The 2009 Hirosaki University International Symposium

The 1st International Symposium on Radiation Emergency Medicine at Hirosaki University

Hirosaki University Press

The 1st International Symposium on Radiation Emergency Medicine at Hirosaki University

Organizer: Hirosaki University Graduate School of Health Sciences
Joint Auspices: National Institute of Radiological Sciences (NIRS)
Institute for Environmental Sciences (IES)
Japan Atomic Energy Agency
Sponsors: Aomori Prefecture
Hirosaki City

PREFACE

Prevention is the most important issue concerning accidents involving radioactive materials, but taking the appropriate measures in the unlikely event of a radiation accident is indispensable. In the field of radiation emergency medicine, it is expected that advanced medical treatment will be needed in the event of such an accident. In Aomori Prefecture, there are many institutions related to atomic energy, including a nuclear fuel reprocessing plant; an important feature of Aomori. Consequently, the development of a radiation emergency medicine system is a pressing issue for advanced medical and higher education facilities in Aomori.

The goal of the project at Hirosaki University is to prepare an advanced tertiary emergency medical center that includes a system for radiation emergency medicine. In conjunction with the preparation of the center, preparations for the development of an advanced professional education program for radiation emergency medicine commenced in the School of Health Sciences in 2008. Currently, special postgraduate programs, such as nursing care for patients exposed to radiation, contamination control, decontamination, radiation dosimetry and specific clinical assay studies are being developed, as well as entry-level undergraduate educational programs.

In line with these initiatives, the 1st International Symposium on Radiation Emergency Medicine at Hirosaki University was held on August 1st, 2009, as part of Hirosaki University's 60th Anniversary celebrations. The purposes of the Symposium were to: (1) share information about the current status of radiation emergency medicine studies worldwide; (2) identify the achievements, challenges and problems related to radiation emergency medicine in each country; and (3) discuss future directions of the field. Distinguished guests from both China and France were invited to the Symposium, and lectures and discussions were held concerning radiation emergency medicine. Presentations comprised six sessions, and 14 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 express our sincerest gratitude for the assistance provided by Japan Nuclear Fuel Limited, the National Institute of Radiological Sciences and the Institute for Environmental Sciences, which contributed to the success of The 1st International Symposium on Radiation Emergency Medicine at Hirosaki University. I would also like to acknowledge the contributions of the guest speakers and the members of the governing board of the Symposium, who made what I hope to be the first of many successful meetings run smoothly.

Hitoshi Tsushima
Chief Chair, The 1st International Symposium on Radiation Emergency Medicine at Hirosaki University
Head of School, Hirosaki University Graduate School of Health Sciences

Symposium

Opening by Professor Masahiko Endo, the President of Hirosaki University

Dr. Kohsei Kudo, Hirosaki University
The 1st presenter

Professor Takashi Kondo, Toyama University
Chair of Symposium I

Dr. Koichi Ito, Hirosaki University
The 2nd presenter

Professor Ikuo Kashiwakura, Hirosaki University
The 3rd presenter

Dr. Yoichi Oghiso, IES
Chair of Symposium II and the 7th presenter

Dr. Makoto Akashi, NIRS
Chair of Symposium VI and the 4th presenter

Dr. Hideo Tatsuzaki, NIRS
The 5th presenter

Dr. Mitsuaki A. Yoshida, NIRS
The 6th presenter

Dr. Kimio Tanaka, IES
The 8th presenter

Professor Mikinori Kuwabara, Hokkaido University
Chair of Symposium III

Dr. Takumaro Momose, Japan Atomic Energy Agency
The 9th presenter

Dr. Kiyomitsu Kawachi, Nuclear Safety Technology Center
Chair of Symposium IV

Dr. Toshiharu Miyakawa, Japan Nuclear Fuel Ltd
The 10th presenter

Professor Xiaohua Chen, Beijin Institute of Radiation Medicine
The 12th presenter

Dr. Jean-Jacques Lataillade
Hôpital d'Instruction des Armées Percy
The 13th presenter

Dr. Eric Bey
Hôpital d'Instruction des Armées Percy
The final presenter

Closing by Professor Hitoshi Tsushima
Director of Hirosaki University Graduate School of Health Siences

Welcome Reception

Welcome speech by Professor Masahiko Endo, the President of Hirosaki University

Toast was proposed by Dr Akihiro Shima
the President of IES

Opening speech by Professor Hitoshi Tsushima
Director of Hirosaki University Graduate School of Health Siences

Speech by Professor Xiaohua Chen
Beijin Institute of Radiation Medicine

Invitation speakers were introduced by Dr. Hideo Tatsuzaki
NIRS

Speech by Dr. Jean-Jacques Lataillade
Hôpital d'Instruction des Armées Percy

Speech by Dr. Eric Bey
Hôpital d'Instruction des Armées Percy

Speech by Dr Kinichi Ishitoya
Director of Hirosaki University Supporting Committee

