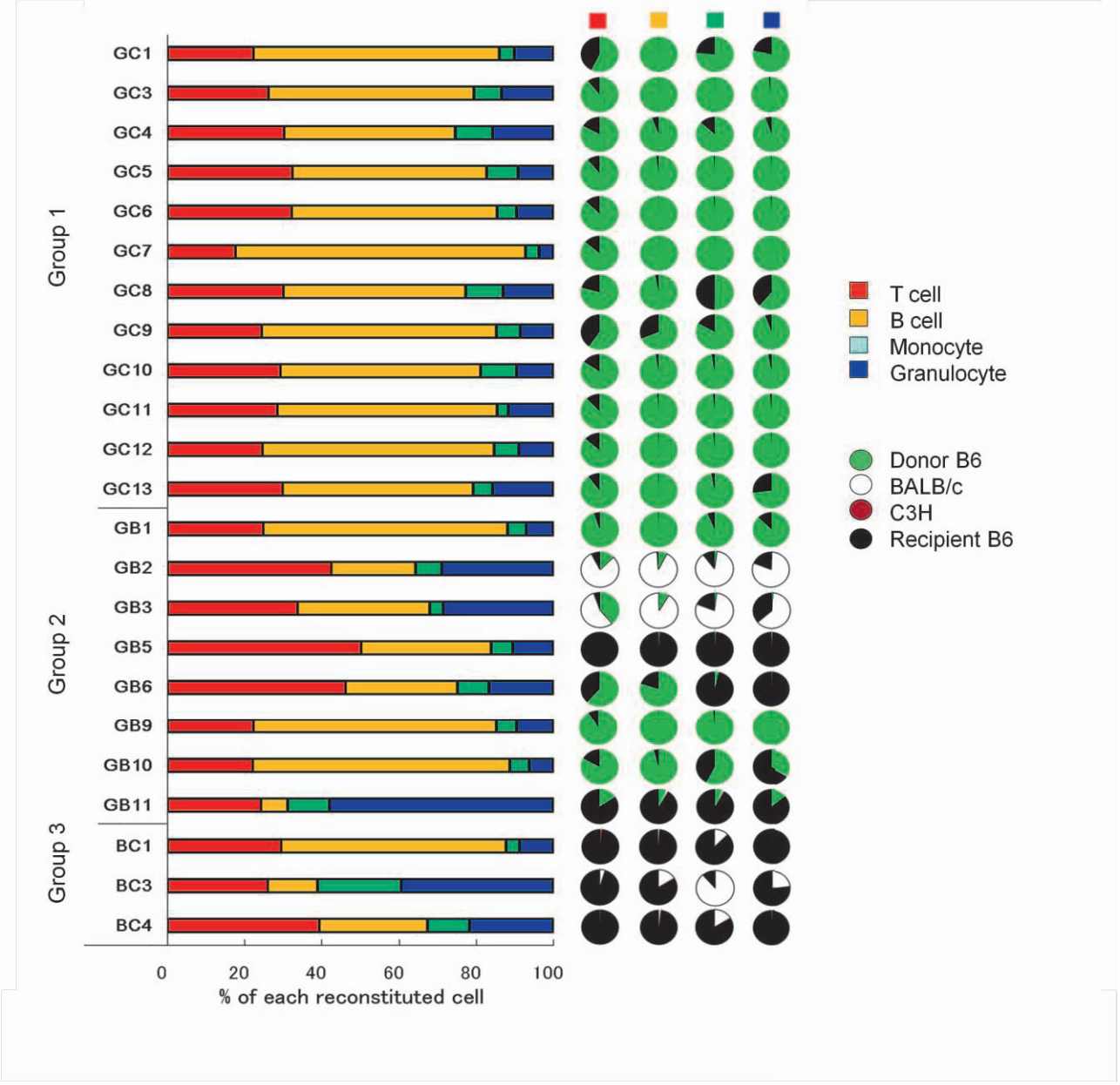


Figure 3. Reconstitution of immune cells by mixed UCBC transplantation

Reconstitution of immune cells by mixed UCBC transplantation

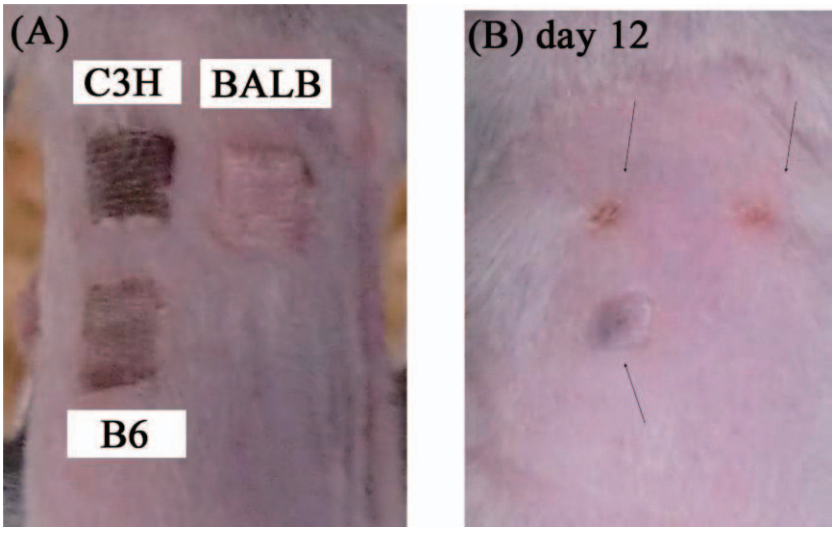
The details of the reconstituted immune cells in recipients were evaluated at 16 weeks after transplantation by flow cytometric analysis using specific antibodies against both lineage markers (for T cells, B cells, macrophages and granulocytes) and major histocompatibility complex (MHC) (for C3H and BALB/c) (Figure 3). The bar graph shows the percentage of each reconstituted lineage population in a recipient. The pie chart displays the proportion of donor-origin cells in each lineage population on the bar graph. All recipients showed recovery of the four major lineage populations, and these were constituted by GFP^+ cells and /or B6

recipients' own HSCs: group (1), 100% (12/12 mice); group (2), 75% (6/8 mice); group (3), 100% (3/3 mice), suggesting predominant engraftment of MHC-matched UCBC-HSCs in the recipients' bone marrow.

Skin grafting to recipient mice

Over 16 weeks after transplantation, a piece of skin harvested from the tail of B6, BALB/c, and C3H mice was simultaneously grafted onto the shaved back of the recipient mice (Figure 4A). For example, GC11 in group (1) shown in Fig 3, in which reconstituted immune cells formed the B6 background, completely rejected the skin grafts from C3H and BALB/c as the allo-antigen on day 12, whereas a skin graft from B6 was accepted as self-antigen, in accordance with the immunological rule of MHC restriction (Figure 4B).

Figure 4 . Skin grafting to recipient mice



In vivo functional analysis of reconstituted lymphocytes

All recipients having a B6 background completely rejected allogeneic skin grafts from C3H and BALB/c mice within 20 days (Table 1). This was observed in both types of recipient whose immune cells were reconstituted by HSC from the GFP^+ donor or recipient self. Unexpectedly, however, several of these recipients also showed rejection of skin grafts from B6 as self-antigen, suggesting functional maturation of the repopulated T and B cells in the recipient mice.

In vitro functional analysis of reconstituted lymphocytes

The splenic T-cell response of each individual recipient that had rejected the skin graft was evaluated by the mixed lymphocyte reaction with B6, BALB/c, and C3H stimulators (Table 2). The T-cell response was expressed as a value relative to the minimum MLR response (obtained as cpm of ^3H incorporation) in three stimulations, which was defined as 1. In addition, the production of allo-antibody in the these mice was examined using the complement-dependent cytotoxicity (CDC) test. Data were expressed as the final serum sample dilution ratio when more than 50% of the target cells had died. Both cellular and humoral immune responses were exerted against BALB/c and C3H as non- self in almost all the recipients, indicating normal T- and B-cell development and function in a B6 background.

Table1. In vivo functional analysis of reconstituted lymphocytes

Recipients	Rejection time (days)		
	B6	BALB	C3H
GC1	>30	20	10
GC3	>30	13	10
GC4	>30	16	11
GC5	>30	11	10
GC6	>30	13	10
GC7	>30	12	14
GC8	>30	13	12
GC9	>30	16	11
GC10	>30	14	15
GC11	>30	12	12
GC12	>30	12	12
GC13	>30	9	9
GB1	12	16	10
GB2	NT	NT	NT
GB3	11	8	8
GB5	>30	9	13
GB6	NT	NT	NT
GB9	>30	9	15
GB10	>30	11	11
GB11	13	16	15
BC1	10	9	10
BC3	NT	NT	NT
BC4	>30	11	11

Table2. In vitro functional analysis of reconstituted lymphocytes

Recipients	MLR response			CDC		
	Stimulator cells			Target cells		
	B6	BALB	C3H	B6	BALB	C3H
GC1	NT	NT	NT	0	>64	>64
GC3	1	1.01	3.11	0	>64	>64
GC4	NT	NT	NT	0	>64	32
GC5	1	13.11	17.33	0	>64	>64
GC6	1	0.79	0.8	0	>64	4
GC7	1	4.28	2.39	0	>64	0
GC8	1	10.96	7.69	0	16	>64
GC9	1	2.19	1.64	0	>64	>64
GC10	NT	NT	NT	0	>64	>64
GC11	NT	NT	NT	0	>64	>64
GC12	1	32.75	33.73	0	>64	32
GC13	NT	NT	NT	0	>64	>64
GB1	1	1.64	1.72	0	>64	>64
GB2	NT	NT	NT	NT	NT	NT
GB3	1	1.32	18.55	0	0	0
GB5	NT	NT	NT	0	>64	4
GB6	NT	NT	NT	NT	NT	NT
GB9	1	30.98	11.18	0	>64	>64
GB10	1	4.83	4.44	0	>64	>64
GB11	NT	NT	NT	NT	NT	NT
BC1	1	86.56	122.47	0	>64	>64
BC3	NT	NT	NT	NT	NT	NT
BC4	NT	NT	NT	0	0	0

Conclusions

Mixed UCBC transplantation clearly rescued mice that had been subjected to lethal X-ray irradiation. Furthermore, our observations indicate that T and B lymphocytes derived from mixed UCBCs transplants are immunologically fully competent with the ability to distinguish self from non-self using different MHC antigens. However, a clear understanding of the mechanisms underlying the predominant engraftment of MHC-matched HSCs in recipients' bone marrow will be necessary for future studies.

Acknowledgements

This work was supported by a Grant for Co-medical Education Program in Radiation Emergency Medicine by the Ministry of Education, Culture, Sport, Science and Technology, Japan, and by a Grant for Hirosaki University Institutional Research.

References

[1] Wang JCY, Doedens M and Dick JE. Primitive human hematopoietic cells are enriched in cord blood compared with adult bone marrow or mobilized peripheral blood as measured by the quantitative in vivo SCID-repopulating cell assay. *Blood* 89: 3919-3924 (1997).

[2] Yahata T, Ando K, Nakamura Y, Ueyama Y, Shimamura K, Tamaoki N, Kato S and Hotta T. Functional human T lymphocytes development from cord blood $CD34^+$ cells in nonobese diabetic/Shi-scid, IL-2 receptor gamma null mice. *J. Immunol.* 169: 204-209 (2002).

[3] Traggiai E, Chicha L, Mazzucchelli L, Bronz L, Piffaretti JC, Lanzavecchia A and Manz MG. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science* 304: 104-107 (2004).

[4] Gluckman E, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N. Engl. J. Med.* 321: 1174-1178 (1989).

[5] Gluckman E, et al. Outcome of cord-blood transplantation from related and unrelated donors. *N. Engl. J. Med.* 337: 373-381 (1997).

[6] Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D, Arny M and Boyse EA. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc. Natl. Acad. Sci. USA* 86: 3828-3832 (1989)

Promising potential of umbilical cord blood cell transplantation in repopulating bacterial-reactive natural IgM-producing B-1a cells

Ayumi WAKAYAMA[1,2], Kyoko ITO[1], Koichi ITO[1*]

[1] Department of Biomedical Sciences,
Hirosaki University Graduate School of Health Sciences, Japan.
[2] Transfusion and Cell Therapy Center,
Hakodate Municipal Hospital, Japan.

Abstract. Murine umbilical cord blood cells (UCBCs) were studied for their ability to reconstitute hematopoietic system. In this study, the extent of B-1a cell reconstitution was compared between UCBC and bone marrow cell (BMC) transplantation. RAG2 (-/-) C57BL/6 (B6) recipient mice were lethally irradiated with 9-Gray and immediately given a transplant of syngeneic UCBCs or BMCs obtained from green fluorescent protein (GFP)-transgenic B6 mice. At 16 weeks after the transplantation, reconstitution of peritoneal B-1a cells in the recipients was detected by multicolor staining with anti-CD45R/B220, anti-CD5, anti-IgM, and anti-CD11b antibodies. Furthermore, production of natural IgM antibodies from B-1a cells in the recipient mice was measured by enzyme-linked immunosorbent assay before and after immunization with bacterial-derived polysaccharide. Flow cytometric analysis revealed that the number of GFP-positive peritoneal B-1a cells was relatively high in UCBC recipients and low in BMC recipients. Bacterial polysaccharide-reactive IgM antibodies were produced predominantly in UCBC recipients, indicating that IgM production is correlated with the presence of B-1a cells. On the basis of our finding, we strongly recommend the use of UCBCs for hematopoietic stem cell transplantation.

Key words: umbilical cord blood, immune reconstitution, B-1a cells, radiation exposure

Introduction

Umbilical cord blood cell (UCBC) transplantation is an effective treatment of not only various hematological diseases [1-3] but also accidental high-dose total-body radiation exposure [4]. UCBC transplantation has several advantages over bone marrow cell (BMC) transplantation: the larger size of the available donor pool, enriched hematopoietic progenitor cells [5], low content of mature T cells causing graft-versus-host reaction [6, 7], and absence of cytomegalovirus infection [8]. Although UCBCs and BMCs essentially have a similar ability of recovering the hematopoietic system, it remains unclear whether UCBC and BMC transplantation reconstitute all immune cells. The B cell population is composed mainly of three different subsets: B-1a, B-1b, and B-2 cells. Among them, B-1a cells spontaneously secrete natural IgM antibodies against pathogens such as *Streptococcus pneumoniae* and influenza virus in the peritoneal and pleural cavities [9, 10]. In this study, we compared the extent of B-1a cell

* Corresponding to: Koich Ito, Associate professor, Hirosaki University, 66-1 Hon-Cho, Hirosaki, 036-8564, Japan. E-mail:kohito@cc.hirosaki-u.ac.jp

reconstitution between UCBC and BMC transplantation. Our results indicate that UCBCs have promising potential to reconstitute functional natural IgM-producing B-1a cells for front-line defense.

Reconstitution of peritoneal B-1a cells in recipients after UCBC or BMC transplantation

RAG2 (-/-) C57BL/6 (H-2^b) recipient mice subjected to 9-Gray irradiation received a transplant of $1x10^6$ UCBCs or BMCs, which had been depleted of mature T cells, obtained from green fluorescent protein (GFP)-transgenic (Tg) C57BL/6 (H-2^b) mice. The experimental procedure was approved by the Animal Research Committee of Hirosaki University, and performed in accordance with the institutional Guidelines for Animal Experimentation. The ability of the UCBCs and BMCs to repopulate natural IgM antibody-producing B-1a cells was then examined in both sets of recipients. At 16 weeks after transplantation, peritoneal cells (PCs) were harvested carefully from the peritoneal cavity of UCBC and BMC recipients by rinsing with 2-3 ml of sterile PBS, avoiding any contamination with peripheral blood, after sacrifice. Harvested PCs were then stained with PE-labeled anti-CD45R/B220, PE-TexasRed-labeled anti-CD5, PerCP-Cy5.5-labeled anti-IgM, and APC-Cy7-labeled anti-CD11b monoclonal antibodies, and the cells were analyzed on a Cytomics FC500 (Beckman Coulter) using CXP software. PCs, which are stained with all these antibodies, were identified as the B-1a cell population. As a representative result, flow cytometric analysis showed that the number of

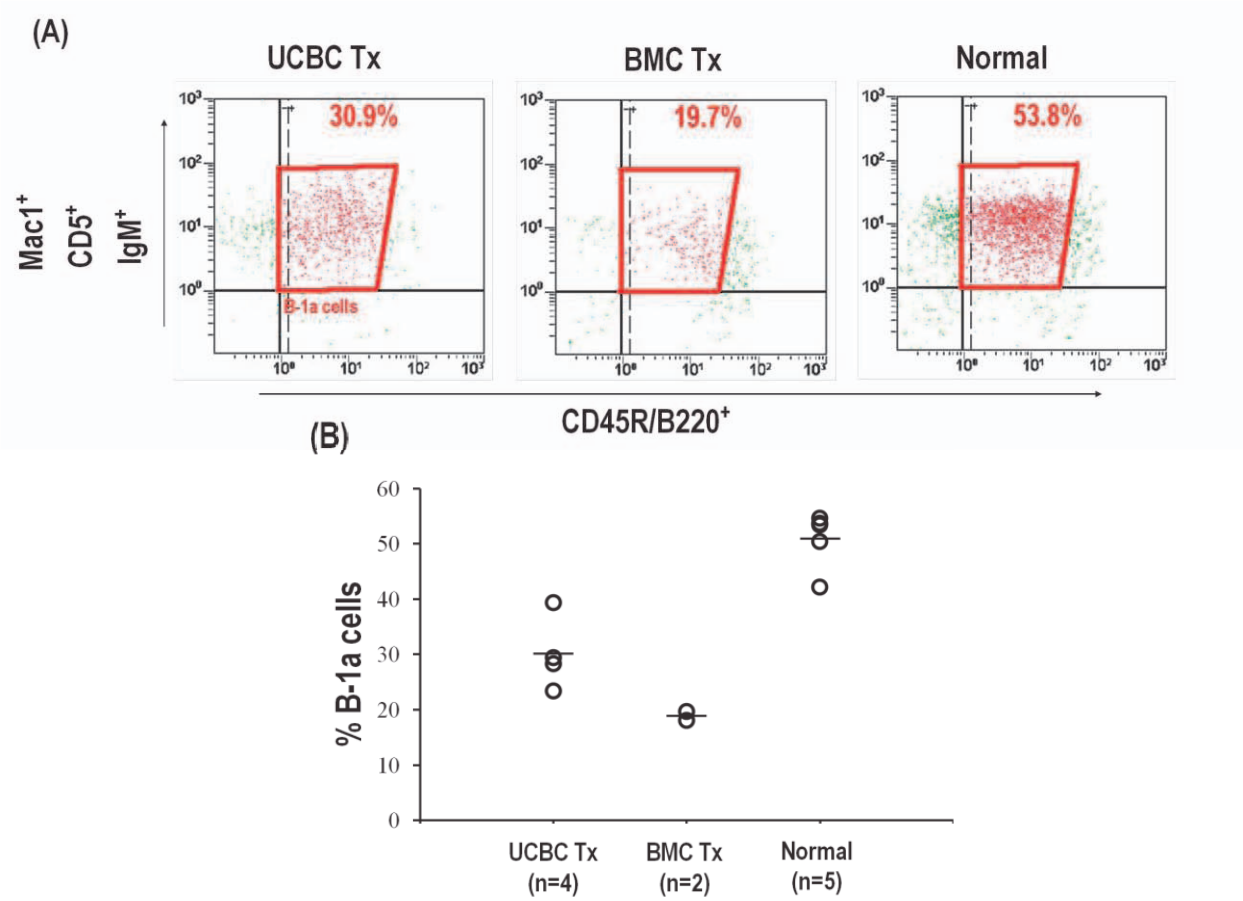


Figure1. Reconstitution of peritoneal B-1a cells in recipients after UCBC or BMC transplantation

B-1a cells was high in an UCBC recipient (30.9%) but low in a BMC recipient (19.7%) (Figure 1A). Although the number of B-1a cells in UCBC recipients did not recover to the level seen in normal C57BL/6 mice, similar results were obtained for a larger number of samples, indicating that UCBCs have promising potential for B-1a cell reconstitution (Figure 1B).