Closing toast was proposed by Professor Mikinori Kuwabara
Hokkaido University

Contents

Effects of mesenchymal stem cell transplantation in preventing radiation-induced intestinal injury in mice

Kohsei KUDO[1*], Yong LIU[2], Kenji TAKAHASHI[1], Kohetsu TARUSAWA[1], Dong-Liang HU[3], Ikuo KASHIWAKURA[1], Hiroshi KIJIMA[4] and Akio NAKANE[3]

[1] Department of Radiological Life Sciences, Hirosaki University Graduate School of Health Sciences, Japan.
[2] Particle Radiation Oncology Research Center, Research Reactor Institute, Kyoto University, Japan.
[3] Department of Microbiology and Immunology, Hirosaki University Graduate School of Medicine, Japan.
[4] Department of Pathology and Bioscience, Hirosaki University Graduate School of Medicine, Japan.

Abstract. The treatment of radiation-induced intestinal injury is difficult and effective treatments are currently unavailable. Developing new treatments for radiation-induced intestinal injury is important. We have previously reported that embryonic stem cells (ESCs) transplanted directly into the wall of irradiated intestine show colonization and differentiation. However, transplantation of ESCs did not influence survival rates or changes in body weights of mice with radiation-induced intestinal injury. The present study investigated whether transplantation of mesenchymal stem cells (MSCs) could prevent radiation-induced intestinal injury. Intestines of female nude mice (ICR *nu*/*nu*) were irradiated at a single dose of 30 Gy (X-ray 150 kV, 5 mA, with 0.5 mm Al filters, at a dose rate of 1.9 Gy/min). Transplantation of male MSCs (C57BL/6n) was then immediately performed into the wall of the irradiated intestine by direct injection for the irradiation + MSCs group. For 13 days after irradiation, mice were weighed daily and survival was recorded. From 13 to 27 days after irradiation, intestines of mice were obtained to assay histological changes by staining with hematoxylin-eosin and Masson trichrome. Mean body weight was significantly higher in the irradiation + MSCs group than in the irradiation-only group after irradiation. In addition, survival rate was significantly higher for the irradiation + MSCs group than for the irradiation-only group after irradiation. Histological observation showed that intestines of MSC-transplanted mice were thick in the submucosal and muscle layers, and had recovered from radiation-induced intestinal injury. These results suggest that transplanted MSCs may play an important role in preventing radiation-induced injury and may offer a new method for treating radiation-induced intestinal injury. The protective mechanisms provided by transplanted MSCs warrant further study.

Key Words: mesenchymal stem cells, transplantation, embryonic stem cells, radiation, intestinal injury

Introduction

Acute ionizing radiation to the whole body induces a range of tissue damage and can result in death, depending on the exposure dose. At extremely high doses, death occurs shortly after irradiation and appears to result from neurologic and cardiovascular breakdown. At intermediate doses, death occurs in a matter of days and is associated with destruction of gastrointestinal mucosa. At low doses, death occurs several weeks after exposure and is caused by bone marrow failure, which is mainly treated by bone marrow

* Corresponding to: Kohsei Kudo, Assistant professor, Hirosaki University, 66-1 Hon-cho, Hirosaki, 036-8564, Japan. E-mail: kohsei@cc.hirosaki-u.ac.jp

transplantation and cytokine therapy [1, 2]. On the other hand, there is no effective treatment against radiation-induced intestinal injury, which is treated based on symptoms. Thus, investigation of novel approaches is required.

Embryonic stem cells (ESCs) have a pluripotent capacity to differentiate into a variety of cell lineages in vitro [3]. Previous studies have shown that the derivation of oocytes and blastocyst-like structure from mouse ESCs could be accomplished with both female and male ESCs [4]. When ESCs are allowed to differentiate in a suspension culture, they form spherical multicellular aggregates, termed embryoid bodies (EBs), and these have been shown to contain a variety of cell populations. Some studies have been successful in inducing mouse ESCs to differentiate into particular types of cells, such as neurons [5], cardiomyocytes [6, 7], hepatocytes [8] and pancreatic islets [9], within an EB environment. In addition, recent studies have suggested that gut-like organs could be formed from ESCs in vitro [10, 11] and have demonstrated that the formation process is basically same as in gut organogenesis. However, transplantation of ESCs in vivo directly in the intestine has not been reported. Little is known about whether ESCs transplanted directly in vivo are able to colonize and differentiate in the radiation-damaged intestine. We previously demonstrated that ESCs transplanted directly into the wall of irradiated intestine exhibit colonization and differentiation [12]. However, transplantation of ESCs did not influence survival rates or changes in body weight in mice with radiation-induced intestinal injury.

Mesenchymal stem cells (MSCs) are pluripotent progenitor cells that contribute to the maintenance and regeneration of various connective tissues, including bone, adipose, cartilage and muscle [13]. Therefore, MSCs are expected to become a source of cells for regenerative therapy. Furthermore, previous studies have reported that MSCs play various roles depending on the situation; for example, suppression of T-cell proliferation [14], distribution in areas of tissue inflammation [15] and promotion of breast cancer metastasis [16].

In this study, we investigated whether transplantation of MSCs instead of ESCs is able to prevent radiation-induced intestinal injury. Mice underwent direct transplantation of bone-marrow-derived mouse MSCs into locally irradiated intestine. For 13 days after irradiation, mice were weighed daily and survival was recorded. At 13 to 27 days after irradiation, intestines were obtained in order to determine histological changes by staining with hematoxylin-eosin (H.E.) and Masson trichrome (M.T.). The results suggest that transplanted MSCs play an important role in preventing radiation-induced injury and may offer a new method for treating radiation-induced intestinal injury.

Materials and Methods

Animals

Female ICR *nu*/*nu* mice (age, 6-12 weeks) were obtained from Japan Charles River Laboratories (Yokohama), and were housed in plastic cages under specific pathogen-free conditions at the Institute for Animal Experiments, Hirosaki University School of Medicine. Mice were kept on a 12 h:12 h light:dark cycle, and food and water were available at all times. All animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Hirosaki University.

MSC culture

MSCs were obtained from C57BL/6 mice (male, 8-10 weeks of age) from Japan Charles River Laboratories (Yokohama). Bone marrow cells were harvested from femurs and tibias using the methods described by Dobson et al. [17], and were then cultivated in a plastic dish according to the protocol for isolation and expansion of MSCs developed by Peister et al [18]. Proliferated cells were MSCs, which were transfected with pEGFP (enhanced green fluorescent protein) using Lipofectamine reagent. MSCs were then prepared for transplantation at a concentration of 1×10^8 cells/ml.

pEGFP transfection

MSCs were transfected with pEGFP using Lipofectamine 2000 (Invitrogen). In a six-well tissue culture plate, cells were seeded at 2×10^5 cells per well in 2 ml of DMEM (Invitrogen) containing 10% FBS. Cells were incubated at 37°C in a CO_2 incubator overnight. Next, 10 μg of pEGFP (kindly provided by the Department of Microbiology and Immunology, Hirosaki

University Graduate School of Medicine, Japan.) and 12 μl of Lipofectamine reagent were each diluted in 375 μl of serum-free DMEM, and the two solutions were mixed gently, incubated at room temperature for 30 min, and added to 750 μl of serum-free DMEM. Cells were washed once with 2 ml of serum-free medium, followed by incubation for 5 h at 37°C in a CO_2 in solution containing pEGFP and Lipofectamine reagent. Cells were then removed from the transfection mixture and were placed in normal growth medium. Cells were used at 24 h following the start of transfection. MSCs transfected with pEGFP were observed on a fluorescence microscope (Olympus) equipped with a digital camera.

Local irradiation and cell transplantation

Female ICR *nu/nu* mice were anesthetized by intraperitoneal (I.P.) injection of pentobarbital at a dose of 50 mg/kg body weight and were fixed in place. Part of the intestine was removed from the peritoneal cavity, and a lead plate (thickness, 2 mm) was used to cover the whole body except for the exposed intestine. Only 15-20 mm of intestine was irradiated with a single dose of 30 Gy. X-irradiation was performed using a Hitachi MBR-1505R2 at 150 kV and 5 mA, with 0.5 mm Al filters, at a dose rate of 1.9 Gy/min. The rest of the body was irradiated at less than 0.1 Gy. After irradiation, the walls of irradiated intestines were immediately subjected to transplantation with MSCs [1×10^7 cells/0.1 ml Dulbecco's phosphate buffered saline (Chemicon)] through a 27-gauge syringe using a manipulator, and intestines were returned to the peritoneal cavity. For 13 days after irradiation, mice were weighed daily and survival was recorded. At 13 to 27 days after irradiation, intestines of mice were obtained to assay histological changes by staining with H.E. and M.T.

Body weight ratio and survival rate

For 13 days after irradiation and transplantation, mice were weighed daily and survival was recorded. At 13 to 27 days after transplantation, intestines were obtained in order to evaluate histological changes by staining with H.E. and M.T. Statistical analyses of mean body weight and survival rates were performed using Student's *t*-test and Kaplan-Meier Log-rank test, respectively.

Histological analysis

At 13 to 27 days after irradiation, mice were sacrificed by cervical dislocation, intestines were removed to be fixed overnight in 10% neutral buffered formalin. Sections were paraffin-embedded, cut at 4 μm and mounted onto slides. H.E. and M.T. staining were performed for histological analysis. Tissue sections were examined using an Olympus microscope and were photographed with a digital camera.

Results

Survival rate and changes in body weight after irradiation and cell transplantation

Survival and mean body weight of mice were observed for 13 days (Table 1 and Table 2). In the irradiation-only group, half of the mice died at 13. In contrast, survival rate in the irradiation +

Table 1. Survival rate

	n	Days after irradiation			
		4d	7d	10d	13d
X-ray only (Control)	14	100%	64.3%	57.1%	50.0%
X-ray + MSC	16	100%	93.8%*	87.5%*	87.5%*

* : $p<0.05$ compared with irradiation-only control mice. n : numbers of sample.