IgM production against bacterial polysaccharide in UCBC and BMC recipients

At 16 weeks after transplantation, UCBC and BMC recipients were immunized once intraperitoneally with 11.5ug Pneumovax-NP (Banyu Pharm. Co., Ltd) containing polysaccharides from *Streptococcus pneumonia* constituted by equal amounts of 23 capsular types, and sera were collected on day 7, 14, 21, and 28 after immunization. Anti-bacterial polysaccharide IgM production in serum was detected by enzyme-linked immunosorbent assay on Pneumovax-NP-coated 96-well plates. Optical density (OD) was measured at 490 nm. Serum samples diluted 1:512 were used in the assay. The mean OD values in each group were plotted. Bacterial polysaccharide-reactive IgM was detectable at one week after immunization in UCBC recipients, but not in BMC recipients (Figure 2A). However, the level of the IgM production in UCBC recipients was obviously lower than in normal C57BL/6 mice. All individual OD values of both recipients in Figure 1A were also shown with small scale of OD. All three UCBC recipients developed a similar level of IgM production at one week after immunization (Figure 2B), whereas IgM production in five BMC recipients was

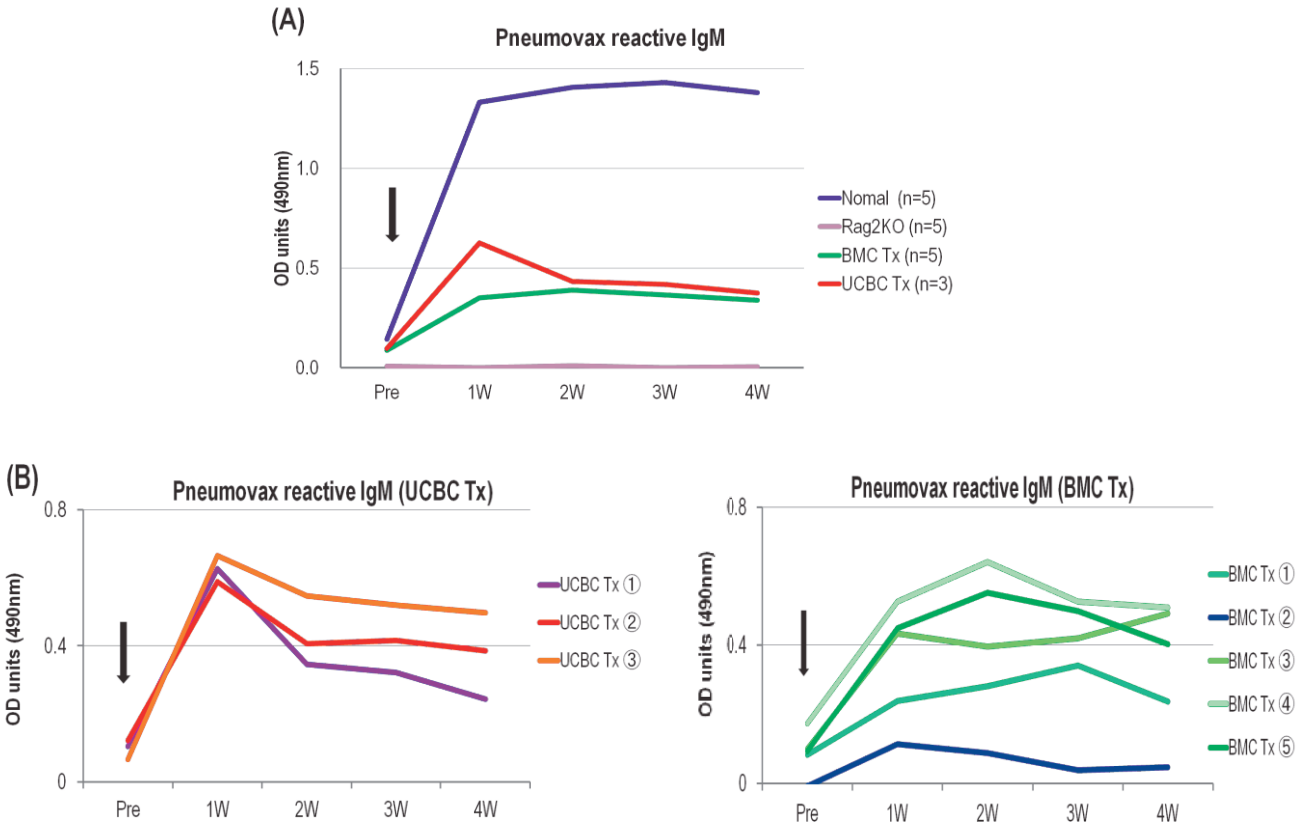


Figure 2. IgM production against bacterial polysaccharide in UCBC and BMC recipients

essentially low and heterogeneous (Figure 2C). These results suggest that bacterial polysaccharide-reactive IgM antibodies appear to be produced predominantly in UCBC recipients, indicating that IgM production is correlated with the number of reconstituted B-1a cells.

Site of residence of B-1a cells in lymphoid organs

B-1a cells were isolated from PCs from GFP-Tg C57BL/6 mice using the magnetic method by negative selection with anti-CD90 antibody, followed by positive selection with anti-CD5 antibody. The cells were then intraperitoneally transferred to RAG2 (-/-) C57BL/6 mouse. At one month after injection,

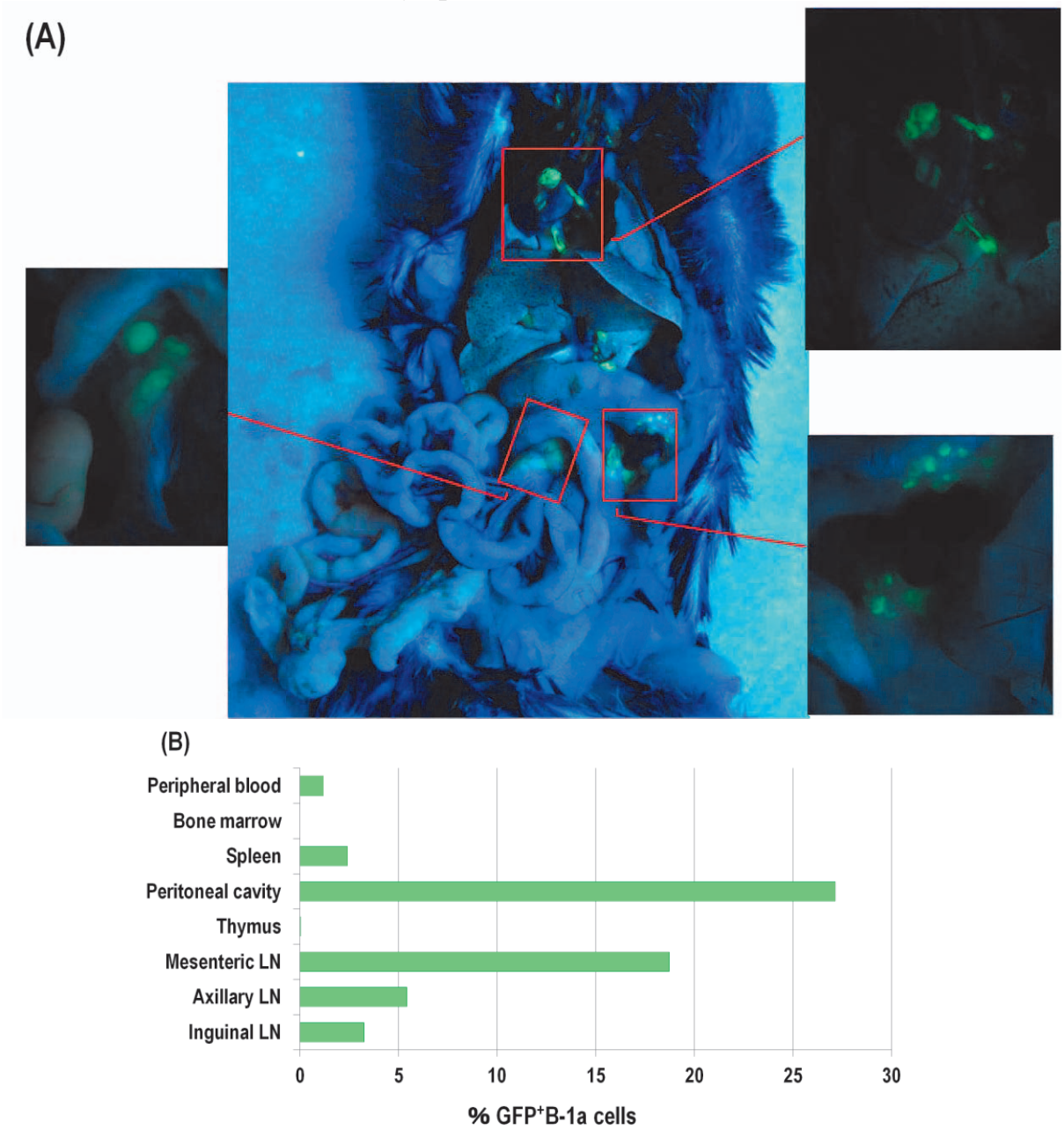


Figure 3. Site of residence of B-1a cells in lymphoid organs

GFP-positive B-1a cells were specifically detected in the pleura and intestine, which is a strict requirement for front-line defense against invading pathogens, under UV exposure in cell-transferred mouse (Figure 3A). Also, the percentage of GFP-positive B-1a cells in various lymphoid tissues was examined in detail by flow cytometry (Figure 3B). Obvious accumulation of GFP-positive B-1a cells was observed in the peritoneal and pleural cavities, and mesenteric lymph nodes, corresponding to the results described in Figure 3A. Essentially similar results were obtained in two other mice (data not shown).

Conclusion

Our results indicate that UCBCs have promising potential in the reconstitution of functional natural IgM-producing B-1a cells involved infront-line innate defense. These finding is clinically valuable for hematopoietic stem cell transplantation.

Acknowledgements

This work was supported by a Grant for Co-medical Education Program in Radiation Emergency Medicine by the Ministry of Education, Culture, Sport, Science and Technology, Japan, and by a Grant for Hirosaki University Institutional Research.

References

[1] Gluckman E, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N. Engl. J. Med.* 321: 1174-1178 (1989).

[2] Gluckman E, et al. Outcome of cord-blood transplantation from related and unrelated donors. *N. Engl. J. Med.* 337: 373-381 (1997).

[3] Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D, Arny M and Boyse EA. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc. Natl. Acad. Sci. USA* 86: 3828-3832 (1989).

[4] Nagayama H, et al. Transient hematopoietic stem cell rescue using umbilical cord blood for a lethally irradiated nuclear accident victim. *Bone Marrow Transplant.* 29: 197-204 (2002).

[5] Wang JCY, Doedens M and Dick JE. Primitive human hematopoietic cells are enriched in cord blood compared with adult bone marrow or mobilized peripheral blood as measured by the quantitative in vivo SCID-repopulating cell assay. *Blood* 89: 3919-3924 (1997).

[6] Bofill M, Akber AN, Salmon M, Robinson M, Burford G and Janossay G. Immature $CD45RA^{low}RO^{low}$ T cells in the human cord blood. *J. Immunol.* 152: 5613-5623 (1994).

[7] Madrigal JA, Cohen SBA, Gluckman E and Charron DJ. Does cord blood transplantation result in lower graft-versus host disease? *Hum. Immunol.* 56: 1-5 (1997).

[8] Tomonari A, Iseki T, Ooi J, Takahashi S, Shindo M, Ishii K, Nagamura F, Uchimaru K, Tani K, Tojo A and Asano S. Cytomegalovirus infection following unrelated cord blood transplantation for adult patients: a single institute experience in Japan. *Br. J. Haematol.* 121: 304-311 (2003).

[9] Montecino-Rodriguez E, Leathers H and Dorshkind K. Identification of a B-1 B cell-specified progenitor. *Nat. Immunol.* 7: 293-301 (2006).

[10] Choi YS and Baumgarth N. Dual for B-1a cells in immunity to influenza virus infection. *J Exp Med.* 205: 3053-3064 (2008).

Radiation-responsive transcriptome analysis in human hematopoietic cells

Mitsuru Chiba[1], Satoru Monzen[2], Kosuke Kasai[3], Tomisato Miura[3], Ikuo Kashiwakura[2], Toshiya Nakamura[1]

[1] Department of Biomedical Sciences, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan
[2] Department of Radiation Life Sciences, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan
[3] Department of Pathologic Analysis, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan

Abstract. Ionizing radiation (IR) causes DNA injury and induces multiple signal mechanisms, including the regulation of DNA repair, cell cycles, and gene expression, by the activation of p53-related pathways. Natural antisense transcripts (NATs) are recognized as important regulators of gene expression, but the effects of IR on NAT expression is unknown. Therefore, we investigated the gene expressions of sense transcripts (mRNA) and NATs after X-ray irradiation in a human B lymphoblast cell line (IM-9). Sense/antisense microarray analysis showed that the mRNA expression of 93 genes and NAT expression of 24 genes were up-regulated more than 1.5 times in irradiated samples (1, 2, and 4 Gy) as compared to non-irradiated samples (0 Gy). Among the mRNA of 93 genes were 5 NAT expression genes, including the *MDM2* and *CDKN1A* genes. In conclusion, radiation-responsive *cis*-NATs were identified for the first time.

Key Words: ionizing radiation, natural antisense transcripts, B lymphoblast cell, microarray

Introduction

Recent studies have revealed that IR generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cells, which leads to DNA injury at random sites. Poly (ADP-ribose) polymerase (PARP) and DNA-dependent protein kinase repair complex (DNA-PK) are activated by double-strand breaks (DSB) of DNA to engage in DNA repair [1]. In addition, Ataxia telangiectasia mutated (ATM) is activated in response to DNA injury to initiate phosphorylation of p53. The p53 was found to up-regulate mRNA expressions of *GADD45*, *MDM2*, *CDKN1A* ($p21^{Cip1}$) and *BAX* to control cell cycle, DNA repair, and apoptosis [1].

Recently, a large number of non-coding RNAs which were not translated into protein have been discovered by transcriptome analysis. Currently, non-coding RNAs are classified in various types of RNA such as microRNA, Piwi-interacting RNA (piRNA), small nuclear RNA (snRNA), and natural antisense transcripts (*cis*-NATs) transcribed from DNA strands opposing the sense strands [2]. The *cis*-NATs were demonstrated to be involved in the control of gene expression in a eukaryote [2, 3].

In the present study, the *cis*-NATs up-regulated by X-ray irradiation were investigated using a human B lymphoblastic cell line IM-9 having a high sensitivity to IR and using a custom-microarray containing human sense/antisense probes for *ca*. 21,000 genes.

Materials and methods

X-ray irradiation.

The IM-9 cells plated on the wells were cultured for 24 h prior to X-ray irradiation. The irradiation by X-ray (1, 2, or 4 Gy) was performed using an X-ray generator MBR-1520R. The irradiated cells

were further cultured for 24 h under the same conditions and then collected by centrifugation.

Microarray analysis.

Cyanine 3 (Cy3)-labeled cDNA was synthesized from 10 μg total RNA of irradiated or non-irradiated samples using a LabelStar Array kit (Qiagen), Cy3-dUTP (GE healthcare), and random nonamer primer. Agilent 44 K x 4 human sense/antisense custom-microarray slides were hybridized with the Cy3-labeled cDNA (2 μg) in a hybridization solution prepared with an In Situ Hybridization Kit Plus (Agilent Technologies), following the manufacturer's instructions.

Strand-specific RT-qPCR.

Total RNAs were subjected to synthesis of the first-strand cDNA only from mRNA or *cis*-NATs using reverse or forward primer and Reverse Transcriptase (Promega) together with *ACTB* reverse primer. Then, the mixtures were incubated at 50°C for 60 min. The first-strand cDNAs derived from mRNA or *cis*-NATs were used as a template for quantitative PCR (qPCR) using a Power SYBR Green PCR Master Mix (Applied Biosystems). The qPCRs were performed using an Applied Biosystems StepOne Plus Real-Time PCR system at 95°C for 10 min followed by 40 cycles each of 95°C for 15 sec and 60°C for 60 sec. In order to compare the PCR results, the values for mRNA and *cis*-NATs were normalized based on the values of *ACTB* mRNA.

Results and discussion

Sense/antisense microarray analysis showed that the mRNA expression of 93 genes and NAT expression of 24 genes were up-regulated more than 1.5 times in irradiated samples (1, 2, and 4 Gy) as compared to non-irradiated samples (0 Gy) (figure 1). Among the mRNA of 93 genes were 5 *cis*-NAT expression genes which had been transcribed from the opposite DNA locus of the gene, including the *MDM2* and *CDKN1A* genes (figure 1, table 1). The mRNA and *cis*-NATs of these 5 genes were up-regulated by X-ray exposure in a dose-dependent fashion (table 1). The mRNAs and *cis*-NATs of *MDM2* and *CDKN1A* were also found to be up-regulated in proportion to X-ray exposure by strand-specific RT-qPCR (figure 2). In conclusion, radiation-responsive *cis*-NATs were identified for the first time. *MDM2* and *CDKN1A* were the p53-related genes involved in the DNA repair and cell cycle checkpoint, and it would be expected that these *cis*-NATs regulate the gene expression of mRNA post-transcription. In the future, IR dose-dependent up-regulation of these mRNAs and *cis*-NATs may be used as a biomarker for evaluating DNA injury.

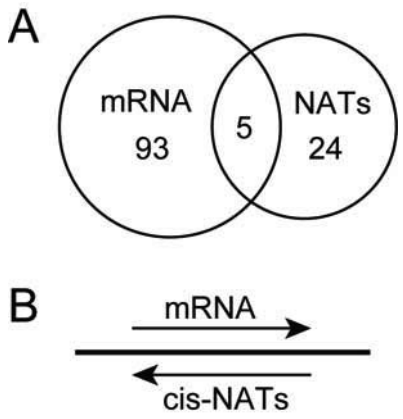


Figure 1. Microarray analysis of sense/antisense transcripts in response to IR.
(A) Venn diagram of mRNAs and NATs up-regulated 1.5 times by X-ray irradiation. The number of mRNAs and NATs were 93 and 24 genes, respectively. (B) Schematic presentation of sense/antisense transcripts. *cis*-NATs were transcribed from opposing DNA strands at the same gene locus.

Acknowledgments

This study was supported by a grant from the Hirosaki University Graduate School of Health Sciences Radiation Emergency Medicine Human Resource Cultivation Project.

References

[1] Bourguignon MH, Gisone PA, Perez MR, et al.: Genetic and epigenetic features in radiation sensitivity Part I: cell signalling in radiation response. Eur J Nucl Med Mol Imaging 32: 229-246, 2005.

[2] Lavorgna G, Dahary D, Lehner B, Sorek R, Sanderson CM and Casari G: In search of antisense. Trends Biochem Sci 29: 88-94, 2004.

[3] Werner A and Berdal A: Natural antisense transcripts: sound or silence? Physiol Genomics 23: 125-131, 2005.

Table 1. The list of mRNAs and NATs up-regulated more than 1.5 times compared to 0 Gy after IR exposure in IM-9.

Gene symbol	Accession no.	Sense/ antisense	Relative expression ratio (vs 0 Gy)				Gene name
			0 Gy	**1 Gy**	**2 Gy**	**4 Gy**	
MDM2	NM002392.2	mRNA	1.00	1.91	2.30	3.49	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)
		NAT	1.00	2.25	2.94	4.37	
CDKN1A	NM078467.1	mRNA	1.00	1.83	2.15	2.96	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
		NAT	1.00	1.76	1.96	2.65	
MS4A1	NM152866.2	mRNA	1.00	1.61	1.68	2.03	membrane-spanning 4-domains, subfamily A, member 1
		NAT	1.00	1.97	2.04	2.62	
PSAP	NM002778.1	mRNA	1.00	1.52	1.72	2.04	prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy)
		NAT	1.00	1.91	2.02	2.92	
TM7SF3	NM016551.1	mRNA	1.00	1.77	1.83	2.32	transmembrane 7 superfamily member 3
		NAT	1.00	1.69	1.66	2.22	

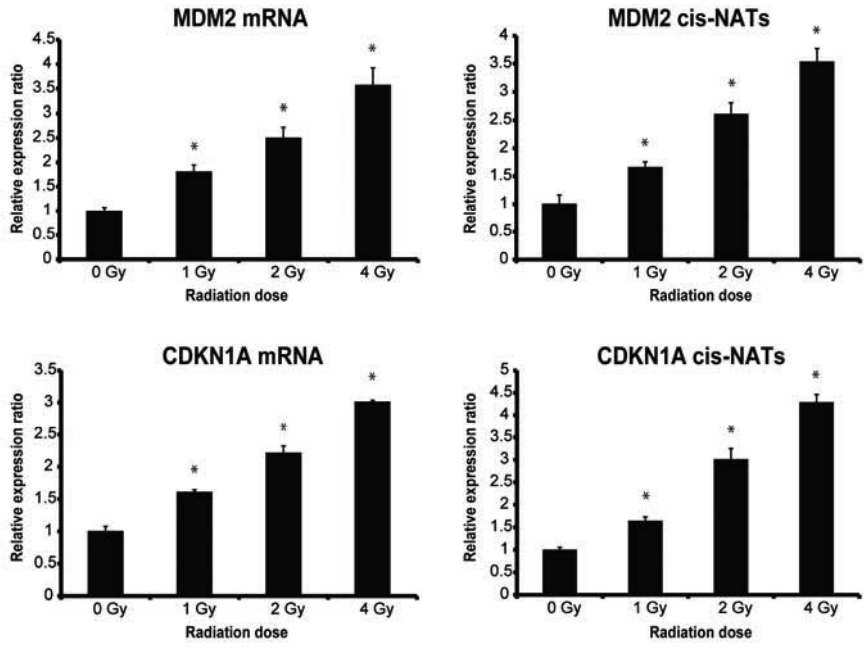


Figure 2. Relative expression of mRNAs and NATs of *MDM2* and *CDKN1A* genes by X-ray irradiated IM-9 cells.
The mRNA and NATs expression levels were determined using a strand-specific RT-qPCR analysis. Values for mRNAs and NATs were normalized based on values of *ACTB* mRNA. Asterisks indicate statistical significance, compared with 0 Gy ($p < 0.05$).

Analysis of the profile and mechanism of radiation-induced hair loss through examination of protein derived from the hair of patients with acute alopecia caused by external radiation exposure

Naoki Nanashima[1], Koichi Ito[1], Hiroyuki Nozaka[1], Takashi Ishikawa[1], Manabu Nakano[1], Asako Ishizawa[2], Honami Narita[2] and Toshiya Nakamura[1]

[1] Department of Biomedical Sciences, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan
[2] Department of Medical Technology, Hirosaki University School of Health Sciences, Hirosaki, Japan

Abstract. A rapid, non-invasive screening test is needed to evaluate the effects of radiation exposure following radiation accidents, acts of nuclear terrorism, or widespread radiation disasters. Acute alopecia is a recognized consequence of radiation exposure, but the mechanism by which radiation causes acute alopecia and chemical changes in hair protein is unknown. Therefore, we analyzed the mechanism underlying acute alopecia following radiation exposure and examined whether hair protein could be used as a new biomarker for evaluating radiation exposure. In order to examine whether hair protein profiles are different between irradiated mice and controls, six-week-old male C57/BL6 mice were exposed to 6 Gy of X-irradiation, and, after 5weeks, their hair and skin were collected. The hair protein was subjected to 2-dimensional electrophoresis and liquid chromatography/electro-ionization mass spectrometry. There was no change in the composition of major hair keratins, such as Krt81, Krt83, and Krt86, but the expression of Krt15, which is a marker of hair stem cells, was detected in the control mice but not in the mice with alopecia. Krt1, Krt5, and Krt10 were detected in the hair of the mice with alopecia. These data suggest hair stem cells were damaged by irradiation, and these keratins are expected to be new biological markers for acute radiation symptoms.