Table 2. Body weight ratio

	n	Days after irradiation			
		4d	7d	10d	13d
X-ray only (Control)	7	0.94±0.02	0.92±0.03	0.90±0.07	0.85±0.12
X-ray + MSC	14	0.93±0.05	0.96±0.05	1.00±0.04*	1.01±0.05**

Data are presented as means ±SD. *: $p<0.05$. **: $p<0.01$ vs. control mice.
n : numbers of sample.

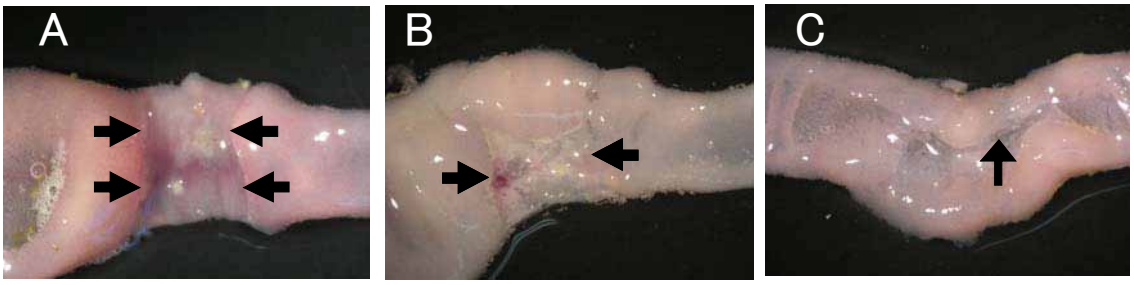


Figure 1. Macroscopic appearance of intestinal damage after irradiation alone, or after irradiation and MSC transplantation. Mice were anesthetized and intestines were irradiated with X-rays at a single dose of 30 Gy. Immediately after irradiation, mouse MSCs were inoculated into the walls of irradiated intestines. On days 13 and 27 after irradiation and transplantation, intestines were removed and observed. A: Control intestines from mice after irradiation alone. B and C: Intestines from mice after irradiation and MSC transplantation (B: on day 13, C: on day 27). Black arrows show radiation-induced intestinal damage. Observed intestines showed signs of recovery after irradiation and transplantation. means ± SD. *: $P<0.05$. **: $P<0.01$ vs. irradiation-only group.

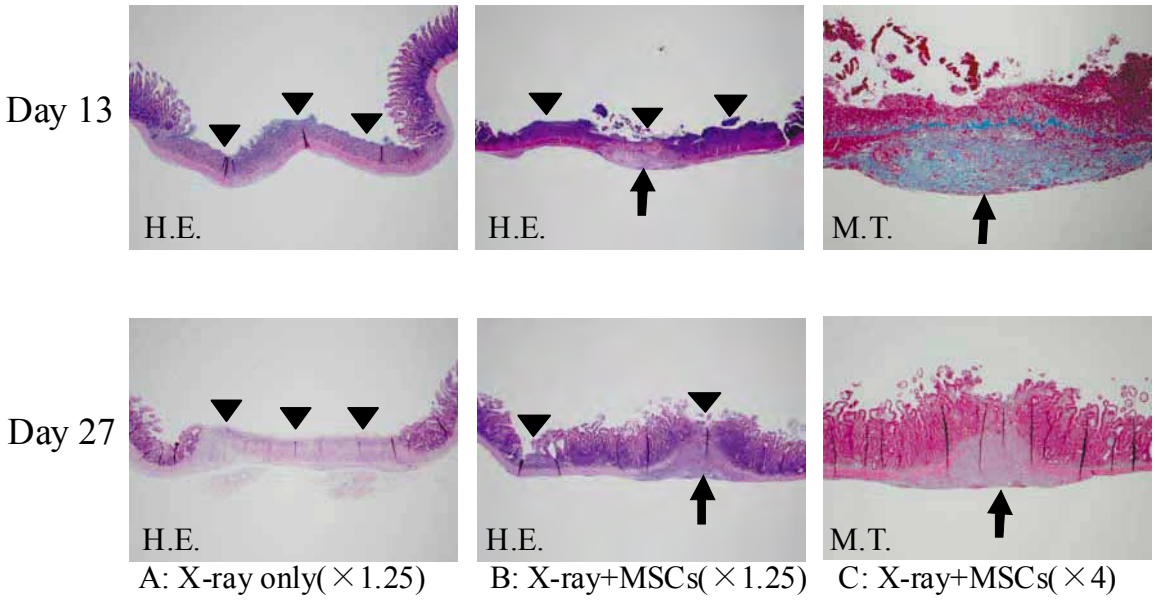
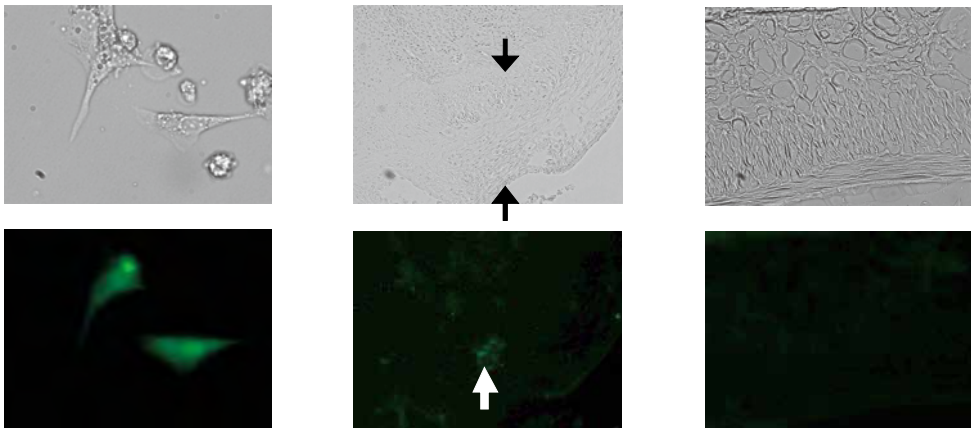


Figure 2. Histological analysis of irradiated and MSC-transplanted intestines. On days 13 and 27 after irradiation alone, or after irradiation and transplantation, intestines were removed and prepared for H.E. or M.T. staining. A: Intestines from irradiation-only control mice. B: Intestines from irradiation + MSC mice. A and B show H.E. staining at ×1.25 magnification (objective lens). Arrow heads (▼) show areas of radiation injury. Areas showing injury were fewer in irradiation + MSC mice at 27 days when compared with irradiation-only mice. The observed thick areas (black arrows) were stained with M.T. (C). C: ×4 magnification (objective lens). Upper photos show samples at day 13 and lower photos show samples at day 27.

MSC group was 87.5% after 13 days. Survival rates were significantly higher for the irradiation + MSC group than for the irradiation-only group at 13 day after irradiation ($P<0.05$) (Table 1). Changes in body weight were also recorded for 13 days (Table 2), and mean body weight was significantly higher in the irradiation + MSC group than in the irradiation-only group at 13 days after irradiation ($P<0.01$). These results suggested that transplantation of MSCs affected survival rate and changes in body weight in mice following radiation-induced intestinal injury.



A : pEGFP-MSCs　　B : Transplantation of pEGFP-MSCs　　C : Un-transplantation

Figure 3. Fluorescence microscope images of intestines after MSC transplantation. A: MSCs transfected with pEGFP (enhanced green fluorescent protein) using Lipofectamine reagent. Magnification: ×40. B: Intestine transplanted with MSCs transfected with pEGFP on day 13 after irradiation. Magnification: × 10. C: Intestine on day 13 after irradiation alone. Upper photos are light microscope images. Magnification: × 40. Black arrows show the thickening area after MSC transplantation. Green fluorescence was found in the thickening areas (white arrow).

Radiation-induced intestinal damage

We further investigated whether transplanted MSCs were able to repair damage in the irradiated intestine. After irradiation and transplantation (Figure 1), ulceration (black arrows) in intestines from irradiated and transplanted mice on day 13 (Figure 1B) was less severe than in irradiated-only control mice (Figure 1A), and on day 27, occupied a very small area (Figure 1C). These results suggest that transplanted MSCs are able to repair radiation-induced intestinal damage by inhibiting ulceration.

Histological analysis of radiation-induced intestinal damage

Intestines were removed on days 13 and 27 after MSC transplantation, and were subjected to H.E. and M.T. staining. Intestines from irradiated-only control mice showed ulceration and granulation tissue formation in the intestinal mucosa, and on day 27, showed no assigns of recovery (Figure 2A). In contrast, on day 13, the intestines from irradiated and MSC-transplanted mice also showed ulceration and granulation tissue formation, but on day 27, numerous regenerating crypts and ulceration healing were observed (Figure 2B). Fibrous thickening areas were observed at ulceration sites (Figure 2B, black arrows), and were thought to be due to MSC transplantation, while no apparent proliferating MSC colonies were found. The fibrous thickening areas were further detected as having numerous collagen bundles on M.T. staining (Figure 2C). To confirm the degree of recovery, the length of ulcerated area after irradiation was measured using an Olympus BX41 microscope equipped with a DP25 digital CCD camera and DP2-BSW software. On day 27, the length ulcerated area in irradiation-only group was 3.55± 1.58 mm. In contrast, on day 27, the length ulcerated area in irradiation + MSC group was 0.76 ± 0.35 mm. Intestines of irradiated and MSC transplanted mice had almost fully recovered from radiation-induced intestinal injury by day 27. These results suggest that transplanted MSCs are able to repair radiation-induced intestinal damage by increasing collagen bundles, as well as inhibiting ulceration.