Key Words: X-ray irradiation, alopecia, keratin,

Introduction

After the Great East Japan Earthquake in March 2011, a large quantity of radioactive material was dispersed by an accident at the Fukushima Daiichi Nuclear Power Plant, where workers and local residents were exposed to radiation (1). Symptoms that occur when the entire body is irradiated with large doses of radioactivity are called acute radiation symptoms. Generally, it is known that acute radiation symptoms such as gastrointestinal disturbances and blood and bone marrow disorders occur within several hours to several weeks after radiation exposure of 1–6 Gy.

Hair loss is also an acute radiation symptom, but little is known about the mechanism that causes it. In humans, hair loss is caused by X-ray irradiation of more than 3 Gy, with almost complete hair loss occurring at exposure to 6 Gy (2). It is thought that the cause of hair loss is stem cell damage due to irradiation, but no studies have investigated this hypothesis. Therefore, we investigated the mechanism of radiation-induced hair loss. There are hair stem cells and many types of keratin protein in hair follicles. To better understand the mechanism of radiation-induced hair loss, we studied the changes in hair proteins in response to irradiation and hoped to identify a new radiation exposure biomarker.

Materials and methods

Mice

The male C57/BL6 mice were irradiated with X-rays (150kV, 20mA) using 0.5-mm aluminum and 0.3-mm copper filters at a distance of 45 cm from the focus with a dose of 0.2 Gy/min (MBR-1520R-3; Hitachi Medical

Corporation, Tokyo, Japan) in the 6 Gy. After 5 weeks, the hair and skin of the mice were collected as they were shed.

Hair protein extraction, two-dimensional electrophoresis and western blotting

Hair proteins were extracted, as described by Winter *et al*. (3), from the hairs of mice. Two-dimensional electrophoresis was carried out by a PROTEAN system (BIO-RAD). Gels were stained with Coomassie Brilliant Blue R-250. Western blotting was performed according to the method of Towbin *et al*. (4).

Liquid Chromatography/Electrospray-Ionization Mass Spectrometry (LC/ESI-MS)

The samples were applied to a Nano Frontier LC column, C18 (75 µm id x 150 mm, Hitachi-Hitec, Tokyo, Japan) and eluted by a gradient flow of acetonitrile and distilled water containing 0.1% formic acid (flow rate 200 nl/min). The peptide fragments eluted were analyzed using an online coupled linear trap electrospray-ionization mass spectrometer (Nano Frontier L, Hitachi-Hitec) at a heated capillary temperature of 140ºC and a voltage of 1.0 kV. Peptide sequence analysis was performed using BioLynx software (Micromass). The sequence information was submitted to the MASCOT programs.

Immunohistochemistry

Skin tissues from the anterior dorsal regions of the mice were fixed in 10% formaldehyde and embedded in paraffin. Immunohistochemical staining for CD34, Krt1, Krt10, and Krt15 was performed by the avidin-biotin-peroxidase complex (ABC) method (5) with the respective antibodies. The biotinylated anti-rabbit antibody and VECTASTAIN ABC kit were obtained from Vector Laboratories. The specimens were examined and photographed using a fluorescence microscope FSX100 (OLYMPUS, Tokyo, Japan).

Results

The X-ray irradiated mice developed alopecia and decreased hair count (Fig. 1). To examine whether hair protein profiles were different between the control mice and the radiation-induced hair-loss mice, hair proteins extracted from the hairs of X-ray irradiated mice were subjected to two-dimensional electrophoresis. We cut X1-3 spot in the mice with alopecia and C1-6 in the control mice. Spots decreased to an extent in the hair protein of the mice with alopecia (Fig. 2).

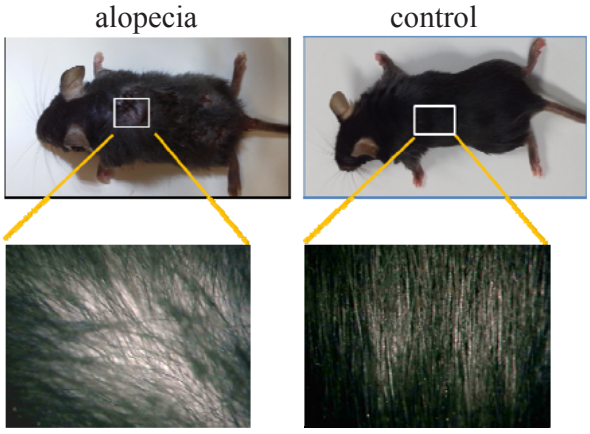


Fig. 1 X-ray Irradiated mouse
A mouse 5 weeks after irradiation with 6 Gy.

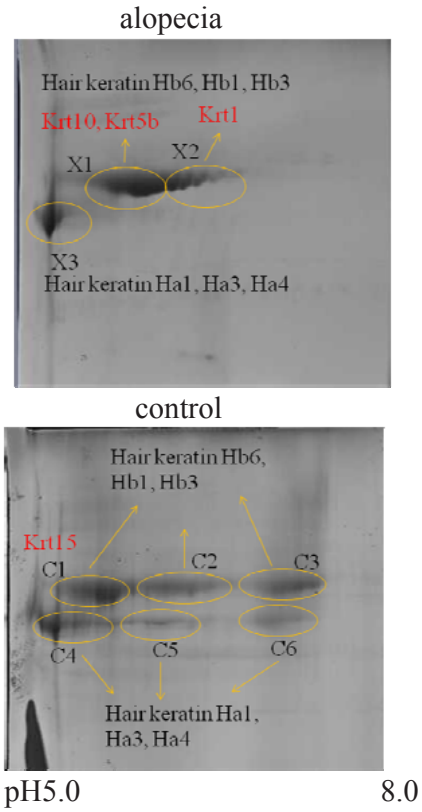


Fig. 2 Two-dimensional electrophoresis of hair protein
Two-dimensional electrophoresis was carried out by a PROTEAN system (BIO-RAD). Protein staining with Coomassie brilliant blue R-250 revealed some spots.

To identify each spot, the sample recovered from the SDS gels was digested with trypsin and the fragments were subjected to LC/ESI-MS analysis. This analysis revealed that most hair proteins in the mice were hair keratins such as Keratin (Krt) 86, Krt81, and Krt83. There was no change in the composition of the major hair

keratins; however, the expression of Krt15 was detected in the control mice, but not in the mice with alopecia. Krt1, Krt5b, and Krt10 were detected in the hair of the mice with alopecia (Table).

Table. Keratins which were detected in either only the X-ray irradiated mice or only the control mice.

alopecia	score	control	score
Krt5b	141	Krt15	129
Krt1	116		
Krt10	108		
Krt77	65		
Krt14	65		
Krt6b	54		
Krt5a	48		

With the anti-Krt15 antibody, the basal layer of the epidermis and the outer root sheath of the hair follicle of the control mice showed a positive reaction, but the staining of the hair follicles from the irradiated mice was negative. Staining with the anti-CD34 antibody revealed a positive reaction in the cortex. Krt1 and Krt10 were expressed in the epidermis of the irradiated mice (Fig.3 arrows).

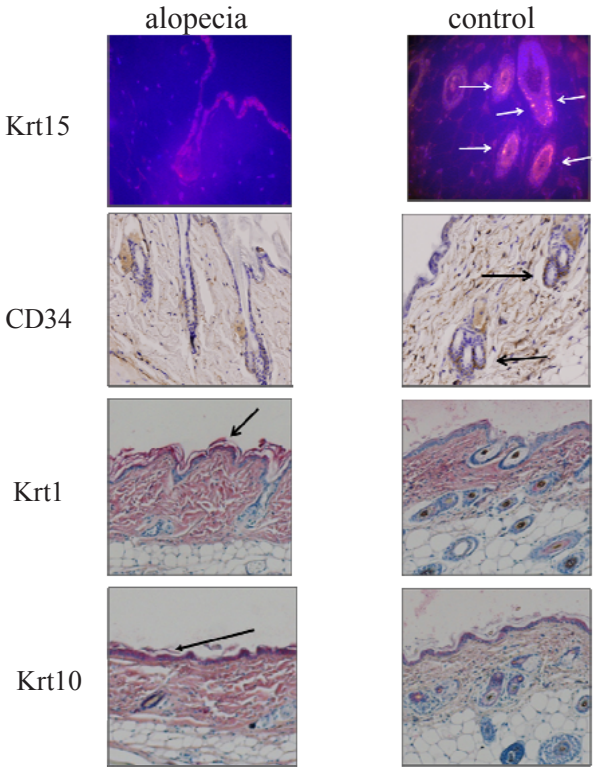


Fig. 3 Immunohistochemical stain of anti -keratin antibodies.
Krt15 and CD34 were expressed in the hair stem cells of the control mice, and Krt1 and Krt10 were expressed in the alopecia mice (arrows).

Discussion

Hair loss is caused when humans are irradiated with X-rays. The cause of hair loss has been thought to be stem cells being damaged by irradiation, but a study has yet to prove this. Therefore, we examined the mechanism of radiation-induced hair loss. In regards to LC/ESI-MS analysis and immunohistochemical staining, Krt15 and CD34 were expressed in the hair follicles of the control mice but not the irradiated mice. This would suggest that hair stem cells were damaged by irradiation.

Because hair keratin did not change, it was thought that any change that did occur was not caused by the radiation. Cytokeratin such as Krt1, Krt5, and Krt10 were detected in the irradiated mice (Table and Fig.3).

These results suggested that expression of Krt1, Krt5, and Krt10 increased in X-ray irradiated epidermis and caused these keratins to be attached to the hair. These keratins are expected to be new biological markers for acute radiation symptoms.

References

[1] Tsuji M, Kanda H, Kakamu T, et al. An assessment of radiation doses at an educational institution 57.8 km away from the Fukushima Daiichi nuclear power plant 1 month after the nuclear accident. Environ Health Prev Med in press (2011).

[2] Stram D O, Mizuno S. Analysis of the DS86 atomic bomb radiation dosimetry methods using data on severe epilation. Radiat Res 117: 93-113 (1989).

[3] Winter H, Hofmann I, Langbein L, Rogers M A, Schweizer J. A splice site mutation in the gene of the human type I hair keratin hHa1 results in the expression of a tailless keratin isoform. J Biol Chem 272: 32345-32352 (1997).

[4] Laemmli U K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685 (1970).

[5] Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 76: 4350-4354 (1979).

Optimization of the protein phosphatase-inhibitors (okadaic acid) treatment in the premature chromosome condensation (PCC)-ringmethod for biodosimetry of accidental high dose exposure

Akifumi Nakata[1], Yasuhiro Satoh[2], Kiyotaka Shibutani[2], Hiromi Ichikawa[2], Tomisato Miura[2], and Mitsuaki A Yoshida[1]

[1]Department of Radiation Biology, Institute of Radiation Emergency Medicine, Hirosaki University (IREM/HU), Hirosaki, Japan
[2]GraduateSchool of Health Sciences, Hirosaki University, Hirosaki, Japan

Abstract. The dicentric chromosome assay (DCA) is very important for dose estimation in acutely irradiated persons. However, this method is not available inthe case of high dose exposure to ionizing radiation over the lethal dose because mitotic delay and apoptosis, resulting in poor mitotic index, are induced in the lymphocytes of such highly irradiated cases. In order to accurately estimate the radiation dose in cases of high dose radiation exposure, the premature chromosome condensation (PCC) method, which induces DNA condensation in the interphase cell, has been developed and a ring chromosome is recommended as the chromosome aberration to be used for biodosimetry. Okadaic acid and calyculin A are protein phosphatase inhibitors that can induce PCC in any phase of the cell cycle. However, the mechanism of PCC and the influence of these reagents on chromosome condensation have yet to be sufficiently elucidated. The aim ofthe present study is to optimize the okadaic acid treatment which induces PCC for biodosimetry in the case of accidental high dose exposure. We investigated the frequency of PCC and the degree of chromosome condensation in both non-irradiated and X-ray irradiated lymphocytes by various treatment times and concentrations of okadaic acid. It was found that the frequency of PCC in cells irradiated by 10 Gy of X-rays was lower than in non-irradiated cells and that the optimal condition was a one hour treatment with 1000 nM of okadaic acid. Furthermore, the highly condensed chromosomes were frequently identified according to the increase of the concentration and treatment time of protein phosphatase inhibitors. These results indicate that the protein phosphatase inhibitors have some influence on the chromosome morphogenesis.

Key Words: biodosimetry, premature chromosome condensation,okadaic acid

Biological dosimetry is very important as the first step in the medical treatment of irradiated persons. Chromosome aberration has been considered to be the most specific indicator for the biological dose estimation in radiation exposure [1]. Therefore, it is important to prepare metaphase spreads suitable for the chromosome analysis. However, the condition of the protein phosphatase inhibitors, such as okadaic acid (OA) and calyculin A (CA), for PCC induction were different between laboratories [2-5]. As shown in Figure 1, both chemicals induced PCC in a dose-dependent manner. Although CA was about 20 times more effective than OA, CA shortened the length of the chromosome more than OA. Therefore, we examined the optimization of the OA treatment for the biodosimetry scoring rings PCC.

At first, we investigated the cultivation time of peripheral blood and found that it is necessary to culture the lymphocytes for 40 hours or more to obtain metaphase cells sufficient for the chromosome analysis.We have also examined both time and concentration of OA treatment based on chromosome length and morphology. OA was added into the culture media at doses of 200 – 1000 nM during the last 30, 60, and 120 min. Then, we measured the length of chromosome 2 at G2/M-PCC. In the result, the highly condensed

chromosomes were frequently identified according to the increase of the concentration and treatment time of OA (Figure 2a). On the other hand, we investigated the morphology of the chromosomes and found that the increase in the yield of fuzzy chromosomesat high doses may have been caused by over-treatment of this chemical (Figure 2b).

In conclusion, the optimal dose and treatment time were determined based not only on the frequency of PCC cells but also on the quality of the chromosome morphology. It was found that treatment with 500 and 1000 nM OA for over 2 h resulted in fuzzy and short chromosomes, which made chromosome analysis difficult. On the other hand, treatment with 1000 nM for1 h was available for the ring analysis as an optimum condition.

References

[1] IAEA, Cytogenetic analysis for radiation dose assessment. A manual, technical report series 405 IAEA, Vienna.(2001).

[2] Hayata I, Kanda R, Minamihisamatsu M, Furukawa M. Sasaki MS. Cytogenetical dose estimation for 3 severely exposed patients in the JCO criticality accident in Tokai-mura. J Radiat Res (Tokyo) 42 Suppl, S149-155 (2001).

[3] Wang ZZ., Li WJ., Zhi DJ, Jing XG, Wei W, Gao QX, Liu B. Biodosimetry estimate for high-LET irradiation. Radiat Environ Biophys 46, 229-235 (2007).

[4] Balakrishnan S, Shirsath K, Bhat N Anjaria K. Biodosimetry for high dose accidental exposures by drug induced premature chromosome condensation (PCC) assay. Mutat Res 699, 11-16, doi:S1383-5718 (2010).

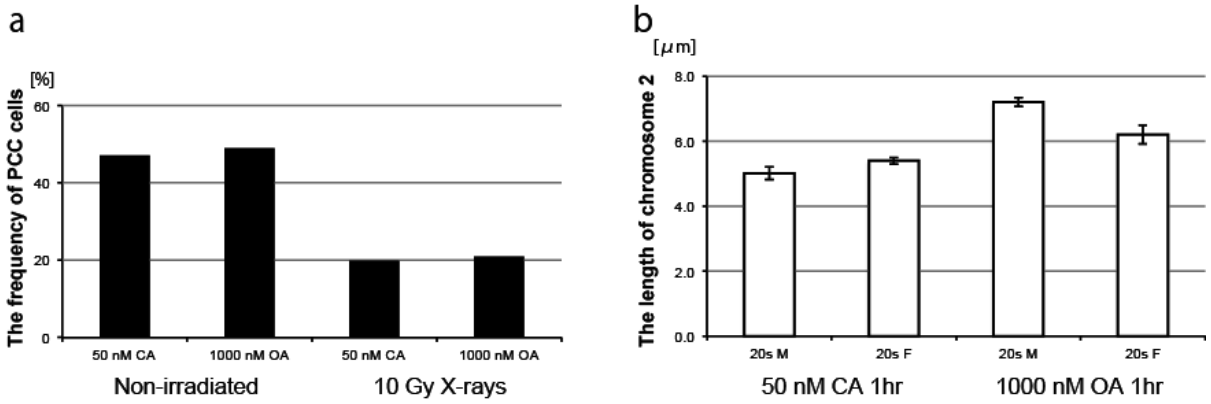


Figure 1. a) The frequency of cells with PCC for non-irradiated and 10 Gy-irradiated human peripheral lymphocytes. Lymphocytes were treated with 50 nM calyculin A (CA) or 1000 nM okadaic acid (OA). b) The length of chromosome 2 at G2/M phase.

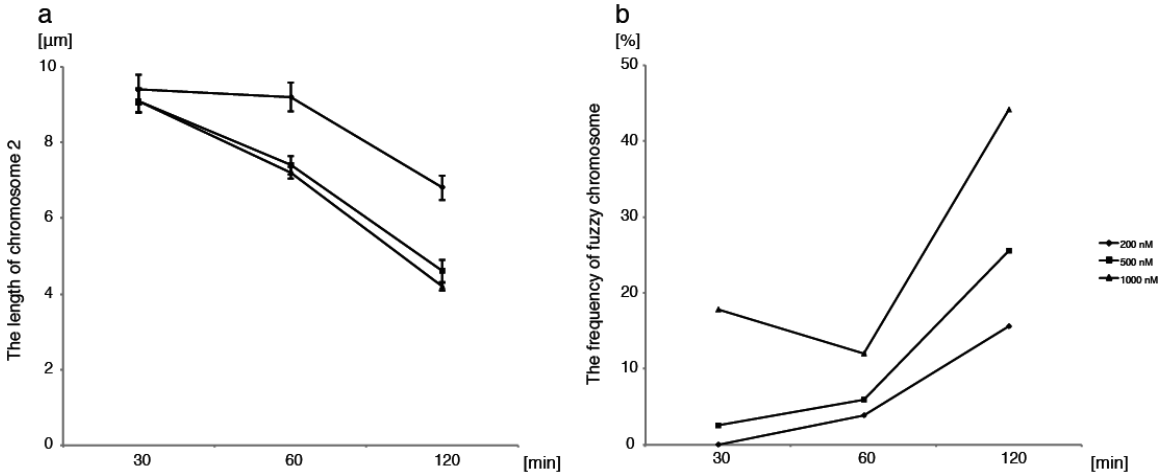


Figure 2. a) The length of chromosome 2 at G2/M phase, and b) the frequency of fuzzy chromosome in each okadaic acid treatment, respectively.

Optimization of calyculin A-induced premature chromosome condensation assay for chromosome aberration studies

T. Miura[1*] and W. F. Blakely[2]

[1] Department of Pathologic analysis, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan
[2] Armed Forces Radiobiology Research Institute (AFRRI) Bethesda, MD, USA

Abstract. Calyculin A (CA)-induced premature chromosome condensation (PCC) assay is a simple and useful method to assess structural chromosome aberrations in interphase cells. PCC assay is the recommended biodosimetry method, vice the gold-standard lymphocyte mitogen-stimulated metaphase-spread dicentric and ring assay (DCA), for use in cases of high-dose (>5 Gy) and partial-body exposures. Treatment with CA (50 nM) for 60 min, which is typically used in these studies, however, results in the induction of fuzzy and shortened chromosomes. In this study, the effect of the CA exposure on chromosome morphology and PCC frequency was investigated in a human peripheral blood ex vivo irradiation (^{60}Co- γ rays; ~0.6 Gy/min) model. Treatment with CA for 60 min increased the frequency of G 2 /M-PCC cells with shortened chromosomes by 71.7 ± 21.5-fold at dose range of 0 – 3 Gy compared with 15- or 30-min treatment. The frequency of G_2/M-PCC cells with fuzzy chromosomes in 60-min treated group was also increased by 3.0 ± 0.5-fold and 1.9 ± 0.2-fold of the value obtained from the 15- and 30-min treated group, respectively. The G_2/M-PCC scoring index in 60-min treated group was decreased by 44.3 ± 5.6% and 42.7 ± 6.0% of the values in 15- and 30-min treated groups, respectively. The G_2/M-PCC efficiency of 30-min treated group was highest in the three conditions of CA exposure to obtain scorable PCCs, those without fuzzy or shortened chromosomes. We conclude that CA treatment for 30 minutes before harvesting is optimum for chemical induced-PCC assay in PHA-stimulated PBL ex vivo radiation model.

Key Words: premature chromosome condensation, calyculin A, human blood culture, radiation, biodosimetry

Introduction

The premature chromosome condensation (PCC) assay was first reported by Johnson and Rao in 1970 [1]. Chromatin of "test" interphase cells in different cell-cycle phases were condensed by mitotic factors derived from "reagent" mitotic cells fused with the test HeLa cells using Sendai virus. In 1983, Pantelias and Maillie [2] reported the PEG-mediated fusion and PCC induction method using Chinese hamster ovary cells and human peripheral lymphocytes (PBL). In 1993, Dyban, et al. reported that okadaic acid, a specific inhibitor of serine/threonine phosphoprotein phosphatases (PP) 1 and 2A, induced PCC cells in mouse embryos [3]. Furthermore, Gotoh, et al. reported that PCC was also inducible by calyculin A in many types of cells with high efficiency in 1995 [4]. In addition, Gotoh, et al. reported that calyculin A-induced PCC method showed a linear dose response for the induction of G2 chromatid breaks after radiation of normal human fibroblast cells [5]. And Kanda

* Corresponding to: Tomisato Miura, Ph. D., Department of Pathologic analysis, Division of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan.
E-mail: Tomisato@cc.hirosaki-u.ac.jp

reported the dose–response relationship of the yield of PCC rings in the irradiated lymphocytes [6].