Fluorescence microscope images of intestine

In order to confirm whether transplanted MSCs are able to survive in irradiated intestines, we observed intestines transplanted with MSCs (Figure 3A) transfected with pEGFP on day 13 by fluorescence microscopy (Figure 3). Green fluorescence was observed in the thick areas (Figure 3B). In contrast, intestines from irradiation-only mice showed no green fluorescence (Figure 3C). These results suggest that transplanted MSCs are able to survive in the irradiated intestine.

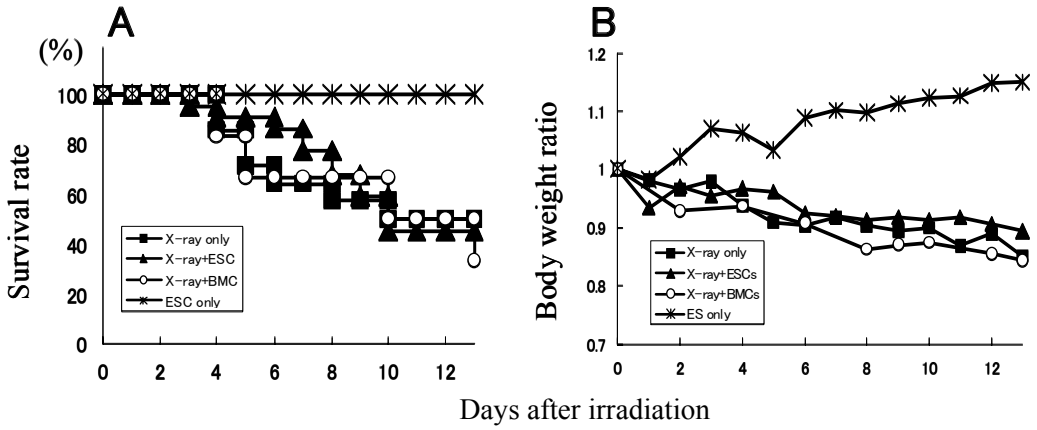


Figure 4. Supplement data of survival and body weights of mice for 13 days after irradiation. Female ICR *nu/nu* mice were anesthetized and intestines were irradiated with X-rays (150 kVp, 5 mA, 0.5 mm Al filter, 1.9 Gy/min) at a single dose of 30 Gy. Immediately after irradiation, ESCs or BMCs were inoculated into the walls of irradiated intestines. Survival and mean body weights of the mice were observed for 13 days after irradiation. A: Survival rates of the ESC transplantation-only group (＊, 6 mice), the irradiation-only group (■, 14 mice), the irradiation + ESC group (▲, 22 mice), and the irradiation + BMC group (○, 6 mice). B: Changes in mean body weight ratio of ESC transplantation-only group (＊, 6 mice), irradiation-only group (■, 7 mice), the irradiation + ESC group (▲, 10 mice), and the irradiation + BMC group (○, 3 mice). There were no significant difference between irradiation-only group and the irradiation +ESC or BMC group.

Discussion

We investigated survival rate and changes in body weight after irradiation and MSC transplantation. Transplantation of MSCs resulted in significantly higher survival rates and clearly higher body weights after irradiation when compared with the irradiation-only group. We previously demonstrated that transplantation of ESCs [1] or bone marrow cells (BMCs) directly into the walls of irradiated intestine had no influence on survival rates or changes in body weight in mice with radiation-induced intestinal injury (We presented supplement data in figure 4).

The present results demonstrate that transplantation of MSCs directly into the walls of high-dose irradiated intestine improves radiation-induced intestinal injury. Recent studies have shown that MSCs transplanted via the tail vein after abdominal irradiation at 13 Gy possessed the capacity to engraft into the enteric mucosa [19], and intestinal structure had recovered with systemic MSC transplantation at 3 days after 3.5-Gy total body irradiation and 4.5-Gy local abdominal irradiation [20]. However, direct transplantation of MSCs with a local irradiation dose of 30 Gy had not previously been reported.

On histological observation, length of ulceration area on day 27 after irradiation was significant different shorter in the MSC transplantation group than in the irradiation-only group, and intestines of irradiated and MSC transplanted mice had almost fully recovered from radiation-induced intestinal injury by day 27. These results suggest that transplanted MSCs are able to repair radiation-induced intestinal damage by inhibiting ulceration. Lorenzi et al. reported that MSC injection improved muscle regeneration and increased contractile function of anal sphincters after injury and repair. In that study, rats underwent sphincterotomy and surgical repair followed by intrasphincteric injection of MSC using a microsyringe [21]. Therefore, MSC transplantation may be a novel treatment for radiation-induced intestinal injury.

In conclusion, we investigated the effects of MSC transplantation in preventing radiation-induced intestinal injury. These results suggest that transplanted MSCs play an important role in preventing radiation-induced injury and may offer a novel approach for treating radiation-induced intestinal injury. The protective

mechanisms provided by transplanted MSCs warrant further study.