PCC assay is the recommended biodosimetry method, vice the gold-standard lymphocyte mitogen-stimulated metaphase-spread dicentric and ring assay, for use in cases of high-dose (>5 Gy) exposure. High radiation doses delay or eliminate cell division resulting in very low yields of metaphase spreads but has negligible effects on PCC yields. Hayata et al. performed biodosimetry on 3 severely exposed patients in the JCO criticality accident in Tokai-mura, Japan using the PCC assay [7]. In addition, assessment of exposure dose and fraction of the body exposed to acute partial-body irradiation (PBI) can be performed using an analysis method (Q_{PCC}) with the PCC assay. Blakely et al. carried out PBI simulation study using fusion-PCC assay in the human peripheral blood lymphocyte ex vivo radiation model, and the usefulness of Q_{PCC} method in the local irradiation was clarified [8]. In addition Darroudi, et al. reported that only the PCC assay was useful on high-dose PBI evaluation, while the dicentric assay and cytokinesiss-blocked micronucleus assay could not accurately distinguished dose and fraction of body exposed to PBI from whole-body-irradiation (WBI) in the monkey irradiation model [9]. It was reported that calyculin A used in the PCC method induced fuzzy and shortened chromosomes [6, 10]. It is estimated that fuzzy and/or shorten chromosome decrease the detection sensitivity of structural chromosome aberrations such as small PCC rings and small excess fragments in PCC assay. The length of treatment with calyculin A also varied among studies using chemical-induced PCC method [11-14].

In this study, the effect of the CA exposure on chromosome morphology and PCC frequency was investigated in a human peripheral blood ex vivo irradiation model. G2/M-PCC are typically used to score structural chromosome aberrations after radiation exposure [15], hence we focused on this cell-cycle stage to optimize the calyculin A exposure in PCC assay.

Experimental design

Blood collection: Whole peripheral blood from healthy human donors was collected by venipuncture into the vacutainers containing lithium heparin. The informed consent form used was approved by the Uniformed Services University of the Health Sciences' IRB. The drown blood was diluted with same volume of RPMI 1640 medium supplemented with 10% FBS, penicillin and streptomycin.

Ex vivo radiation and blood culture: Whole blood was irradiated with 0, 1, 3, 5, 7, 10, 20 and 30 Gy 60Co γ -rays (0.56 Gy/min) at 37° C using a Robertson Tank. Two hours after irradiation (DNA repair), lymphocytes were isolated using a density gradient. Lymphocytes equivalent to 0.5 ml whole blood were cultured in 5 ml of PB-MAX Karyotyping Medium (Invitrogen), which contained a mitogen (phytohemagglutinin, PHA) in a 5% CO2 at 37°C for 48 h.

PCC induction, harvest, and spread: Calyculin A (Calbiochem) was used to induce PCC cells. Calyculin A (50 nM) was added into culture media 15, 30 or 60 min before 48-h culture was completed. After hypotonic treatment, cells were fixed in cold 3:1 methanol: glacial acetic acid. Hanabi metaphase spreader (ADSTEC, Japan) was used to prepare PCC spreads. The slides were then stained with 10 % Wright Giemsa (pH 6.8).

G_2/M-PCC scoring index and efficiency: One hundred well-spreading G_2/M-PCC spreads were scored to analyze chromosome morphologies under a light microscope using 100 x objective lens. 1,000 cells were scored to determine the G_2/M-PCC index, calculated by using the formula: G2/M-PCC index = (number of G_2/M-PCC cells) / (number of lymphocytes) x 100. Scorable G_2/M-PCC scoring index was calculated as a parameter for evaluation of calyculin A treatment and it was given as frequencies of G_2/PCC cells without shortened chromosomes and/or fuzzy chromosomes.

Summary

- Studies were performed using calyculin A to induced PCC spreads suitable for the evaluation of structural chromosome aberrations induced by exposure to ionizing radiation.
- 60-min calyculin A (50 nM) exposure produced fuzzy and shortened PCC based on repeated experiments (3 experiments with radiation; 6 experiments without radiation).
- Quantitative morphological criteria was established to optimize calyculin A-induced PCC in G_2/M cells based on chromosome length (Figure 6, upper panel) and compactness.
- Prolonged treatment (60 min) with calyculin A caused an increased yield of PCC with relative shortening of chromosome length compared with the 15- or 30-min treatment (Figure 1, left).

- Radiation caused a dose-dependent increase in the yield of G_2/M-PCC with extended chromosome length, which is likely the result of the accumulation of G_2 cells due to radiation-induced G_2 cell arrest (Figure 1, right).
- Prolonged calyculin A treatment (15 to 60 min) decreased the yield of PCCs with sharp compactness chromosomes in non-irradiated cells while increasing the fraction of G_2/M-PCC with intermediate and fuzzy chromosome morphology.
- This effect of prolonged exposure with calyculin A to increase fuzzy chromosome morphology was independent of radiation exposure over a broad dose range.
- The length of CA exposure had little to no significant effect on the yield of sharp and intermediate PCC compactness in irradiated cells over a broad dose range (1 – 30 Gy), however, increasing the number of scored cells might demonstrate a slight progressive decrease of sharp PCC yields with prolonged calyculin A treatment at doses from 1 to 10 Gy.
- Calyculin A exposure for 15- or 30-min treatment produced significantly higher yields of scorable G_2/M- PCC than 60-min treatment on a per PCC basis.
- Calyculin A treatment for 30 min before harvesting was optimum for this chemical induced-PCC assay in PHA-stimulated PBL ex vivo radiation model based on a per lymphocyte basis.
- Small acentric excess fragments and small ring chromosomes were able to be identified not only in cells irradiated with high doses but also in cells irradiated with sub-lethal doses in cells prepared with the optimum 30-min calyculin A treatment.
- This optimized CA exposure provide an improved method for assessment structural chromosome aberration in interphase that have broad implication for use in studies on mechanism of chromosome damage, screening of medical countermeasures for radiological protection, and radiation biodosimetry.

References

[1] Johnson RT, Rao PN. Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei. Nature. 226(5247):717-722 (1970).

[2] Pantelias GE, Maillie HD. A simple method for premature chromosome condensation induction in primary human and rodent cells using polyethylene glycol. Somatic Cell Genet. 9(5):533-547 (1983).

[3] Dyban AP, De Sutter P, Verlinsky Y. Okadaic acid induces premature chromosome condensation reflecting the cell cycle progression in one-cell stage mouse embryos. Mol Reprod Dev. 34(4):402-415 (1993).

[4] Gotoh E, Asakawa Y, Kosaka H. Inhibition of protein serine/threonine phosphatases directly induces premature chromosome condensation in mammalian somatic cells. Biomed Res. 16:63-68 (1995).

[5] Gotoh E, Kawata T, Durante M. Chromatid break rejoining and exchange aberration formation following gamma-ray exposure: analysis in G2 human fibroblasts by chemically induced premature chromosome condensation. Int J Radiat Biol. 75(9):1129-1135 (1999).

[6] Kanda R, Hayata I, Lloyd DC. Easy biodosimetry for high-dose radiation exposures using drug-induced, prematurely condensed chromosomes. Int J Radiat Biol. 75(4):441-446 (1999).

[7] Hayata I, Kanda R, Minamihisamatsu M, et al. Cytogenetical dose estimation for 3 severely exposed patients in the JCO criticality accident in Tokai-mura. J Radiat Res (Tokyo). 42:S149-155 (2001).

[8] Blakely WF, Prasanna PG, Kolanko CJ, et al. Application of the premature chromosome condensation assay in simulated partial-body radiation exposures: evaluation of the use of an automated metaphase-finder. Stem Cells. 13(S1):223-230 (1995).

[9] Darroudi F, Natarajan AT, Bentvelzen PA, et al. Detection of total- and partial-body irradiation in a monkey model: a comparative study of chromosomal aberration, micronucleus and premature chromosome condensation assays. Int J Radiat Biol. 74(2):207-215 (1998).

[10] Kanda R, Shang Y, Tsuji S, et al. An improved culture system of mouse peripheral blood lymphocytes for analysis of radiation-induced chromosome aberrations. Biosci Rep. 24(6):641-650 (2004).

[11] Gotoh E, Tanno Y, Takakura K. Simple biodosimetry method for use in cases of

high-dose radiation exposure that scores the chromosome number of Giemsa-stained drug-induced prematurely condensed chromosomes (PCC). Int J Radiat Biol. 81(1):33-40 (2005).
[12] Wang ZZ, Li WJ, Zhi DJ, et al. Biodosimetry estimate for high-LET irradiation. Radiat Environ Biophys. 46(3):229-235 (2007).
[13] George K, Wu H, Willingham V, et al. High- and low-LET induced chromosome damage in human lymphocytes: a time-course of aberrations in metaphase and interphase. Int J Radiat Biol. 77(2):175-183 (2001).
[14] Lee R, Nasonova E, Ritter S. Chromosome aberration yields and apoptosis in human lymphocytes irradiated with Fe-ions of differing LET. Adv Space Res. 35(2):268-275 (2005).
[15] Durante M, Furusawa Y, Gotoh E. A simple method for simultaneous interphase-metaphase chromosome analysis in biodosimetry. Int J Radiat Biol. 74(4):457-462 (1998).

Acknowledgments

The Armed Forces Radiobiology Research Institute supported this research under intramural grant RAB4AM. The first author performed these studies as a National Research Council (NRC) senior fellow at The Armed Forces Radiobiology Research Institute (AFRRI), Uniformed Services University (USU) and wishes to acknowledge and thank both NRC and AFRRI for the opportunity to perform this project. We also thank Professor Mitsuaki Yoshida, research center for radiation medicine, Hirosaki University for advice of PCC classification, Dr. Arifur Rahman, Sergio Gallego and David J. Sandgren for their technical expertise in conducting the radiation-PCC assay studies, and Dr. Vitaly Nagy and his colleagues in the Radiation Science Department, AFRRI, for their excellent dosimetry support.

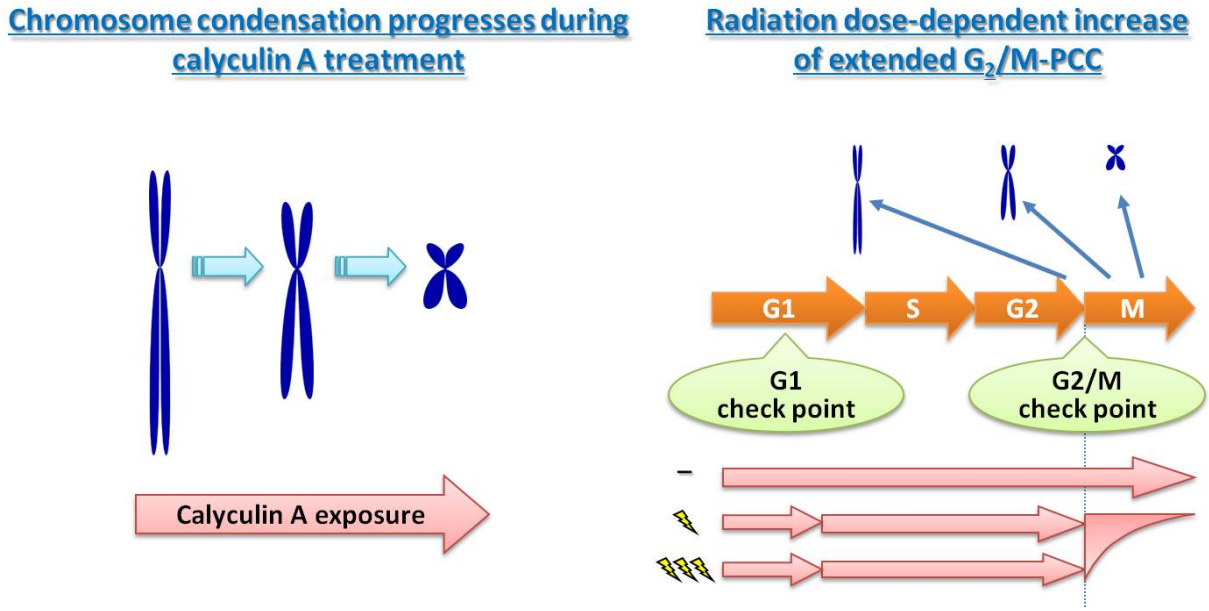


Figure 1. Hypothesis of the induction of shortened G_2/M-PCC by calyculin A.

Radiation mitigators: Compounds, which are effective when administered immediately after exposure to radiations

Kazunori Anzai[1*], Megumi Ueno[2], Ken-ichiro Matsumoto[2], Jiro Takata[3]

[1]Nihon Pharmaceutical University, Japan
[2]National Institute of Radiological Sciences, Japan
[3]Faculty of Pharmaceutical Sciences, Fukuoka University, Japan

Abstract. We can classify the compounds that modulate radiation injuries to three categories with respect to the timing of administration: the compounds for prophylaxis, mitigation, and treatment of radiation injuries. Most of radiation protectors so far reported are for prophylaxis and should be present in the appropriate position in the living body before exposure. In contrast, for countermeasure to accidental overexposure, it is necessary to use the compounds that are effective when administered after exposure. These compounds are called mitigator and relatively few agents have been reported so far. Ameliorating effects of three compounds when administered after whole-body X-irradiation are shown. They are mineral-yeast, and two vitamin E analogs, 2-(α- D-glucopyranosyl)methyl-2,5,7,8-tetramethylchroman-6-ol (TMG), and γ-Tocopheryl *N,N*-dimethylglycine (γ -TDMG).

Key Words: radiation mitigator, bone marrow death, mineral-yeast, vitamin E anlog

Introduction

Radiotherapy is one of the most effective treatments for cancer and its importance has increased in recent years. For effective treatment, the target tissue should be irradiated with a sufficient dose, which always increases the risk of radiation injury to surrounding normal tissues other than the target cancer cells. Therefore, finding ways to decrease the risk to normal tissues is a fundamental requirement to improve the outcome of radiation therapy. Using radioprotective agents is one way of decreasing damage to normal tissues, and various radioprotective compounds have been reported [1-3].

We can classify the compounds that modulate radiation injuries to three categories with respect to the timing of administration: the compounds for prophylaxis, mitigation, and treatment of radiation injuries (Fig. 1). Most of radiation protectors so far reported are for prophylaxis and should be present in the appropriate position in the living body before exposures. In contrast, for countermeasure to accidental overexposure, it is necessary to use the compounds that are effective when administered after exposure. These compounds are called mitigator and relatively few agents have been reported so far.

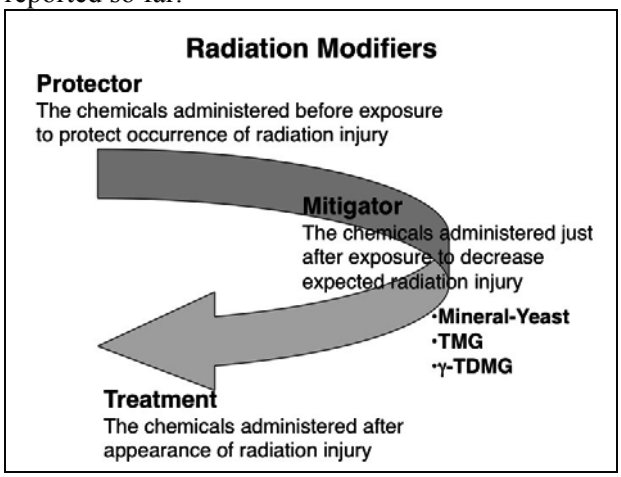


Figure 1. Classification of radiation modifiers with respect to timing of administration

Here, we will show three mitigators, mineral-yeast, TMG, and γ-TDMG, which are effective to

save mice from bone marrow death caused by lethal dose of whole-body irradiation.

Mineral-Yeast [4]

A variety of mineral-containing heat-killed yeast powders were obtained from Omnica Co. Ltd. (Tokyo, Japan). According to the supplier's information, *Saccharomyces cerevisiae* was cultured in medium containing steam-sterilized syrup, wort, phosphate, ammonium sulfate, potassium chloride, and zinc sulfate (in the case of Zn-yeast). The cultured yeast suspension was purified by repeated centrifugation and resuspension in water to yield pure yeast suspension. The yeast suspension was sterilized at 95°C for 1-3 h. After ultra-sonic drying of the heat-killed yeast suspension, it was pulverized by a spray dryer. Selenium methionate, manganese sulfate, or copper gluconate were added instead of zinc sulfate for Se-yeast, Mn-yeast, and Cu-yeast, respectively. The content of Zn, Mn, Cu, and Se in Zn-, Cu-, Mn-, and Se-yeast was reported by the supplier to be more than 10%, 5%, 5%, and 0.2%, respectively. We examined the content of Zn and Cu in Zn- and Cu-yeast, respectively, by X-ray absorption analysis and confirmed their content. Methylcellulose was obtained from Wako Co. Ltd. (Osaka, Japan). Other reagents were of analytical grade and used without further purification. The yeast powder was pulverized finely with a mortar and pestle, 0.5% methylcellulose solution was added to the powder, and the suspension was mixed well. The turbid suspension was directly administered i.p. to mice.

The 30-day survival rate of the control group of C3H mice was about 6% when mice were whole-body X-irradiated at 7.5 Gy. The survival rate of mice to which mineral-yeast was injected at immediately after the irradiation was 81%, 91%, and 90% for Mn-, Cu-, and Zn-yeast, respectively. The mice administered with yeast of non-additive-mineral also showed high survival rate (73%). The survival rate of the Zn-yeast treated group was significantly higher than that of yeast treated group ($p<0.05$, Breslow-Gehan-Wilcoxon test).

The survival rate depended on the timing of administration after X-irradiation. The survival of mice to which Zn-yeast was injected at immediately (0 h), 4 h, 10 h, and 24 h after the irradiation was 90%, 73%, 73%, and 27%, respectively. The survival rate of the groups treated with Zn-yeast at 0, 4, 10, and 24 h after irradiation is significantly higher than that of the control group ($p<0.001$ for 0, 4, 10 h and $p<0.01$ for 24 h, Breslow-Gehan-Wilcoxon test).

The maximum effect of Zn-yeast was observed at the dose of 50 mg/kg body weight of mice. Dose reduction factor (DRF) was about 1.2 for 30-day survival of C3H mice of Zn-yeast administered 100 mg/kg at immediately after 7.5 Gy whole-body X-irradiation.

To investigate the effective fraction in the suspension of Zn-yeast, the suspension in 0.5 % methylcellulose was centrifuged (1,500 x g, 10 min). The supernatant fraction was removed and the precipitate fraction was re-suspended with the same volume of the methylcellulose solution. The supernatant fraction or the re-suspended precipitated fraction was i.p. administered to mice immediately after 7.5 Gy whole body X-irradiation. The insoluble fraction showed significantly higher activity than the soluble fraction. The soluble fraction showed only moderate radioprotection, which was about a half that of the insoluble fraction.

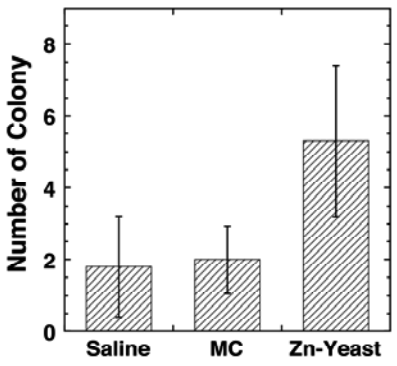


Figure 2 Comparison of the formation of endogenous spleen colony.

Endogenous spleen colony assay was performed to study the possible mechanism of Zn-yeast to prevent bone marrow death of mice after lethal dose of X-irradiation. Mice were whole-body X-irradiated at 7.5 Gy and saline solution, methylcellulose solution, or Zn-yeast suspension was administered i.p. immediately after the irradiation. At the day 11 after the irradiation, spleen of each mouse was removed and immersed in Bouin's solution. As shown in figure 2, the

number of endogenous spleen colony of Zn-yeast treated group was significantly higher than that of control groups (Saline and MC). This result suggests that Zn-yeast may stimulate proliferation and/or settlement of survived bone marrow cells after irradiation [5, 6].

TMG [6]

TMG (2-(α- D-glucopyranosyl)methyl - 2,5,7,8-tetramethylchroman-6-ol) is a water-soluble vitamin E analog containing chroman ring and glucose moiety (figure 3). Radioprotection of mice by i.p. administration of TMG from whole-body γ-irradiation was already reported [7]. Here, we extended the investigation.

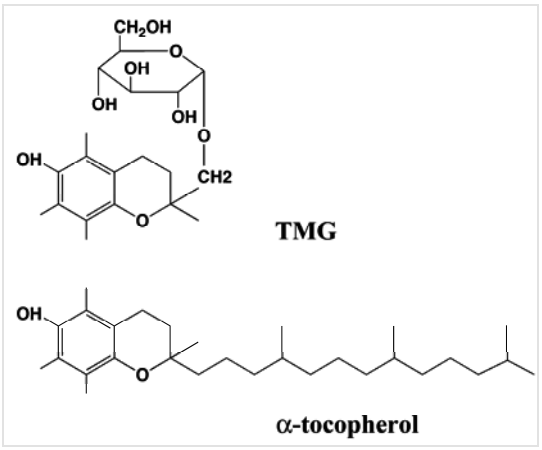


Figure 3 Structure of TMG and α-tocopherol

When mice was i.p. injected with TMG (650 mg/kg body weight) immediately before or immediately after whole-body 7.0 Gy X-irradiation, 30-day survival rate of mice significantly increased. The survival rates of the groups of mice injected with TMG immediately before (–), immediately after (+), and 15, 30, and 60 min after irradiation were 83, 75, 65, 60 and 52 %, respectively, whereas the 30-day survival rate of the control group of mice was about 24 %. The survival curves for (+), (–), 15 min, and 30 min were significantly high compared to the control ($p < 0.001$ by the Mantel-Cox test and Breslow-Gehan-Wilcoxon test). The survival curves for 60 min, and 240 min were also significantly high compared to that of the control ($p < 0.01$). These findings show that TMG is effective to prevent bone marrow death of mice when administered even at 6 h after X-irradiation. Subcutaneous injection of TMG immediately after 7.0 Gy X-irradiation also significantly prevented bone marrow death of mice.

γ-TDMG

γ-TDMG (γ-Tocopheryl *N,N*-dimethylglycine) is also a water-soluble vitamin E analog (figure 4). It exists as a powder at room temperature and is resistant to oxidation.