Acknowledgments

We would like to thank the late Yoshinao Abe of Hirosaki University for providing guidance in the planning and performing of this study. This work was supported by a Grant-in-Aid for Exploratory Research (19659299).

References

[1] Gale RP, Butturini A. Medical response to nuclear and radiation accidents. Occup Med. 6(4):581-596(1991).

[2] Hérodin F, Mayol JF, Mourcin F, Drouet M. Which place for stem cell therapy in the treatment of acute radiation syndrome? Folia Histochem Cytobiol. 43(4):223-227(2005).

[3] Donovan PJ, Gearhart J. The end of the beginning for pluripotent stem cells. Nature. 414(6859):92-97(2001).

[4] Hübner K, Fuhrmann G, Christenson LK et al. Derivation of oocytes from mouse embryonic stem cells. Science. 300(5623):1251-1256(2003).

[5] Bain G, Kitchens D, Yao M, Huettner JE, Gottlieb DI. Embryonic stem cells express neuronal properties in vitro. Dev Biol. 168(2):342-357(1995).

[6] Sachinidis A, Fleischmann BK, Kolossov E, Wartenberg M, Sauer H, Hescheler J. Cardiac specific differentiation of mouse embryonic stem cells. Cardiovasc Res. 58(2):278-291(2003).

[7] Zandstra PW, Bauwens C, Yin T et al. Scalable production of embryonic stem cell-derived cardiomyocytes. Tissue Engineering. 9(4):767-778(2003).

[8] Cui S, Schwartz L, Quaggin SE. Pod1 is required in stromal cells for glomerulogenesis. Dev Dyn. 226(3):512-522(2003).

[9] Blyszczuk P, Wobus AM. Stem cells and pancreatic differentiation in vitro. Journal of Biotechnology. 113(1-3):3-13(2004).

[10] Yamada T, Yoshikawa M, Takaki M et al. In vitro functional gut-like organ formation from mouse embryonic stem cells. Stem Cells. 20(1):41-49(2002).

[11] Kuwahara M, Ogaeri T, Matsuura R, Kogo H, Fujimoto T, Torihashi S. In vitro organogenesis of gut-like structures from mouse embryonic stem cells. Neurogastroenterol Motil. 16 Suppl 1:14-18(2004).

[12] Kudo K, Abe Y, Hu DL, Kijima H, Nakane A. Colonization and differentiation of transplanted embryonic stem cells in the irradiated intestine of mice. Tohoku J Exp Med. 212:143-150(2007).

[13] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science. 284:143-147(1999).

[14] Sato K, Ozaki K, Oh I, Meguro A, Hatanaka K, Nagai T, Muroi K, Ozawa K. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood. 109:228-234(2007).

[15] Sokolova IB, Zin'kova NN, Shvedova EV, Kruglyakov PV, Polyntsev DG. Distribution of mesenchymal stem cells in the area of tissue inflammation after transplantation of the cell material via different routes. Bull Exp Biol Med. 143:143-146(2007).

[16] Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature. 449:557-563(2007).

[17] Dobson KR, Reading L, Haberey M, Marine X, Scutt A. Centrifugal isolation of bone marrow from bone: an improved method for the recovery and quantitation of bone marrow osteoprogenitor cells from rat tibiae and femurae. Calcif Tissue Int. :411-413(1999).

[18] Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ. Adult stem cells from bone marrow (MSCs) isolated

from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood. 103:1662-1668(2004).

[19] Zhang J, Gong JF, Zhang W, Zhu WM, Li JS. Effects of transplanted bone marrow mesenchymal stem cells on the irradiated intestine of mice. J Biomed Sci. 15:585-594(2008).

[20] Sémont A, François S, Mouiseddine M, François A, Saché A, Frick J, Thierry D, Chapel A.Mesenchymal stem cells increase self-renewal of small intestinal epithelium and accelerate structural recovery after radiation injury. Adv Exp Med Biol. 585:19-30(2006).

[21] Lorenzi B, Pessina F, Lorenzoni P, Urbani S, Vernillo R, Sgaragli G, Gerli R, Mazzanti B, Bosi A, Saccardi R, Lorenzi M. Treatment of experimental injury of anal sphincters with primary surgical repair and injection of bone marrow-derived mesenchymal stem cells. Dis Colon Rectum. 51:411-420(2008).

Recovery of the hematopoietic system after murine allogeneic umbilical cord blood transplantation

Hideaki Sato, Kyoko Ito, and Koichi Ito*

Department of Biomedical Sciences, Hirosaki University Graduate School of Health Sciences, Japan.