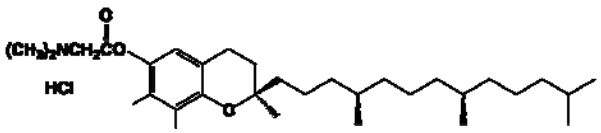


Figure 4. Structure of γ-TDMG

When 100 mg/kg of γ-TDMG was administered i.p. to mice 30 min before or immediately after whole-body exposure to 7.5 Gy X-rays, the 30 day survival rate was significantly increased as shown in figure 5. Interestingly, the effect was higher for the administration at immediately after exposure compared to that before exposure.

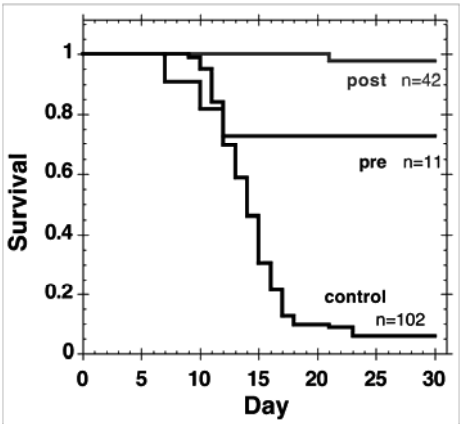


Figure 5. Survival curve of mice after 7.5 Gy whole body X-irradiation. γ-TDMG was i.p. administered immediately before (pre) or after (post) irradiation.

The maximum effect was observed at the dose of 100 mg/kg body weight. The ameliorating effect

was observed even when γ-TDMG was administered at 24 h after the irradiation. Subcutaneous injection was also effective. Similar to the results of mineral-yeast, endogenous spleen colony formation was significantly increased by the treatment with γ-TDMG.

Conclusion

Several reagents were found to be effective to rescue mice from bone marrow death caused by lethal dose of whole-body X-irradiation by post-irradiation administration. Although exact mechanism is not clear yet, a possible mechanism is stimulating proliferation of survived bone marrow (stem) cells. Some vitamin E analogs may become potential lead compounds for the radiation mitigator.

References

[1] Poggi, MM, Coleman, CN, Mitchell, JB. Sensitizers and protectors of radiation and chemotherapy. *Curr. Probl. Cancer* 25:334-411 (2001).

[2] Nair, CKK, Parida, DK, Nomura, T. Radioprotectors in radiotherapy. *J. Radiat. Res.* 42: 21-37 (2001).

[3] Patchen, ML. Immunomodulators and cytokines: Their use in the mitigation of radiation-induced hemopoietic injury. In Bump, EA, Malaker, K (eds): Radioprotectors: Chemical, biological, and clinical perspectives, Boca Raton: CRC Press, pp. 213-236 (1997).

[4] Anzai, K, Ikota, N, Ueno, M, Nyui, M, Kagiya, TV. Heat-treated mineral-yeast as a potent post-irradiation radioprotector. *J. Radiat. Res.* 49:425-430 (2008).

[5] Ueno, M, Imadome, K, Iwakawa, M, Anzai, K, Ikota, N, Imai, T. Vascular homeostasis regulators, Edn1 and Agpt2, are upregulated as a protective effect of heat-treated zinc yeast in irradiated murine bone marrow. *J. Radiat. Res.* 51:519-525 (2010).

[6] Ueno, M, Inano, H, Onoda, M, Murase, H, Ikota, N, Kagiya, TV, Anzai, K. Modification of mortality and tumorigenesis by tocopherol-mono-glucoside (TMG) administered after X-irradiation in mice and rats. *Radiat. Res.* 172:519-524 (2009).

[7] Satyamitra, M, Uma Devi, P, Murase, H, Kagiya, VT. In vivo postirradiation protection by a vitamin E analog, alpha-TMG. *Radiat. Res.* 160:655-661 (2003).

Biomarkers for early-response assessment of radiation exposure

Natalia I Ossetrova* **and David J Sandgren**

Armed Forces Radiobiology Research Institute (AFRRI),
Scientific Research Department,
Biological Dosimetry Research Group
Bethesda, Maryland, USA

Abstract. Acts of nuclear or radiological terrorism could expose large numbers of people to ionizing radiation. Early treatment of populations exposed to ionizing radiation requires accurate and rapid biodosimetry with a precision as high as possible to determine an individual's risk for life-threatening exposures. Early-phase and tissue-specific (i.e., parotid glands, skin, bone marrow, liver, small bowel, etc.) protein biomarkers detected in peripheral blood can provide rapid estimation of an individual's exposure dose as well as diagnostic information of organ-specific radiation injury. Their combination with hematological and salivary amylase activity changes has potential triage biodosimetry applications to help in early-phase medical management of radiation accidents. These biomarkers need to be validated in suitable radiation model systems. We established animal (i.e., *Mus musculus*, *Macaca mulatta*) radiation models to transition this research platform for application to humans. We recently reported results from a murine total-body irradiation (TBI) model demonstrating for the first time that a protein expression profile measured in samples collected 1 and 2 d after exposure can predict mice exposed to radiation and distinguish the level of photon-radiation exposure, ranging from 1 to 7 Gy. Combination of protein and hematological biomarkers demonstrated an enhanced separation of 1-Gy irradiated animals from controls and an improvement of the threshold for exposure detection compared to the selected protein profile only. We also reported results from several nonhuman primate (NHP) TBI studies and demonstrated dose- and time-dependent hematological and blood chemistry changes along with early-phase and organ-specific plasma protein biomarkers. Herein, we present preliminary summary results for two animal irradiation models demonstrating proof-of-concept that proteomics shows promise as a complimentary approach to conventional biodosimetry for early assessment of radiation exposure as well as prognostic indicators of acute radiation sickness outcome.

Key Words: Biological dosimetry, hematological and protein biomarkers, salivary amylase activity, total-body irradiation, mice, nonhuman primates.

1. Introduction

Development and validation of early-response radiation injury biomarkers are critical for triage as well as to assess efficacy of treatment and follow-up of irradiated individuals. The early medical-management situation requires quantitative indications for early initiation (within one day after radiation exposure) of cytokine therapy in individuals exposed to life-threatening radiation doses and/or bone marrow acute radiation syndrome (ARS) as well as effective triage tools for first-responders in mass-casualty radiological incidents [1, 2]. Terrorist attacks or nuclear accidents could expose large numbers of people to ionizing radiation, and early biomarkers of radiation injury would be critical for triage, treatment, and follow-up of such individuals. Casualty estimates after a nuclear event predicts that radiation alone will affect only 15-20% of the injured. Approximately

*Address for correspondence: Armed Forces Radiobiology Research Institute, 8901 Wisconsin Ave., Bldg. 42, Bethesda, MD, 20889; e-mail: ossetrova@afrri.usuhs.mil

65-70% of the casualties are expected to receive both radiation and a traumatic injury [3, 4]. Past experiences (i.e., nuclear detonations in Hiroshima and Nagasaki; Chernobyl reactor incident) have shown that with a critically-associated explosion traumatic injury such as burns and wounds will occur in combination with radiation exposure.

Hematological biomarkers of exposure to ionizing radiation are well characterized and used in medical management of radiological casualties [3]. Measurements of lymphocyte depletion kinetics [5, 6] and time- and radiation severity changes in neutrophil cell counts observed after irradiation [7] provide clinical information soon after exposure.

Estimation of radiation exposure doses by lymphocyte depletion kinetics or counting chromosome aberrations is problematic after doses close to the LD_{50}, and certainly for higher doses. Peripheral lymphocytes significantly decline (< 200 cells/mm^3) by 24 h, hence limiting accurate assessment of total-dose assessment using lymphocyte depletion kinetics methodology. Normalization of the inter-individual variations in the ratio of neutrophils to lymphocytes has been evaluated and used along with lymphocyte depletion kinetics to get an enhanced discrimination index of radiation exposure [8, 9].

While the traditional radiation exposure biomarkers based on cytogenetic assays and lymphocyte depletion kinetics serve as the standard, the development of rapid and non-invasive tests for radiation exposure is needed. Proteomics is an area offering hope for potential new biological indicators of radiation exposure. Radiation responsive protein levels show considerable potential as biodosimeters; biological measures of a suspected exposed individual's absorbed radiation dose. Tissue specific protein biomarkers detected in peripheral blood can provide diagnostic information of organ-specific radiation injury [10 – 13]. The level of specific proteins may be useful to provide early diagnostic information for acute radiation exposures [14 – 16]. Multiparametric approaches combining Flt-3 ligand levels and hematology have been compared to cytogenetic analysis to demonstrate a clinical utility in assessing a hematopoietic syndrome in radiation accident victims [11].

An increase in serum amylase activity (hyperamylasemia) from the irradiation of salivary tissue has been proposed as a biochemical measure of early radiation effect in normal tissue. These findings have been reported in radiation therapy patients [17, 18] as well as in a recent radiation accident [19]. Radiation-induced increases of serum amylase in 41 patients following either whole-body irradiation or irradiation of the head and neck region were reported by Hofmann and colleagues [20]. Serum amylase activity generally shows early increase and peak values between 18–30 h after exposure, returning to normal levels within a few days. Based on these findings, serum amylase was advocated to be used as a biochemical dosimeter for prolonged spaceflights [21, 22].

The physiological response to radiation injury initiates wide-ranging systemic response events. Shortly after the injury, macrophages produce a wide range of cytokines and growth factors. Cytokines, via the central nervous system and blood circulation, initiate a wide range of systemic responses including fever, increasing production and differentiation of bone marrow cells, and dramatic expression of acute-phase proteins C-reactive protein (CRP) and Serum Amyloid A (SAA) [23]. Acute-phase proteins, whose concentrations are significantly increased during the acute-phase response, have been extensively investigated and shown to play an essential role in injuries caused by radiation in animals [14, 24 – 27] and humans [15, 28]. Additional studies in nonhuman primates [14, 24] and rabbits [26] show that dynamics and content of CRP exactly reflect the course and severity of ARS, as well as demonstrating its ability to play a role as a prognostic ARS indicator. Periods of appearance of CRP in the blood of irradiated animals correlate with periods of expressed development of the cytolytic and destructive processes induced by irradiation [14, 24, 26]. Indexes of CRP content in peripheral blood of 147 Chernobyl nuclear power plant accident patients during primary reaction to the irradiation (1 - 2 d after) and in the latent period of radiation disease (3 - 9 d after) were correlated with a prognosis of ARS outcome [15].

Our research is focused to investigate the diagnostic utility of proteomic biomarkers combined with hematological bioindicators for radiation dose assessment using murine and nonhuman primate radiation models. Our expectations are that this research will contribute to bridging a gap that exists in the current capabilities to rapidly and effectively identify and assess radiation

exposure early after a radiation event, especially after a mass-casualty radiological incident. In particular, these efforts contribute to validating an early test to distinguish individuals exposed and injured by radiation in order to assist the physicians to choose the appropriate medical treatments, hence reducing the adverse acute effects or long-term risks associated with radiation exposure. We expect that this research will also shorten the gap between radiation dose and injury assessment in the case of combined injury (i.e., ionizing radiation in combination with trauma). The advancement in this type of research might also provide a powerful tool for the accurate assessment of an individual's radiation risk response early after an incident, especially after a mass-casualty radiological event.

Herein, we present preliminary summary results from the on-going mouse (*Mus musculus*) and nonhuman primate (NHP), total-body irradiation (TBI) studies demonstrating dose- and time-dependent changes in amylase activity and protein content in serum, plasma levels of CRP and SAA, along with hematology parameters (i.e., lymphocyte counts and neutrophil to lymphocyte ratio).

2. Animal models and methods

Table 1 illustrates the animal models established at the Armed Forces Radiobiology Research Institute (AFRRI) and used in these studies. Male and female mice (8-12 weeks old) from three strains (i.e., BALB/c, CD2F1, and B6D2F1) and adult nonhuman primates (NHPs), rhesus macaques, were used in these studies [9, 29 – 34]. Animals were housed in cages in conventional holding rooms in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International at the AFRRI's Veterinary Research Department or the University of Maryland's Veterinary Resources Department. Research was conducted according to the principles enunciated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Institute of Laboratory Animal Resources, National Research Council, and under Institutional Animal Care and Use Committee (IACUC) approved protocols.

TBI of animals was carried out in the AFRRI's ^{60}Co facility or in the University of Maryland's x-irradiation facility. The bilateral irradiation of mice was performed in well-ventilated Plexiglas boxes. Mice in dose cohorts (n=8-12) received TBI at dose rates of 0.1 – 0.6 Gy/min to a midline tissue over a broad dose range (1 – 14 Gy) [30, 31]. In partial-body irradiation (PBI) studies, mice were irradiated at different body fractions to a dose of 6 Gy with a 250-kVp x-ray source at 0.55 Gy/min in the AFRRI's irradiation facility [34]. The ketamine anesthetized NHPs were placed in a Plexiglass restraint chair and irradiated bi-laterally. Ten NHPs received TBI dose of 6 Gy 250-kVp x-rays at dose rate of 0.13 Gy/min in the University of Maryland's irradiation facility [29]. Eight NHPs in each dose-rate cohort (0.4 and 0.6 Gy/min) received TBI dose of 6.5 Gy in the AFRRI's ^{60}Co facility [9, 33]. In a recent dose-response study, NHPs in dose cohorts (n=6), received TBI to a midline tissue dose of 1.0, 3.5, 6.5, and 8.5 Gy ^{60}Co γ-rays at 0.55 Gy/min [32]. Animals representing sham were also treated the same except they were not exposed to radiation.

At designated sampling time points after exposure, peripheral blood was drawn from mice and NHPs while under anesthesia. Drawn blood was collected into a serum separator tube and potassium EDTA vacutainer tubes. Blood in EDTA tubes for complete blood cell counts and differentials (CBC/diff) was analyzed within several hours after biosampling using a clinical hematology analyzer (Bayer Advia 120, Bayer, Tarrytown, NY). Amylase activity was measured using a hematology chemical analyzer (Vitros 250, Orthos Clinical Diagnostics, Rochester, NY), as previously described [9]. Radiation responsive protein biomarkers were measured in newly developed enzyme linked immunosorbent assays (ELISA) or using commercially available ELISA kits, as previously described [29 – 32].

Statistical software, PC SAS (SAS Institute Inc., Cary, NJ), was used for statistical data analysis. Multivariate analysis of variance (MANOVA) Wilks' Lambda statistics was used when comparing more than two groups and two-sided Student's *t* test was used when comparing two groups to determine significant difference among sampling time- and dose-points. Values of $P < 0.05$ were considered statistically significant. Values were expressed as means ± standard error (SE).

Table 1. Animal Radiation Models					
Model	**Species/ Strain/ Gender**	**Type of Radiation**	**Dose, Dose rate**	**Research Study**	**Project status/ References**
Mouse TBI and PBI	*Mus musculus,* BALB/c, ♂	^{60}Co γ-rays	1 – 7 Gy 0.1 Gy/min	Discovering of novel biomarkers; TBI study	2005-2008 Ossetrova et al. 2009, 2010
	Mus musculus, CD2F1, ♂	^{60}Co γ-rays	1 – 14 Gy 0.57 Gy/min	Further discovering and validation of novel biomarkers;	ongoing
		250-kVp x-rays	6 Gy 0.55 Gy/min	TBI and PBI studies	ongoing Blakely et al. 2011
	Mus musculus, B6D2F1/J, ♀	^{60}Co γ-rays	1 – 14 Gy 0.57 Gy/min	Further discovering and validation of novel biomarkers; TBI in combination with trauma	ongoing Ledney et al. 2010
NHP TBI	*Macaque mulata*, ♂	250-kVp x-rays	6 Gy 0.13 Gy/min	Collaboration with Dr. MacVittie; Discovering of novel biomarkers; TBI study with testing of the efficacy of supportive care	2005-2006 Ossetrova et al. 2007
	Macaque mulata, ♂	^{60}Co γ-rays	6.5 Gy 0.4 Gy/min	Collaboration with Dr. Ledney; Further discovering of novel biomarkers; TBI study with testing of the efficacy of supportive care and treatment	2005-2006 Blakely et al. 2007
	Macaque mulata, ♂♀	^{60}Co γ-rays	6.5 Gy 0.6 Gy/min	Collaboration with Dr. Whitnall; Further discovering of novel biomarkers; TBI study with testing of the countermeasure	2006-2008 Blakely et al. 2010
	Macaque mulata, ♂♀	^{60}Co γ-rays	1 – 8.5 Gy 0.6 Gy/min	Collaboration with Dr. Blakely; Further discovering and validation of novel biomarkers; TBI study with a limited supportive care	2008-2010 Ossetrova et al. 2011

Multiple linear regression analysis was used to develop dose-response relationships for combination of hematological and protein biomarkers for radiation dose assessment. Multivariate discriminant function analysis was used to demonstrate accurate radiological detection of the proposed protein and hematological biomarkers from biological samples into quartiles of doses 0-1 Gy, 1-3 Gy, 3-6 Gy, 6-10 Gy, and greater than 10 Gy as previously described [29 – 32].

3. Results and discussion

We have reported results from murine (BALB/c, males) *in vivo* total-body irradiation (TBI,

^{60}Co γ-rays, 0.10 Gy/min) model studies to analyze simultaneously multiple proteins selected from distinctly different pathways (i.e., Growth Arrest and DNA Damage Inducible Gene 45 protein or GADD45α, IL-6, and SAA) and showed, for the first time, that a protein expression profile can be developed not only to predict radiation exposure in mice but also to distinguish the level of radiation exposure, ranging from 1 to 7 Gy. The use of multiple protein targets was evaluated using multiple linear regression analysis to provide dose-response calibration curves for dose assessment with a threshold for γ-exposure detection of 2 Gy. Multivariate discriminant analysis demonstrated enhanced dose-dependent separation of irradiated animals from control as the number of biomarkers increased [30].

The time-course up to 4 days post-TBI for absolute lymphocyte counts (ALC) and ratio of neutrophils (ANC) to lymphocytes in CD2F1 mice (n=8 per group) irradiated to 8 Gy {new data] and NHPs (n=8 per group) irradiated to 6.5 Gy [9] with ^{60}Co γ- rays at dose rates 0.55 and 0.4 Gy/ min, respectively, are shown in figure 1 [30]. In mice, baseline for peripheral blood ALC was in the range (1.68 – 4.14) $\times 10^9$/L with a pooled (n=8) cohort value of 2.64 (±0.34) $\times 10^9$/L. Baseline for ANC to ALC ratio was in the range from 0.12 to 0.27 with a pooled cohort value of 0.20 (±0.02). In mice irradiated to 8 Gy, early decline of ALC to 0.81 (±0.08) $\times 10^9$/L was observed at 4 h that represents a 3.26 (±0.08)-fold decrease compared to pre-TBI level. ALC declined dramatically at 1 d after irradiation to 0.12 (±0.03) $\times 10^9$/L and continued to decrease to 0.08 (±0.03) $\times 10^9$/L at 3 d that represent a 22.41 (±2.84)- and 35.55 (±3.23)-fold decrease, respectively, compared to pre-TBI level (figure 1). The ANC to ALC ratio early increase to 1.05 (±0.12) was also observed at 4 h. A strong time-dependent increase was found peaking at 2 d and reached the level of 17.41 (±2.87), which represents an 87.47 (±14.42)-fold increase compared to pre-TBI level. In NHPs, baseline for ALC is in the range (0.63 – 3.49) $\times 10^9$/L with a pooled cohort (n=8) value of 1.92 (±0.28) $\times 10^9$/L. Baseline for ANC to ALC ratio ranged from 1.15 to 3.46 with a pooled cohort value of 2.00 (±0.95) (figure 1). In NHPs irradiated to 6.5 Gy, ALC declined dramatically to 0.28 (±0.12) $\times 10^9$/L and 0.31 (±0.12) $\times 10^9$/L at 1 and 2 d after irradiation, respectively. The ANC to ALC ratio values increased to 25.97 (±8.68) and 8.47 (±3.61), which represent a 13 (±2.66)- and 4.23 (±0.95)-fold increase at 1 and 2 d after irradiation, respectively, compared to pre-TBI levels [9].

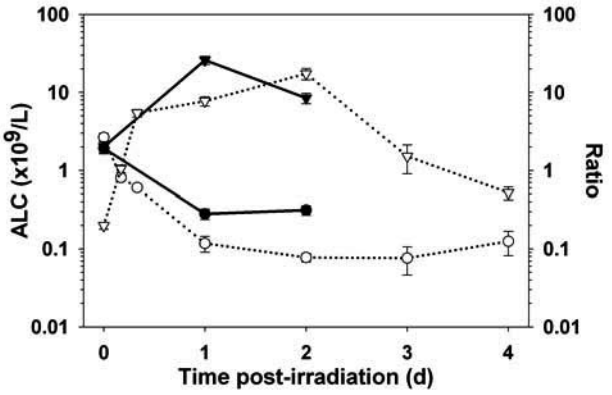


Figure 1. The time-course up to 4 days post TBI for absolute lymphocyte counts (ALC) and ratio of neutrophils (ANC) to lymphocytes (ANC to ALC) in CD2F1 mice (n=8 per group) irradiated to 8 Gy (new data) and NHPs (n=8 per group) irradiated to 6.5 Gy [9] with ^{60}Co γ-rays at dose rates 0.55 and 0.4 Gy/min, respectively. Symbols for ALC (○) and ANC to ALC ratio (▽) in mice, ALC (●) and ANC to ALC ratio (▼) in NHPs represent the mean ± SEM.

The combination of only one protein (SAA) with hematological biomarkers (ALC and ratio of ANC to ALC) demonstrated: (1) enhanced separation of 1-Gy irradiated animals from controls and between different combinations of doses, and (2) improvement of the threshold for γ-exposure detection up to ~1 Gy compared to the selected protein profile only (figure 2) [31]. While hematological parameters were only 73% successful to discriminate control group of mice from 1-Gy irradiated cohort 1 d post TBI (table 2), their combination with proteins demonstrated 100% separation as well as enhanced discrimination after comparing irradiated groups for all combinations of doses (1, 2, 3.5, 5, or 7 Gy). Discrimination power values to separate 3.5- and 5-Gy animal cohorts are show (table 2) [31].

A murine (CD2F1 males) partial-body radiation exposure model was established for studies supporting the identification and validation of novel biological dosimetry diagnostic assays. Mice irradiated to 6 Gy with a 250-kVp x-ray source at 0.55 Gy/min. Hematological biomarkers

Table 2. Discrimination power (%) between 0 vs 1 and 3.5 vs 5-Gy irradiated animals 1 d post TBI										
Groups (Gy)	SAA	GADD45a	IL6-4h	IL6-24h	For protein profile	ANC	ALC	ANC/ALC	For hematology profile	For protein and hematology profiles
0 vs 1	50.0	75.0	68.8	62.5	81.3	72.7	72.7	95.0	90.9	100.0
3.5 vs 5	62.5	75.0	87.5	62.5	100.0	57.1	57.1	57.1	64.3	100.0

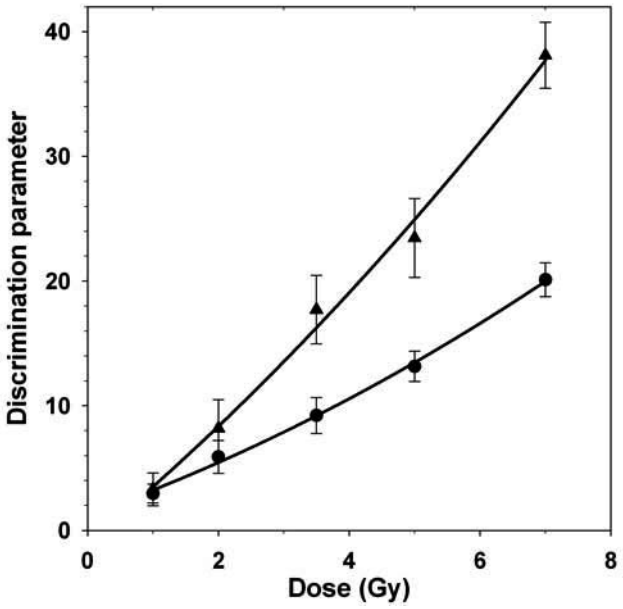


Figure 2. Discriminant analysis results for dose-dependent separation of non-irradiated (0 Gy) from irradiated groups of mice (2-7 Gy) for three protein biomarkers (GADD45α, SAA, and IL-6) (●) [30] and for combination of protein biomarker SAA with lymphocytes and neutrophils to lymphocytes ratio (▲) [31] at 1 d post TBI. Discrimination parameter represents a distance (in canonical score) between medians of distributions of irradiated and control animals. Error bars represent the means of widths of these distributions at 95% confidence level. P values are 0.00154 and 0.0005 for doses of 1 and 2 Gy respectively, and less than 0.0001 for doses of 3.5, 5, and 7 Gy. P value associated with comparison of animal groups irradiated to 2 and 3.5 Gy is 0.004. Highly significant difference ($p<0.001$) was found for pair-wise comparison of irradiated animal groups for other combinations of doses.

(ALC, ANC, and ratio of ANC to ALC) along with plasma protein biomarkers (Flt-3 ligand and SAA) have been evaluated at 1 and 2 d post exposure. As expected, multiple biomarkers provided an enhancement in early-phase partial-body radiation exposure assessment reflecting the fraction of the body exposed [34].

Recently, we demonstrated greater efficacy for exposure assessment using multiple biomarkers. We reported results from a study in a nonhuman primate (*Macaca mulatta*) total-body irradiation model and showed that the protein expression profile (i.e., p53, p21 WAF1, IL-6, salivary α-amylase, and CRP) measured in blood of 10 animals irradiated to 6 Gy 250-kVp x-rays (0.13 Gy/min) and 8 animals to 6.5 Gy ^{60}Co γ-rays (0.4 Gy/min and 0.6 Gy/min) analyzed together along with changes in serum amylase activity and blood cell counts established very successful separation of samples at 1 and 2 d from exposed animals versus samples from the same animals before irradiation. An enhanced separation was observed as the number of biomarkers increased [29, 33].

Time-course of relative amylase activity in NHPs (n=8) and amylase protein content in NHPs (n=8) exposed to 6.5 Gy with ^{60}Co γ-rays at dose rate 0.4 Gy/min [9] and amylase protein content in NHPs (n=10) exposed to 6 Gy with 250-kVp x-rays at dose rate 0.13 Gy/min [29] are shown in figure 3. Relative amylase activity increase was observed to 12.44 (±3.23) and 2.59 (±0.56) fold at 1 and 2 d after irradiation, respectively. For the same time points, the relative amylase protein content increased to 4.48 (±0.54) and 1.91 (±0.17) in NHPs irradiated to 6 Gy with 250-kVp x-rays and to 2.73 (±0.44) and 1.55 (±0.19) in NHPs irradiated to 6.5 Gy with ^{60}Co γ-rays. Two-fold difference in salivary amylase protein increase in animals exposed to different irradiation sources might be explained by different means of the absorbed dose distribution of the lineal energy, which are 1.9 keV/μm and 4.0 keV/μm for ^{60}Co γ-irradiation and 250-kVp x-irradiation, respectively [35].

We recently presented results from 30 rhesus macaques total-body irradiated to a broad dose range of 1 to 8.5 Gy with ^{60}Co γ-rays (0.55 Gy/min) and demonstrated dose- and time-dependent changes in blood of CRP, SAA, and IL-6

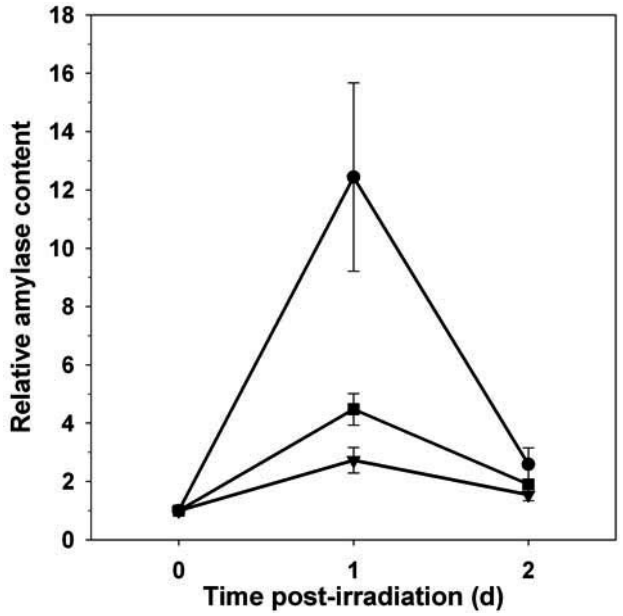


Figure 3. Time-course of relative amylase activity (●) in NHPs (n=8) and amylase protein content (▼) in NHPs (n=8) exposed to 6.5 Gy with ^{60}Co γ-rays at dose rate 0.4 Gy/min [9] and amylase protein content (■) in NHPs (n=10) exposed to 6 Gy with 250-kVp x-rays at dose rate 0.13 Gy/min [29]. Symbols represent the biomarker mean values in animal cohort ± SEM.

measured by ELISA [32]. CRP and SAA dose-response results are consistent with ~1 Gy and ~0.2 Gy thresholds for photon-exposure at 1 d after TBI, respectively. Highly significant elevations of CRP and SAA ($p=0.00017$ and $p=0.0024$, respectively) were found in animal plasma at 6 h after all TBI doses suggesting their potential use as early-phase biodosimeters. The mean relative SAA content normalized on pre-TBI level was found ~400- fold elevated in 1-Gy animal cohort 1 d after TBI [32]. Time-course of relative acute phase proteins SAA and CRP normalized on pre-TBI level in BALB/c mice (n=8) exposed to 7 Gy and one NHP exposed to 6.5 Gy with ^{60}Co γ-rays are shown in figure 4 [30, 32]. The serum concentration of the rapid reacting of SAA and CRP increases within four hours. The acute phase response is detectable for several days after the stimulus, but the kinetics of the response depends on the species in question and on the extent of tissue damage. For example, we have demonstrated the SAA dose- and time-dependent changes in mice total-body irradiated to doses 1 – 7 Gy with ^{60}Co γ-rays. However, CRP is not radiation-responsive protein in mice [30].

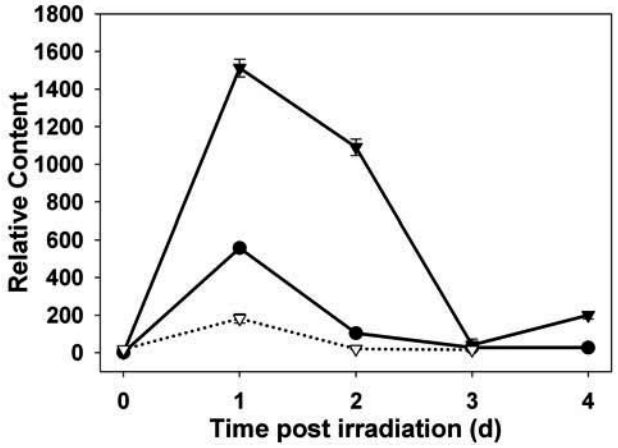


Figure 4. Time-course of relative acute phase proteins SAA and CRP normalized on pre-TBI level in BALB/c mice exposed to 7 Gy [30] and NHP exposed to 6.5 Gy [32] with ^{60}Co γ-rays. Symbols for monkey CRP (●), SAA (▼), and mouse SAA (▽) represent the mean from three replicate measurements per sample ± SEM.

Baseline level of SAA in plasma of non-exposed mice (n=8) ranges from 8.06 (±1.84) to 12.93 (±1.56) μg/mL. Highly significant elevation of SAA level to 181.95 (±24.83) μg/mL has been found in mice irradiated to dose of 7 Gy at 1 d post TBI (figure 4). In NHP irradiated to 6.5 Gy with ^{60}Co γ-rays, CRP was elevated from 0.79 (±0.36) to 334.75 (±11.05) μg/mL, which represents a 423.73 (±13.98)-fold increase compared to pre-TBI level. SAA level in the same animal was elevated from 0.03 (±0.03) to 70.14 (±2.49) μg/mL, which represents a 2447.42 (±86.96) -fold increase compared to pre-TBI level (figure 2). In this study, two phases in CRP and SAA expressions have been found in NHPs after TBI to the lethal doses 6.5 and 8.5 Gy. Our findings for CRP are in good agreement with earlier reports [14, 24, 27]. During the first phase, a progressive dose- and time-dependent increase in CRP levels was observed peaking at 1 d and returning to a baseline level at 2-3 d and 3-5 d in 1- and 3.5-Gy cohorts, respectively. In 6.5- and 8.5-Gy cohorts, CRP remained elevated until the beginning of the second phase at 8-9 d and 6-7 d, respectively. We demonstrated for the first time two phases in SAA expression in NHPs following TBI to the lethal doses 6.5 and 8.5 Gy with a similar but more dramatic response compared to CRP [32]. During the first phase, a progressive dose- and time-dependent increase in

SAA levels was observed peaking at 1 d and 2 d post TBI with sustained expression at 4-7 d and 7-9 d in 1- and 3.5-Gy cohorts, respectively. SAA returned to a baseline level at 7 d and 9-11 d in 1- and 3.5-Gy cohorts, respectively. In 6.5- and 8.5-Gy cohorts, SAA remained elevated until the beginning of the second phase at 7-9 d and 4-7 d, respectively. While the first phase in SAA expression is strongly dose-dependent, the second one is mostly dominated by individual variability in radiation response which relates to ARS outcome. We found that the duration of the latency period separating these two phases correlates with radiation dose. Results also show that the dynamics and content of CRP and SAA levels reflect the course and severity of the acute radiation sickness (ARS) and may function as prognostic indicators of ARS outcome [32].

We have earlier suggested the use of CRP as a triage assay in the case of mass casualty radiological incidents based on nonhuman TBI studies [29, 33]. We have also extended this biodosimetry concept of operations for dose assessment applications. Currently, there are FDA approved home-use and hand-held devices for measurement of CRP based on ELISA methodology, latex-enhanced immunonephelometric methods, automated immunoturbidimetric and immunoluminometric assays, or the latex-agglutination turbidimetric immunoassay, which are sensitive and fast. SAA might be detected in plasma (potentially in whole blood) using similar methodologies. In the case of a mass casualty incident, it would be useful to employ a triage diagnostic screening approach to rapidly separate the concerned population (i.e., worried well) from severely radiation-exposed individuals. The primary triage diagnostic assay must be rapid, ideally self-administered, and readily identify all (i.e., no false negatives) radiation-over-exposed individuals. Since the desired results of this primary triage diagnostic assay are to identify candidate individuals for high-priority further diagnostic testing, a small percentage of false positives are acceptable. An individual with baseline levels of CRP and or SAA within 1 to 2 d of a suspected radiation exposure is indicative that they were not likely exposed to a life-threatening radiation dose. Based on dose-response studies, we demonstrated additional proof-of-concept results for use of early response biomarkers as a contributor along with other diagnostic endpoints for radiation dose assessment. Validation of the use of acute phase and inflammatory biomarkers for radiation injury and dose assessment await rigorous specificity and sensitivity studies. These studies should use appropriate animal models and human populations, when possible, and address potential relevant confounding effects (i.e., burns, inflammation, infection, wounding, partial-body exposures, and dose- and time-response relationships).

Use of multiple protein targets and blood markers, along with classical biodosimetric methodologies, is expected to enhance the specificity and diagnostic utility of a protein-based biomarker approach for early assessment of severe radiation exposure. Our preliminary results demonstrate proof-in-concept that plasma radiation-responsive proteins show promise as a complimentary approach to conventional biodosimetry for early assessment of radiation exposures. We expect that such proteins coupled with peripheral blood cell counts may provide an enhanced discrimination index of radiation exposure to effectively manage radiation casualty incidents. Proteomic studies have revealed sentinel radiation- and organ-specific protein biomarkers that show promise for use as diagnostic tools for early assessment of radiation exposures and a complimentary approach to conventional biodosimetry. Use of this multiple blood protein biomarkers biodosimetry approach, especially when coupled with peripheral blood cell counts, may provide an enhanced discrimination index of radiation exposure to effectively manage radiation casualty incidents [31].

Acknowledgements

Research supported by Armed Forces Radiobiology Research Institute (AFRRI), Biomedical Advanced Research and Development Authority (BARDA), and Defense Threat Reduction Agency (DTRA) We would like to thank Dr. William F. Blakely, Dr. Arifur Rahman, Katya Krasnopolsky, Donald Condliffe, HM2 (SW) Sergio Gallego, and HM1 (SW) Lynette Lowery for scientific and technical contributions.

References

[1] MacVittie, T.J., Farese, A. M., Jackson, William III. 2005. Defining the full therapeutic potential of recombinant growth factors in the post radiation-accident environment: the effect of supportive care plus administration of G-CSF. Health Physics 89(5): 546-555.

[2] Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley AL, Dickerson WE, Tsu H, Confer DL, Coleman N, Seed T, Lowry P, Armitage, JO, Dainiak N: Strategic National Stockpile Radiation Working Group. 2004. Medical management of the acute radiation syndrome: Recommendations of the Strategic National Stockpile Working Group. Annals of Internal Medicine 140(12): 1037–1051.

[3] Dainiak N, Waselenko JK, Armitage JO, MacVittie TJ, Farese AM. 2003. The hematologist and radiation casualties. Hematology - the Education Program of the American Society of Hematology. American Society of Hematology. Education Program 2003: 473-496.

[4] Pellmar TC and Ledney GD. 2005. Combined Injury: Radiation in Combination with Trauma, Infectious Disease, or Chemical Exposures. NATO RTG-099 2005 "Radiation Bioeffects and Countermeasures" meeting, held in Bethesda, Maryland, USA, June 21-23, 2005, and published in AFRRI CD 05-2.

[5] Baranov AE, Guskova AK, Nadejina NM, Nugis VY. Chernobyl experience: biological indicators of exposure to ionizing radiation. Stem Cells. 13(Suppl 1): 69-77; 1995.

[6] Goans RE, Holloway EC, Berger ME, Ricks RC. Early dose assessment following severe radiation accidents. Health Physics 72:513-518; 1997.

[7] Fliedner TM, Friesecke I, Beyrer K. Medical management of radiation accidents–manual on the acute radiation syndrome. The British Institute of Radiology, London; 2001.

[8] Zhang A, Azizova TV, Wald N, Day R. Changes of ratio of peripheral neutrophils and lymphocytes after radiation exposure may serve as a prognostic indicator of accident severity. Found in "Final Program, 49th Annual Meeting of the Health Physics Society, Health Physics Society", McLean, VA, Abstract. P8, p.17; 2004.

[9] Blakely WF, Ossetrova NI, Manglapus GL, Levine IH, Jackson WE, Grace MB, Prasanna PGS, Sandgren DJ, and Ledney GD. Amylase and Blood Cell-Count Hematological Radiation-Injury Biomarkers in a Rhesus Monkey Radiation Model – Use of Multiparameter and Integrated Biological Dosimetry. Radiation Measurements. 2007. 42: 1164-1170.

[10] Bertho JM, Demarquay C, Frick J, Joubert C, Arenales S, Jacquet N, Sorokine-Durm I, Chau Q, Lopez M, Aigueperse J, Gorin NC, Gourmelon P. 2001. Level of Flt3-ligand in plasma: a possible new bio-indicator for radiation-induced aplasia. International Journal of Radiation Biology 77(6): 703-12.

[11] Bertho JM, Roy L, Souidi M, Benderitter M, Gueguen Y, Lataillade JJ, Prat M, Fagot T, De Revel T, Gourmelon P. 2008. New biological indicators to evaluate and monitor radiation-induced damage: an accident case report. Radiation Research 169(5): 543-50.

[12] Lutgens LC HW, Deutz NEP, Gueulette J, Cleutjens JPM, Berger MPF, Wouters BG, Von Meyenfeldt MF, and Lambin P. 2003. Citrulline: A Physiologic Marker Enabling Quantitation and Monitoring of Epithelial Radiation-Induced Small Bowel Damage. Int. J. Radiation Oncology Biol. Phys., Vol. 57(4):1067–74.

[13] Guipaud O, Holler V, Buard V, Tarlet G, Royer N, Vinh J, Benderitter M. 2007. Time-course analysis of mouse serum proteome changes following exposure of the skin to ionizing radiation Proteomics 7: 3992-4002.

[14] Mal'tsev, V.N., Strel'nikov, V.A., Ivanov, A.A., 1978. C-reactive protein in the blood

serum as an indicator of the severity of radiation lesion. Doklady Akademii Nauk SSR 239(3), 750-752.

[15] Mal'tsev, V.N., Ivanov, A.A., Mikhailov, V.F., Mazurik, V.K. 2006. The individual prognosis of the gravity of the outcome of radiation disease on immunological indexes. Journal of Radiation Biology 46(2): 152-158.

[16] Marchetti F, Coleman MA**,** Jones IM, Wyrobek AJ. 2006. Candidate protein biodosimeters of human exposure to ionizing radiation. International Journal of Radiation Biology 82(9): 605-639.

[17] Chen, W., Kereiakes, J.G., Silberstein, E.B., Aron, B.S., Saenger, E.L., 1973. Radiation induces changes in serum and urinary amylase levels in man. Radiat. Res. 54, 141–151.

[18] Dubray, B., Girinski, T., Thames, H.D., Becciolini, A., Porciani, S., Hennequin, C., Socie, G., Bonnay, M., Cosset, J.M., 1992. Post-irradiation hyperamylasemia as a biological dosimetry. Radiother. Oncol. 24 (1), 21–26.

[19] Akashi, M., Hirama, T., Tanosaki, S., Kuroiwa, N., Nakagawa, K., Tsuji, H., Kato, H., Yamada, S., Kamata, T., Kinugasa, T., Ariga, H., Maekawa, K., Suzuki, G., Tsujii, H., 2001. Initial symptoms of acute radiation syndrome in the JCO criticality accident in Tokai-mura. Radiat. Res. (Tokyo) 42 (Suppl.), S157–S166.

[20] Hofmann, R., Schreiber, G.A., Willich, N., Westhaus, R., Bogi, K.W., 1990. Increased serum amylase as a probable bioindicator for radiation exposure. Strahlenther Onkology 166(10), 688-695.

[21] Becciolini, A., Porciani, S., Lanini, A., Balzi, M., and Faroani, P., 2001. Proposal for biochemical dosimeter for prolonged space flights. Physica Medica 17 Supplement 1, 185-6.

[22] Horneck, G., 1998. Biological monitoring of radiation exposure. Advances in Space Research 22(12), 1631-41.

[23] Steel, D. M. and Whitehead, A. S. 1993 in The Natural Immune System: Humoral Factors (Sim, E., ed.), IRL Press, Oxford, pp. 1–29.

[24] Petrov, R. 1963, Immunology of acute radiation injury, Joint Publications Research Service Arlington VA, pp.305.

[25] Wood, H.F., Anderle, S., Hammond, C.W., Miller, C.P. 1960. Studies on the Cx-reactive protein. The effect of irradiation on the acute phase protein system. Journal of Experimental Medicine 111: 601-609.

[26] Tukachinski, S.E. and Moiseeva, V.P. 1961. Cx-reactive protein in radiation injury. Bulletin of Experimental Biology and Medicine 52: 48-52.

[27] Agay, D., Chancerelle, Y., Herodin, F., Mathieu, J., Multon, E., Van Uye, A., Mestries, J.C. 1997. The inflammatory response plays a major role in the acute radiation syndrome induced by fission radiation. Radioprotection 32: C47–49.

[28] Koc, M., Taysi, S., Sezen, O., Bakan, N., 2003. Levels of some acute-phase proteins in the serum of patients with cancer during radiotherapy. Biology Pharmaceutical Bulletin 26(10), 1494-1497.

[29] Ossetrova NI, Farese AM, MacVittie TJ, Manglapus GL, and Blakely WF. The use of discriminant analysis for evaluation of early-response multiple biomarkers of radiation exposure using non-human primate 6-Gy whole-body radiation model. Radiation Measurements. 2007. 42:1158-1163.

[30] Ossetrova NI**,** Blakely WF. Multiple blood-proteins approach for early-response exposure assessment using an *in vivo* murine radiation model. International Journal of Radiation Biology. 2009. 85(10): 837-850.

[31] Ossetrova NI, Sandgren DJ, Gallego S, Blakely WF. Combined approach of hematological biomarkers and plasma protein SAA for improvement of radiation dose assessment in triage biodosimetry applications. Health Physics Journal. 2010. 98(2): 204-208.

[32] Ossetrova NI, Sandgren DJ, Blakely WF. C-reactive Protein and Serum Amyloid A as Early-phase and Prognostic Indicators of Acute Radiation Exposure in Nonhuman Primate Total-body Irradiation Model. 2011. Radiation Measurements Journal (in press; available online).

[33] Blakely WF, Ossetrova NI, Whitnall MH, Sandgren DJ, Krivokrysenko VI, Shakhov A, Feinstein E. (2010). Multiple parameter radiation injury assessment using a nonhuman primate radiation model—biodosimetry applications. Health Physics Journal. 2010. 98(2): 153-159.

[34] Blakely WF, Sandgren DJ, Nagy V, Kim S-Y, Ossetrova NI**.** Murine Partial-Body Radiation Exposure Model for Biodosimetry Studies — Preliminary Report. Radiation Measurements Journal 2011 (in press; available online).

[35] Prasanna PGS, Loats H, Gerstenberg HM, Torres BN, Shehata CW, Duffy KL, Floura RS, Khusen AW, Jackson WE, Blakely WF. 2002. AFRRI's gamma-ray, x-ray, and fission-neutron calibration curves for the dicentric assay. Application of a metaphase finder system. Fort Belvoir, VA, USA: Defense Technical Information Center, Fort Belvoir, VA.

United States Armed Forces Radiobiology Research Institute countermeasures program and related research prioritization

Mark H Whitnall*, Lynnette H Cary, Maria Moroni, Barbara F Ngudiankama, Michael R Landauer, Vijay K Singh, Sanchita P Ghosh, Shilpa Kulkarni, Alexandra C Miller, Juliann G Kiang, Merriline Satyamitra, Venkataraman Srinivasan, and Mang Xiao

Radiation Countermeasures Program, Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda MD, USA

Abstract. A nuclear detonation produces mixed neutrons and gamma rays initially, and gamma rays from fallout. Hence there is a pressing need to develop radiation countermeasures to prepare for radiation disaster scenarios. Major themes of countermeasure development have been free radical scavengers, stimulating hematopoietic progenitors, enhancing DNA repair and blocking cell death pathways. Four agents have US Food and Drug Administration Investigational New Drug (IND) status, allowing safety testing in humans. These are 5-androstenediol (5-AED), genistein (BIO300), Ex-RAD® and CBLB502. G-CSF (Neupogen®) has Emergency Use IND status. The Armed Forces Radiobiology Research Institute (AFRRI) was involved in developing all of these agents. Neupogen®, 5-AED and BIO300 were conceived and initially developed as countermeasures at AFRRI. AFRRI collaborated at early stages of CBLB502 and Ex-RAD® development. AFRRI is also evaluating hematopoietic progenitor cells, which could be given days after irradiation. Other AFRRI agents under investigation are tocols (Vitamin E), and dual use drugs addressing ARS and late effects such as cancer. The mechanisms and countermeasures relevant to radiation combined injury are also under investigation. Additional large animal models are needed for advanced drug development. Policy issues include planning for particular disaster scenarios, timing and routes of drug administration, and priorities of various classes of agents.

Key Words: radiation countermeasures, 5-androstenediol, genistein, Ex-RAD®, CBLB502

* Correspond to: Mark H Whitnall, Program Advisor, Radiation Countermeasures, Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, 8901 Wisconsin Ave, Bethesda MD 20889-5603, USA. Email: mark.whitnall@usuhs.edu. The views expressed here are those of the authors; no endorsement by the U.S. Department of Defense or the U.S. government has been given or inferred.

Introduction

The 1945 detonations of nuclear weapons in Japan and resulting observations of "radiation sickness" had the effect of spurring research on radiation injury, radiation countermeasures and hematology. In the late 1940s and 1950s, it was discovered that thiols protected animals from the acute radiation syndrome (ARS) via their free radical-scavenging properties [1]. The Walter Reed Army Institute of Research embarked on a program in the 1950s that lasted through the 1980s to develop phosphorylated aminothiols as radiation countermeasures. A number of "WR" compounds caused significant enhancement of survival when administered to animals shortly before total body irradiation (TBI) [1]. The prototype member of this series, WR-2721 (amifostine, Ethyol®) has been approved by the US Food and Drug Administration FDA) for use in the clinic to minimize injury to salivary glands during head and neck irradiation [1]. However, its side

effects of hypotension and nausea, investigated at AFRRI, make it unsuitable for use outside the clinic [2].

In the 1950s, microbial constituents were explored as immunomodulators that would induce an immune system response beneficial to irradiated animals suffering from loss of white blood cells and platelets (hematopoietic syndrome) [3-5]. Also in the 1950s and 1960s, research was conducted on how best to control infections in these animals with antibiotics [6-7]. This led to work at AFRRI in the 1980s-1990s on the management of radiation-induced sepsis based on studies of gastrointestinal (GI) flora and specific classes of antimicrobials [8-11].

During this period, immunomodulators were extensively studied at AFRRI [4,12-13]. Realizing the effects of immunomodulators were mediated by cytokine induction, Ruth Neta at AFRRI introduced cytokines as radiation countermeasures [14-17]. The roles and inter-relationships of a number of cytokines were explored by testing the effects of neutralizing antibodies to the cytokines on radiation countermeasure efficacy in mice [17], followed by AFRRI studies on the use of cytokines as radiation countermeasures in large animal models [18]. This work led to the introduction of granulocyte colony-stimulating factor (G-CSF, Neupogen®) as a standard countermeasure for use in humans exposed to ionizing radiation, and its inclusion in the US Strategic National Stockpile [19]. The FDA could authorize its use during a radiation disaster via an Emergency Use Authorization [20]. The US Centers for Disease Control and Prevention (CDC) currently holds both the Emergency Use IND and Emergency Use Authorization applications for the use of Neupogen® in patients after a radiation incident [21].

AFRRI's Current Countermeasure Research Program

AFRRI conducts basic and applied research in an integrated manner to further our mission of identifying and developing radiation countermeasures. The focus is on radiation countermeasure candidates with a realistic chance of successful development, meaning practical issues such as route of administration, storage and toxicity must be taken into account.

In addition to cobalt-60 γ- and X-ray sources, AFRRI maintains a 1.0 megawatt research nuclear reactor that can provide mixed neutron/gamma fields (MF) designed to produce fields with an energy distribution similar to those of nuclear detonations [22-23]. AFRRI has published studies on cultured cells, rodents, canines, non-human primates and minipigs using this source. MF exposures in the reactor can range from almost pure gamma to almost pure neutrons, depending on the shielding used. Dose rates can range from 0.5 mG/min in steady-state mode to 10^5 Gy/sec in pulse mode. The ability to perform pulse irradiations mimicking nuclear detonations, combined with the large exposure rooms for animal experiments, make AFRRI a unique facility.

To facilitate research and policy regarding identification and development of radiation countermeasures, AFRRI participates in extensive interactions with other military and civilian government agencies, academia and industry.

Radiation Countermeasures with INDs

Five radiation countermeasures have FDA IND or Emergency Use IND status. AFRRI was involved in the development of all five. Neupogen®, 5-AED and BIO300 were conceived and initially developed as countermeasures at AFRRI. AFRRI collaborated at early stages of CBLB502 and Ex-RAD® development.

5-AED was conceived, initiated and developed as a radiation countermeasure by AFRRI [24]. AFRRI recruited Hollis-Eden Pharmaceuticals for advanced development of 5-AED as Neumune [25]. It became the prototype small molecule radiation countermeasure with low toxicity for the current era of radiation countermeasure studies. 5-AED elevates circulating granulocytes and platelets in animals and humans [25-27], and enhances survival in γ-irradiated mice and non-human primates (NHP) [28-29]. Human hematopoietic progenitors have been shown to be a target of the steroid [30]. Its mechanism of action in progenitor cells involves induction

of Nuclear Factor-κB (NFκB)-dependent Granulocyte Colony-Stimulating Factor (G-CSF) expression [30], consistent with observations of plasma cytokine levels in mice given 5-AED [31-32]. 5-AED displays an excellent safety profile in humans [25], and was granted IND status in 2005.

Genistein was discovered as a radiation countermeasure with low toxicity at AFRRI [33], and obtained IND status in 2007. Humanetics was recruited to assist in advanced development of genistein as BIO300. Genistein, a kinase inhibitor [34], enhances survival of irradiated mice in association with stimulation of multilineage hematopoietic progenitor recovery [35-36]. The hematopoietic factors G-CSF and interleukin 6 (IL-6) are elevated in irradiated mice treated with genistein [37-39]. Genistein also protects against radiation-induced delayed lung injury [40].

Ex-RAD® is being developed as a radiation countermeasure at AFRRI in collaboration with Onconova Therapeutics [41-42]. It has a good safety profile in clinical testing [42], and received an IND in 2008. Like genistein, it is a small molecule kinase inhibitor [41]. Ex-RAD® enhances DNA repair, reducing signaling molecules in apoptotic pathways [41].

CBLB502, granted IND status in 2008, was developed as a radiation countermeasure by Cleveland BioLabs [43]. At an early stage of testing, AFRRI entered into collaboration with the company, which continues to this day. CBLB502 binds to toll-like receptor 5 (TLR5) and stimulates NFκB [43]. CBLB502 increases survival of mice and NHP exposed to TBI, and minimizes radiation-induced thrombocytopenia and neutropenia [43]. CBLB502 is being evaluated for safety in Phase I clinical trials [44]. AFRRI has shown that cytokine induction by CBLB502 in irradiated mice and NHP can be used as a non-invasive biomarker related to drug dose, reduction of cytopenia and survival improvement [45].

Other AFRRI efforts

Other promising countermeasures at AFRRI in earlier stages of development are the tocols [46-54], myeloid progenitor cells being developed with Cellerant Therapeutics [55], and a toll-like receptor 2/6 agonist being investigated in a collaboration with Cleveland BioLabs [56-57].

In addition, AFRRI conducts studies to appraise countermeasures in the context of MF and radiation combined with other injuries, to mimic more closely the conditions that are expected in terrorist scenarios.

A nuclear criticality results in MF during the first moments with significant risk of exposure to individuals beyond the zone of blast and burn fatalities, for detonations less than 10 KT [58]. Unfortunately, radiation countermeasures have been evaluated almost exclusively using low linear energy transfer (LET) radiation such as γ- and X-rays, rather than high LET radiation such as the neutron component of MF. AFRRI has tested various countermeasures against MF [59-64], and recently demonstrated that while G-CSF was effective as a radiation mitigator against both γ-photons and MF [65], ALXN4100TPO, a thrombopoietin mimetic, was effective against only γ-irradiation [65-66]. Hence radiation countermeasures should be studied with the radiation qualities appropriate for the exposure scenarios being considered in response plans.

Radiation combined injury has long been known to produce a synergistic induction of morbidity compared to the effects of radiation or wounds/burns alone [67]. Because radiation combined injury induces complex pathophysiological responses, a number of countermeasures that are effective against radiation alone fail to mitigate the effects of combined injury [68] and vice versa [69]. AFRRI investigators continue to study mechanisms and countermeasures relevant to combined injury [70-72].

Radiation countermeasures are needed to address not only ARS, but also late effects such as mucositis; cataracts; pathologies such as fibrosis in lungs, skin and other organs; kidney damage; and increased risk of cancers [73-74]. AFRRI scientists are investigating the mechanisms of radiation-induced late effects, as well as dual-use agents such as the histone deactylase inhibitor phenylbutyrate, which shows promise as a countermeasure to both ARS and carcinogenesis [75]. Genistein can also be considered a dual-use agent, increasing

both 30-day survival and delayed lung injury after TBI [40].

According to the FDA's Animal Efficacy Rule, radiation countermeasures may be tested in animal models where pathogenesis, pharmacokinetics and pharmacodynamics are as analogous to the human experience as possible, in order to predict efficacy in humans [74,76]. In order for a drug to be approved by the FDA, the mechanisms of injury and drug action, and the comparative pharmacology and toxicology of a countermeasure, must be well-understood in the animal model [74,76]. The only established large animal models for ARS are canines and NHP [74], which is likely to be a major bottleneck in the development of radiation countermeasures [77]. Swine display close similarity to humans in terms of anatomy and physiology, and are widely accepted by industry and regulatory agencies for development of pharmaceuticals [78-79]. We are developing a minipig model of radiation-induced multi-organ dysfunction syndrome and failure [80]. At the LD50/30 radiation dose (hematopoietic syndrome), the time of nadir and the duration of cytopenia resembled those observed for the corresponding LD50 doses for NHP and canines, and mimicked closely the kinetics of blood cell depletion and recovery in irradiated humans with reversible hematopoietic damage (METREPOL H3 category) [81-82].

Research Prioritization

Pre-irradiation agents (radioprotectants) vs. post-irradiation agents (mitigators)

There is a pressing need for radioprotectants for civilian first responders, civilians emerging into fallout fields after sheltering in place, and military personnel. Post-irradiation agents of course would also be needed for both civilian and military personnel. The funding for radiation countermeasure research has swung almost completely over to post-irradiation measures, for reasons that have not been clearly articulated. Should more support be given to identification and development of prophylactic measures? This is a question that needs to be seriously addressed.

Timing of post-irradiation countermeasures

Civilian funding agencies in the US have instituted a policy that development of post-irradiation measures will not be supported if treatment must be initiated earlier than 24 h after exposure. The rationale is that medical support will not be available earlier than 24 h post-irradiation during a mass casualty scenario. However, how likely is it that hundreds of thousands of casualties will be evacuated from a city to surrounding medical support facilities within 24 h [83-88]? The 24 h limit extends to animal research, despite the fact that different species display different time courses of ARS [89]. Also, most mitigators are more effective when administered early after irradiation, before extensive waves of apoptosis have removed target cells capable of stimulated regeneration [90-95]. Experts recommend that hematopoietic factors should be given as early as possible after exposure [19,96]. Should radiation countermeasure development for civilian use include agents to be given within hours after irradiation?

Far-forward fielding

If radiation countermeasures are to be given within hours after irradiation, how would they be made available during massive breakdowns in communication and transportation during a mass casualty scenario [83-88]? Storage of countermeasures in hospitals, police stations would allow for control of agents by local authorities and release to the community during a disaster. This system (CHEMPACK) is in place for countermeasures to chemical weapons [97-98] and a similar solution for radiological disasters (RADPACK) has been discussed [99]. Hence far-forward fielding of countermeasures to ARS should be considered.

Administration route

It is often assumed that the most practical route of administration would be oral. However, subcutaneous delivery is relatively easy, and there are situations where oral administration would be problematic, such as in patients suffering from nausea and vomiting, common side effects of radiation. Also, personnel wearing protective gear in a contaminated zone would not be advised to

remove their head protection in order to take a pill. In such cases, the recommended route would be an intramuscular injection delivered through the protective clothing. For small scale scenarios or at medical support facilities well after a detonation has taken place, intravenous administration would be possible. This would be necessary for cellular therapies such as myeloid progenitor cells, which can be administered days after irradiation with no diminishment of the survival-enhancing effect [55].

Small molecule vs. large molecule, gene therapy, cellular therapies

Large molecules, gene therapy, and cellular therapies may require intravenous administration and refrigeration or freezing, posing logistical difficulties. Should there be an emphasis on developing small molecule radiation countermeasures? What are the places of gene therapy and cellular therapies in response plans? Response plans should take into consideration all scales and phases of exposure scenarios. These therapies may not be feasible during the early days after a mass casualty incident, but may become useful during the later stages of the response, and after small scale scenarios.

GI Syndrome issues

There are two fundamental issues that should be considered by researchers and policy makers when considering the GI syndrome. First, the traditional GI syndrome in radiobiology has been characterized by early death (before 6-10 days) due to fluid and electrolyte imbalance and bacterial translocation [100-102]. A GI syndrome countermeasure was considered successful if it extended survival beyond that period. However, extension of survival from 10 days to 15 days may not have a significant clinical impact. Longer term survival-enhancement may be required. Hence a treatment may need to cover the hematopoietic syndrome to be approved by the FDA.

Secondly, the traditional concepts of "hematopoietic syndrome" and "GI syndrome" are now being replaced by concepts of multiorgan dysfunction and multiorgan failure [103-104]. It is clear that GI damage contributes to the "hematopoietic syndrome" and bone marrow damage contributes to the "GI syndrome." This is reflected in the fact that the survival monitoring period in studies of the GI syndrome has been creeping up in recent years until now it extends into the beginning (10-14 days) of the period of mortality for the traditional hematopoietic syndrome. Rotolo et al. reported that early deaths can occur without marked GI histopathology [105], and it is known that apoptosis can occur in GI crypts and villi after radiation doses that do not cause the "GI syndrome" [106]. In minipig, radiation doses that cause the "hematopoietic syndrome" are associated with signs of regeneration in GI villi and mild changes in crypts [82]. Also, shielding 5% of bone marrow reveals an influence of bone marrow on the course of the "GI syndrome" [107]. Hence the field needs to be clear about definitions of ARS when planning research and developing countermeasures. It may be necessary to rethink commonly used terminology when describing different degrees of severity of ARS, to frame observations and plans in the context of multi-organ involvement [103-104].

Funding

Funding for research and development of radiation countermeasures is one component of the many efforts addressing weapons of mass destruction in general. The relative proportions of resources devoted to radiological/nuclear emergencies, chemical weapons, and biological warfare should be considered in light of their relative probabilities of occurrence and the relative scope of their consequences.

References

[1] Weiss JF, Landauer MR. History and development of radiation-protective agents. *Int J Radiat Biol*. 85:539-73 (2009).

[2] Bogo V, Jacobs AJ, Weiss JF. Behavioral toxicity and efficacy of WR-2721 as a radioprotectant. *Radiat Res*. 104:182-90 (1985).

[3] Patchen ML, MacVittie TJ. Use of glucan to enhance hemopoietic recovery after exposure to cobalt-60 irradiation. *Adv Exp Med Biol*. 155:267-72 (1982).

[4] Peterson VM, Adamovicz JJ, Elliott TB, Moore MM, Madonna GS, Jackson WE, III, Ledney GD, Gause WC. Gene expression of hematoregulatory cytokines is elevated endogenously after sublethal gamma irradiation and is differentially enhanced by therapeutic administration of biologic response modifiers. *J Immunol*. 153:2321-2330 (1994).

[5] Smith WW, Alderman IM, Gillespie RE. Increased survival in irradiated animals treated with bacterial endotoxins. *Am J Physiol*. 191:124-30 (1957).

[6] Moss WT, Haddy FJ, Sweany SK. Some factors altering the severity of acute radiation pneumonitis: variation with cortisone, heparin, and antibiotics. *Radiology*. 75:50-54 (1960).

[7] Linkenheimer WH, Berger H. The effect of continuous antibiotic feeding on x-irradiation mortality in mice. *Proc Soc Exp Biol Med*. 108:676-80 (1961).

[8] Elliott TB, Madonna GS, Ledney GD, Brook I. Combined therapy for postirradiation infection. *Microecology and Therapy*. 19:105-108 (1989).

[9] Madonna GS, Ledney GD, Moore MM, Elliott TB, Brook I. Treatment of mice with sepsis following irradiation and trauma with antibiotics and synthetic trehalose dicorynomycolate (S-TDCM). *J Trauma*. 31:316-325 (1991).

[10] Patchen ML, Brook I, Elliott TB, Jackson WE, III. Adverse effects of pefloxacin in irradiated C3H/HeN mice: correction with glucan therapy. *Antimicrob Agents Chemother*. 37:1882-9 (1993).

[11] Ledney GD, Elliott TB, Landauer MR, Vigneulle RM, Henderson PL, Harding RA, Tom SP, Jr. Survival of irradiated mice treated with WR-151327, synthetic trehalose dicorynomycolate, or ofloxacin. *Adv Space Res*. 14:(10)583-586 (1994).

[12] Patchen ML, MacVittie TJ, Jackson WE, III. Postirradiation glucan administration enhances the radioprotective effects of WR-2721. *Radiat Res*. 117:59-69 (1989).

[13] Patchen ML. Immunomodulators and cytokines: their use in the mitigation of radiation-induced hemopoietic injury. In Bump EA, Malaker K (eds): Radioprotectors Chemical, Biological and Clinical Perspectives. Boca Raton: CRC Press, pp 213-236 (1998).

[14] Neta R, Vogel SN, Oppenheim JJ, Douches SD. Cytokines in radioprotection. Comparison of the radioprotective effects of IL-1 to IL-6, GM-CSF and IFN-g. *Lymphokine Res*. 5, Suppl. 1:S105-S110 (1986).

[15] Neta R, Oppenheim JJ. Cytokines in therapy of radiation injury. *Blood*. 72:1093-1095 (1988).

[16] Neta R, Oppenheim JJ, Douches SD. Interdependence of the radioprotective effects of human recombinant interleukin 1 alpha, tumor necrosis factor alpha, granulocyte colony-stimulating factor, and murine recombinant granulocyte-macrophage colony-stimulating factor. *J Immunol*. 140:108-11 (1988).

[17] Neta R. Modulation of the radiation response by cytokines. In Bump EA, Malaker K (eds): Radioprotectors Chemical, Biological and Clinical Perspectives. Boca Raton: CRC Press, pp 237-252 (1998).

[18] MacVittie TJ, Farese AM, Jackson W, 3rd. Defining the full therapeutic potential of recombinant growth factors in the post radiation-accident environment: the effect of supportive care plus administration of G-CSF. *Health Phys*. 89:546-555 (2005).

[19] Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley AL, Dickerson WE, Tsu H, Confer DL, Coleman CN, Seed T, Lowry P, Armitage JO, Dainiak N. Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. *Ann Intern Med*. 140:1037-51 (2004).

[20] Food and Drug Administration. Emergency use authorization of medical products. www.fda.gov/RegulatoryInformation/Guidances/ucm125127.htm#intro (2007).

[21] Centers for Disease Control and Prevention. Public health planning for radiological and nuclear terrorism. www.bt.cdc.gov/radiation/pdf/transcript_PH_PlanningDVD_March2008.pdf (2008).

[22] Moore ML. The TRIGA Reactor Facility at the Armed Forces Radiobiology Research Institute: A Simplified Technical Description. Bethesda: AFRRI. (1994)

[23] Verbinski VV, Ferlic K, Cassapakis CC, Daxon E, Hagan WK. Radiation field characterization for the AFRRI TRIGA reactor. Volume I. Baseline measurements and evaluation of calculational data. (1981)

[24] Whitnall MH, Elliott TB, Harding RA, Inal CE, Landauer MR, Wilhelmsen CL, McKinney L, Miner VL, Jackson WE, 3rd, Loria RM, Ledney GD, Seed TM. Androstenediol stimulates myelopoiesis and enhances resistance to infection in gamma-irradiated mice. *Int J Immunopharmacol*. 22:1-14 (2000).

[25] Stickney DR, Groothuis JR, Ahlem C, Kennedy M, Miller BS, Onizuka-Handa N, Schlangen KM, Destiche D, Reading C, Garsd A, Frincke JM. Preliminary clinical findings on NEUMUNE as a potential treatment for acute radiation syndrome. *J Radiol Prot*. 30:687-698 (2010).

[26] Stickney DR, Dowding C, Garsd A, Ahlem C, Whitnall M, McKeon M, Reading C, Frincke J. 5-androstenediol stimulates multilineage hematopoiesis in rhesus monkeys with radiation-induced myelosuppression. *Int Immunopharmacol*. 6:1706-13 (2006).

[27] Whitnall MH, Inal CE, Jackson WE, 3rd, Miner VL, Villa V, Seed TM. *In vivo* radioprotection by 5-androstenediol: Stimulation of the innate immune system. *Radiat Res*. 156:283-293 (2001).

[28] Whitnall MH, Villa V, Seed TM, Benjack J, Miner V, Lewbart ML, Dowding CA, Jackson WE, 3rd. Molecular specificity of 5-androstenediol as a systemic radioprotectant in mice. *Immunopharmacol Immunotoxicol*. 27:15-32 (2005).

[29] Stickney DR, Dowding C, Authier S, Garsd A, Onizuka-Handa N, Reading C, Frincke JM. 5-androstenediol improves survival in clinically unsupported rhesus monkeys with radiation-induced myelosuppression. *Int Immunopharmacol*. 7:500-5 (2007).

[30] Xiao M, Inal CE, Parekh VI, Chang CM, Whitnall MH. 5-Androstenediol promotes survival of gamma-irradiated human hematopoietic progenitors through induction of nuclear factor-kappaB activation and granulocyte colony-stimulating

factor expression. *Mol Pharmacol*. 72:370-9 (2007).

[31] Singh VK, Grace MB, Jacobsen KO, Chang CM, Parekh VI, Inal CE, Shafran RL, Whitnall AD, Kao TC, Jackson WE, 3rd, Whitnall MH. Administration of 5-androstenediol to mice: pharmacokinetics and cytokine gene expression. *Exp Mol Pathol*. 84:178-88 (2008).

[32] Singh VK, Shafran RL, Inal CE, Jackson WE, III, Whitnall MH. Effects of whole-body gamma irradiation and 5-androstenediol administration on serum G-CSF. *Immunopharmacol Immunotoxicol*. 27:521-34 (2005).

[33] Landauer MR, Srinivasan V, Seed TM. Genistein treatment protects mice from ionizing radiation injury. *J Appl Toxicol*. 23:379-85 (2003).

[34] Raffoul JJ, Wang Y, Kucuk O, Forman JD, Sarkar FH, Hillman GG. Genistein inhibits radiation-induced activation of NF-kappaB in prostate cancer cells promoting apoptosis and G2/M cell cycle arrest. *BMC cancer*. 6:107 (2006).

[35] Davis TA, Clarke TK, Mog SR, Landauer MR. Subcutaneous administration of genistein prior to lethal irradiation supports multilineage, hematopoietic progenitor cell recovery and survival. *Int J Radiat Biol*. 83:141-51 (2007).

[36] Davis TA, Mungunsukh O, Zins S, Day RM, Landauer MR. Genistein induces radioprotection by hematopoietic stem cell quiescence. *Int J Radiat Biol*. 84:713-26 (2008).

[37] Grace MB, Parekh V, Chang C-M, Jackson WE, III, Srinivasan V, Whitnall MH, Landauer MR. Isoflavone genistein alters cytokine gene expression in mice after ionizing radiation injury. *Proceedings of FASEB Summer Conference: Nutrient Control of Gene Expression & Signaling*. (2005).

[38] Grace MB, Blakely WF, Landauer MR. Genistein-induced alterations of radiation-responsive gene expression. *Radiat Meas*. 42:1152-1157 (2007).

[39] Singh VK, Grace MB, Parekh VI, Whitnall MH, Landauer MR. Effects of genistein administration on cytokine induction in whole-body gamma irradiated mice. *Int Immunopharmacol*. 9:1401-10 (2009).

[40] Day RM, Barshishat-Kupper M, Mog SR, McCart EA, Prasanna PG, Davis TA, Landauer MR. Genistein protects against biomarkers of delayed lung sequelae in mice surviving high-dose total body irradiation. *J Radiat Res*. 49:361-372 (2008).

[41] Ghosh SP, Perkins MW, Hieber K, Kulkarni S, Kao TC, Reddy EP, Reddy MV, Maniar M, Seed T, Kumar KS. Radiation protection by a new chemical entity, Ex-Rad: efficacy and mechanisms. *Radiat Res*. 171:173-9 (2009).

[42] Onconova Therapeutics Inc. Ex-RAD® (ON 01210.Na). www.onconova.com/exrad.shtml (2012).

[43] Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, Kurnasov OV, Fort FL, Osterman AL, Didonato JA, Feinstein E, Gudkov AV. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science*. 320:226-30 (2008).

[44] Cleveland BioLabs Inc. Radiation antidote for defense. http://www.cbiolabs.com/applications_bio.php (2012).

[45] Singh VK, Krivokrysenko V, Shakhov A, Bone F, Whitnall MH, Pellmar TC, Feinstein E. Cytokines

as biomarkers for radiation countermeasures. *Abstracts, Radiation Research Society Meeting*. September, 2008 (2008).

[46] Ghosh SP, Kulkarni S, Hieber K, Toles R, Romanyukha L, Kao TC, Hauer-Jensen M, Kumar KS. Gamma-tocotrienol, a tocol antioxidant as a potent radioprotector. *Int J Radiat Biol*. 85:598-606 (2009).

[47] Kulkarni S, Ghosh SP, Satyamitra M, Mog S, Hieber K, Romanyukha L, Gambles K, Toles R, Kao TC, Hauer-Jensen M, Kumar KS. Gamma-tocotrienol protects hematopoietic stem and progenitor cells in mice after total-body irradiation. *Radiat Res*. 173:738-47 (2010).

[48] Li XH, Fu D, Latif NH, Mullaney CP, Ney PH, Mog SR, Whitnall MH, Srinivasan V, Xiao M. Delta-tocotrienol protects mouse and human hematopoietic progenitors from gamma-irradiation through extracellular signal-regulated kinase/mammalian target of rapamycin signaling. *Haematologica*. 95:1996-2004 (2010).

[49] Singh VK, Brown DS, Kao TC. Alpha-tocopherol succinate protects mice from gamma-radiation by induction of granulocyte-colony stimulating factor. *Int J Radiat Biol*. 86:12-21 (2010).

[50] Singh VK, Singh PK, Wise SY, Seed TM. Mobilized progenitor cells as a bridging therapy for radiation casualties: a brief review of tocopherol succinate-based approaches. *Int Immunopharmacol*. 11:842-47 (2011).

[51] Srinivasan V, Weiss JF. Radioprotection by vitamin E: injectable vitamin E administered alone or with WR-3689 enhances survival of irradiated mice. *Int J Radiat Oncol Biol Phys*. 23:841-845 (1992).

[52] Singh PK, Wise SY, Ducey EJ, Brown DS, Singh VK. Radioprotective efficacy of tocopherol succinate is mediated through granulocyte-colony stimulating factor. *Cytokine*. 56:411-21 (2011).

[53] Singh PK, Wise SY, Ducey EJ, Fatanmi OO, Elliott TB, Singh VK. alpha-Tocopherol Succinate Protects Mice against Radiation-Induced Gastrointestinal Injury. *Radiat Res*. 177:133-45 (2012).

[54] Singh VK, Wise SY, Singh PK, Ducey EJ, Fatanmi OO, Seed TM. alpha-Tocopherol succinate- and AMD3100-mobilized progenitors mitigate radiation-induced gastrointestinal injury in mice. *Exp Hematol*. in press (2012).

[55] Singh VK, Christensen J, Fantanmi OO, Gille D, Ducey EJ, Wise SY, Karsunky H, Sedello AK. Myeloid progenitors: A radiation countermeasure that is effective when initiated days after irradiation. *Radiat Res*. in press (2012).

[56] Shakhov AN, Singh VK, Bone F, Cheney A, Kononov Y, Krasnov P, Toshkova T, Shakhova VV, Young J, Weil MM. Prevention and mitigation of acute radiation syndrome in mice by synthetic lipopeptide agonists of Toll-like receptor 2 (TLR2). *PLoS ONE*. in press (2012).

[57] Singh VK, Ducey EJ, Fatanmi OO, Singh PK, Brown DS, Purmal A, Shakhova VV, Gudkov AV, Feinstein E, Shakhov A. CBLB613: a TLR 2/6 agonist, natural lipopeptide of Mycoplasma arginini, as a novel radiation countermeasure. *Radiat Res*. in press (2011).

[58] National Security Staff, Interagency Policy Coordination Subcommittee for Preparedeness & Response to

Radiological and Nuclear Threats. Planning Guidance for Response to a Nuclear Detonation, 2nd Edition. (2010)

[59] Landauer MR, McChesney DG, Ledney GD. Synthetic trehalose dicorynomycolate (S-TDCM): behavioral effects and radioprotection. *J Radiat Res*. 38:45-54 (1997).

[60] McChesney DG, Ledney GD, Madonna GS. Trehalose dimycolate enhances survival of fission neutron-irradiated mice and Klebsiella pneumoniae-challenged irradiated mice. *Radiat Res*. 121:71-75 (1990).

[61] Brook I, Tom SP, Ledney GD. Quinolone and glycopeptide therapy for infection in mouse following exposure to mixed-field neutron-gamma-photon radiation. *Int J Radiat Biol*. 64:771-7 (1993).

[62] Elliott TB, Ledney GD, Harding RA, Henderson PL, Gerstenberg HM, Rotruck JR, Verdolin MH, Stille CM, Krieger AG. Mixed-field neutrons and gamma photons induce different changes in ileal bacteria and correlated sepsis in mice. *Int J Radiat Biol*. 68:311-20 (1995).

[63] Ledney GD, Elliott TB, Harding RA, Jackson WE, III, Inal CE, Landauer MR. WR-151327 increases resistance to induced *Klebsiella pneumoniae* infection in mixed-field- and gamma-photon-irradiated mice. *Int J Radiat Biol*. 76:261-271 (2000).

[64] Ledney GD, Madonna GS, Elliott TB, Moore MM, Jackson WE, III. Therapy of infections in mice irradiated in mixed neutron/photon fields and inflicted with wound trauma: a review of current work. *Radiat Res*. 128:S18-S28 (1991).

[65] Cary LH, Ngudiankama BF, Salber RE, Ledney GD, Whitnall MH. Efficacy of radiation countermeasures depends on radiation quality. *Radiat Res*. in press (2012).

[66] Satyamitra M, Lombardini E, Graves J, 3rd, Mullaney C, Ney P, Hunter J, Johnson K, Tamburini P, Wang Y, Springhorn JP, Srinivasan V. A TPO receptor agonist, ALXN4100TPO, mitigates radiation-induced lethality and stimulates hematopoiesis in CD2F1 mice. *Radiat Res*. 175:746-58

[67] Dicarlo AL, Hatchett RJ, Kaminski JM, Ledney GD, Pellmar TC, Okunieff P, Ramakrishnan N. Medical Countermeasures for Radiation Combined Injury: Radiation with Burn, Blast, Trauma and/or Sepsis. Report of an NIAID Workshop, March 26-27, 2007. *Radiat Res*. 169:712-21 (2008).

[68] Ledney GD, Jiao W, Elliott TB, Kiang JG. Combined injury: therapeutic studies. *Abstracts, Radiation Research Society Meeting*. (2009).

[69] Gorbunov NV, Jiao W, Elliott TB, Ledney GD, Kiang JG. Mesenchymal stem cells administered intravenously into mice ameliorate radiation combined injury. *Abstracts, Radiation Research Society Meeting*. (2010).

[70] Jiao W, Kiang JG, Cary L, Elliott TB, Pellmar TC, Ledney GD. COX-2 inhibitors are contraindicated for treatment of combined injury. *Radiat Res*. 172:686-97 (2009).

[71] Ledney GD, Elliott TB. Combined injury: factors with potential to impact radiation dose assessments. *Health Phys*. 98:145-52 (2010).

[72] Kiang JG, Jiao W, Cary LH, Mog SR, Elliott TB, Pellmar TC, Ledney GD. Wound trauma increases radiation-induced mortality by activation of iNOS pathway and elevation of cytokine concentrations and bacterial

infection. *Radiat Res*. 173:319-32 (2010).

[73] Miller AC, Ainsworth EJ, Lui L, Wang TJ, Seed TM. Development of chemopreventive strategies for radiation-induced cancer: targeting radiation-induced genetic alterations. *Military Med*. 167:54-6 (2002).

[74] Williams JP, Brown SL, Georges GE, Hauer-Jensen M, Hill RP, Huser AK, Kirsch DG, Macvittie TJ, Mason KA, Medhora MM, Moulder JE, Okunieff P, Otterson MF, Robbins ME, Smathers JB, McBride WH. Animal models for medical countermeasures to radiation exposure. *Radiat Res*. 173:557-78 (2010).

[75] Miller AC, Cohen S, Stewart M, Rivas R, Lison P. Radioprotection by the histone deacetylase inhibitor phenylbutyrate. *Radiat Environ Biophys*. 50:585-96 (2011).

[76] Snoy PJ. Establishing efficacy of human products using animals: the US food and drug administration's "animal rule". *Vet Pathol*. 47:774-8 (2010).

[77] Augustine AD, Gondre-Lewis T, McBride W, Miller L, Pellmar TC, Rockwell S. Animal models for radiation injury, protection and therapy. *Radiat Res*. 164:100-9 (2005).

[78] Scientific Committee on Health and Environmental Risks. The need for non-human primates in biomedical research, production and testing of products and devices. *European Commission Report*. (2009).

[79] Bode G, Clausing P, Gervais F, Loegsted J, Luft J, Nogues V, Sims J. The utility of the minipig as an animal model in regulatory toxicology. *J Pharmacol Toxicol Methods*. 62:196-220 (2010).

[80] Moroni M, Coolbaugh TV, Mitchell JM, Lombardini E, Moccia KD, Shelton LJ, Nagy V, Whitnall MH. Vascular Access Port Implantation and Serial Blood Sampling in a Gottingen Minipig (Sus scrofa domestica) Model of Acute Radiation Injury. *J Am Assoc Lab Anim Sci*. 50:65-72 (2011).

[81] Moroni M, Coolbaugh TV, Lombardini E, Mitchell JM, Moccia KD, Shelton LJ, Nagy V, Whitnall MH. Hematopoietic radiation syndrome in the Gottingen minipig. *Radiat Res*. 176:89-101 (2011).

[82] Moroni M, Lombardini E, Salber R, Kazemzedeh M, Nagy V, Olsen C, Whitnall MH. Hematological changes as prognostic indicators of survival: similarities between Gottingen minipigs, humans, and other large animal models. *PLoS ONE*. 6:e25210 (2011).

[83] British Medical Association Board of Science and Education. The Medical Effects of Nuclear War. New York: John Wiley & Sons. (1983)

[84] Bell WC, Dallas CE. Vulnerability of populations and the urban health care systems to nuclear weapon attack - examples from four American cities. *International Journal of Health Geographics*. 6:5 (2007).

[85] Holdstock D, Waterston L. Nuclear weapons, a continuing threat to health. *Lancet*. 355:1544-7 (2000).

[86] Alt LA, Forcino CD, Walker RI. Nuclear events and their consequences. In Walker RI, Cerveny TJ (eds): Medical Consequences of Nuclear Warfare. Falls Church, Virginia: TMM Publications, Office of the Surgeon General, pp 1-14 (1989).

[87] Davids MS, Case C, Jr., Hornung R, 3rd, Chao NJ, Chute JP, Coleman CN, Weisdorf D, Confer DL, Weinstock DM. Assessing surge capacity for radiation victims with marrow toxicity. *Biol Blood Marrow Transplant*.

[88] Flynn DF, Goans RE. Nuclear terrorism: triage and medical management of radiation and combined-injury casualties. *Surg Clin North Am*. 86:601-36 (2006).

[89] Fliedner TM, Nothdurft W, Heit H. Biological factors affecting the occurrence of radiation syndromes. In Broerse JJ, MacVittie TJ (eds): Response of Different Species to Total Body Irradiation. Boston: Martinus Nijhoff Publishers, pp 209-219 (1984).

[90] Potten CS, Grant HK. The relationship between ionizing radiation-induced apoptosis and stem cells in the small and large intestine. *Br J Cancer*. 78:993-1003 (1998).

[91] Mouthon MA, Van der Meeren A, Vandamme M, Squiban C, Gaugler MH. Thrombopoietin protects mice from mortality and myelosuppression following high-dose irradiation: importance of time scheduling. *Can J Physiol Pharmacol*. 80:717-21 (2002).

[92] Herodin F, Grenier N, Drouet M. Revisiting therapeutic strategies in radiation casualties. *Exp Hematol*. 35:28-33 (2007).

[93] Radford IR, Murphy TK. Radiation response of mouse lymphoid and myeloid cell lines. Part III. Different signals can lead to apoptosis and may influence sensitivity to killing by DNA double-strand breakage. *Int J Radiat Biol*. 65:229-39 (1994).

[94] Tanikawa S, Nose M, Aoki Y, Tsuneoka K, Shikita M, Nara N. Effects of recombinant human granulocyte colony-stimulating factor on the hematologic recovery and survival of irradiated mice. *Blood*. 76:445-9 (1990).

[95] Neelis KJ, Visser TP, Dimjati W, Thomas GR, Fielder PJ, Bloedow D, Eaton DL, Wagemaker G. A single dose of thrombopoietin shortly after myelosuppressive total body irradiation prevents pancytopenia in mice by promoting short-term multilineage spleen-repopulating cells at the transient expense of bone marrow-repopulating cells. *Blood*. 92:1586-97 (1998).

[96] International Atomic Energy Agency. Acute radiation syndrome clinical picture, diagnosis and treatment. www.pub.iaea.org/MTCD/publications/PDF/eprmedt/Day_2/Day_2-9.pps (2002).

[97] Centers for Disease Control and Prevention. Chempack Program Description. www.bt.cdc.gov/planning/continuationguidance/docs/chempack-attachj.doc (2004).

[98] Nolin K, Murphy C, Ahern JW, McBride K, Corriveau M, Morgan J. Chempack program: role of the health-system pharmacist. *Am J Health Syst Pharm*. 63:2188, 2190 (2006).

[99] Koenig KL, Bey T, Bradley D, Kahn CA, Schultz C The RADPACK: a new concept for stockpiling medical countermeasures for a radiation disaster at the local level. *Western Journal of Emergency Medicine*. 9: http://escholarship.org/uc/item/7rt8b6mr (2008).

[100] Connor AM, Sigdestad CP. Chemical protection against gastrointestinal radiation injury in mice by WR 2822, WR 2823, or WR 109342 after 4 MeV X ray or fission neutron irradiation. *Int J Radiat Oncol Biol Phys*. 8:547-51 (1982).

[101] Potten CS, Merritt A, Hickman J, Hall P, Faranda A. Characterization of radiation-induced apoptosis in the small intestine and its biological implications. *Int J Radiat Biol*. 65:71-8 (1994).

[102] Farrell CL, Bready JV, Rex KL, Chen JN, DiPalma CR, Whitcomb

KL, Yin S, Hill DC, Wiemann B, Starnes CO, Havill AM, Lu ZN, Aukerman SL, Pierce GF, Thomason A, Potten CS, Ulich TR, Lacey DL. Keratinocyte growth factor protects mice from chemotherapy and radiation-induced gastrointestinal injury and mortality. *Cancer Res*. 58:933-9 (1998).

[103] Fliedner TM, H DD, Meineke V. Multi-organ involvement as a pathogenetic principle of the radiation syndromes: a study involving 110 case histories documented in SEARCH and classified as the bases of haematopoietic indicators of effect. *BJR supplement / BIR*. 27:1-8 (2005).

[104] Riecke A, Ruf CG, Meineke V. Assessment of radiation damage-the need for a multiparametric and integrative approach with the help of both clinical and biological dosimetry. *Health Phys*. 98:160-7 (2010).

[105] Rotolo JA, Kolesnick R, Fuks Z. Timing of lethality from gastrointestinal syndrome in mice revisited. *Int J Radiat Oncol Biol Phys*. 73:6-8 (2009).

[106] Haegebarth A, Perekatt AO, Bie W, Gierut JJ, Tyner AL. Induction of protein tyrosine kinase 6 in mouse intestinal crypt epithelial cells promotes DNA damage-induced apoptosis. *Gastroenterology*. 137:945-54 (2009).

[107] van Bekkum DW, Schotman E. Protection from haemopoietic death by shielding versus grafting of bone-marrow. *Int J Radiat Biol Relat Stud Phys Chem Med*. 25:361-72 (1974).

The 2011 Hirosaki University International Symposium

The Proceedings of the 3rd International Symposium on Radiation Emergency Medicine at Hirosaki University

平成２４年　３月１４日　　印刷
平成２４年　３月２１日　　発行

編　集　　弘前大学大学院保健学研究科
〒036-8564　弘前市本町 66-1

印刷所　　やまと印刷株式会社