Abstract. The ability of murine allogeneic umbilical cord blood cells (UCBCs) to reconstitute the hematopoietic system was studied. In this study, C57BL/6 (H-2^b) and BALB/c (H-2^d) background mice were used as donors and recipients, respectively. After UCBC transplantation, phenotypically matured immune cells of donor origin, including T cells, B cells, monocytes and granulocytes, were observed in recipients' peripheral blood by using flow cytometry analysis. Functional analysis demonstrated that allogeneic UCBC-transplanted mice accepted skin grafts from both C57BL/6 and BALB/c mice. However, these chimeric mice completely rejected third-party skin grafts from C3H/HeJ (H-2^K) mice, indicating that both the $CD8^+$ killer and $CD4^+$ helper T cells were functionally mature. Furthermore, 2,4,6-trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH)-immunized UCBC-transplanted mice produced both TNP-specific immunoglobulin (Ig)M and IgG antibodies. These results indicated that recipient mice were capable of generating antibody responses to T-dependent antigen, and the Ig class switching confirmed that both B cells and $CD4^+$ helper T cells derived from allogeneic UCBCs were immunologically competent. In terms of potential clinical application, our observations indicate that allogeneic UCBC transplantation can allow recovery of the normal hematopoietic system in patients who have been accidentally exposed to radiation.

Key words: umbilical cord blood, hematopoietic system, radiation exposure

Introduction

Umbilical cord blood cell (UCBC) transplantation has been applied as a strategy for the treatment of various hematological diseases [1-3] and accidental total-body radiation exposure [4]. UCBC transplantation has several advantages over bone marrow (BM) transplantation, including the much larger size of the available donor pool, the rich proportion of hematopoietic progenitor cells [5], the low content of mature T cells that might cause a graft-versus-host reaction [6, 7], and the low risk of cytomegalovirus infection [8]. However, in clinical situations, perfect major histocompatibility complex (MHC) matching cannot be expected in UCBC transplantation, which is based essentially on non-related donor-recipient combinations. Although the low content of mature T cells allows the use of even MHC-mismatched UCBCs, the extent to which lymphocytes derived from MHC-mismatched UCBC transplantation recover their immune function is still unclear due to the lack of convenient animal models. In this study, we examined the differentiation, maturation, and function of lymphocytes derived from UCBC hematopoietic stem cells (HSCs) in a fully MHC-mismatched combination by using a murine model for UCBC transplantation developed in a previous study [9, 10]. The clinical application of allogeneic UCBC transplantation aims not only to expand donor numbers but also to provide faster emergency medicine without MHC matching for patients who suffer accidental radiation exposure.

Animals

Female C57BL/6 (B6: H-2^b), BALB/c (H-2^d), and C3H/HeJ (H-2^k) mice were purchased from CLEA Japan Inc. (Tokyo). C57BL/6-TgN (act-EGFP) OsbY01 (green fluorescent transgenic mice on a B6 background, H-2^b that were

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

Table 1. Characteristics of 2 donor sources used for transplantation

	UCBCs		BM cells	
	T-cell depletion			
(%)	Before	After	Before	After
Lineage negative cells	23.2 ± 1.3	26.9 ± 1.3	5.8 ± 2.4	7.3 ± 3.0
$CD3e^+$ cells	0.9 ± 0.4	0.8 ± 0.4	3.8 ± 1.7	0.8 ± 0.2
$CD45R/B220^+$ cells	10.2 ± 2.9	10.4 ± 2.7	25.4 ± 6.3	25.0 ± 10.6
$CD11b^+$ cells	64.1 ± 4.8	61.4 ± 4.1	65.5 ± 3.8	67.6 ± 7.6
$Gr\text{-}1^+$ cells	31.8 ± 2.1	31.9 ± 1.5	50.1 ± 3.8	47.2 ± 9.5
$TER119^+$ cells	11.6 ± 2.3	9.7 ± 4.7	2.5 ± 0.5	0.4 ± 0.0

denoted as GFP.B6 in this paper) [11] and RAG2 knockout BALB/c mice (T cell- and B cell-deficient mice on a BALB/c background, $H\text{-}2^d$ that were denoted as RAG2 (-/-) BALB/c in this paper) were provided by Dr. Masaru Okabe (Osaka University) and Dr. Michio Shimamura (Mitsubishi Kagaku Institute of Life Sciences), respectively. Mice were maintained in a specific pathogen-free facility at Hirosaki University. The experimental procedure was approved by the Animal Research Committee of Hirosaki University, and all animal experiments were conducted following the guidelines of the committee.

Preparation of UCBCs and BM cells

For comparison, BM cells were also prepared. UCBCs were collected from fetuses at 18.5 days of gestation, and BM cells were collected from the femur and pelvic bones of adult GFP.B6 mice. Both HSCs were obtained from (GFP.B6 × B6) F1 fetuses and adults, because the strong fluorescence emitted by GFP homozygous cells was not observed with GFP negative cells in the same plotting field on flow cytometry.

Profile of UCBCs and BM cells used for transplantation

To deplete mature T cells, UCBCs or BM cells were subjected to complement-dependent cytotoxicity using anti-CD4 and anti-CD8 antibodies described previously [12]. Then, the non-treated and treated cells were stained separately with biotinylated antibodies against lineage markers, including T cell receptor (CD3e; T cells), B220 (CD45R; B cells), Mac-1 (CD11b; monocytes), Gr-1 (Ly-6G; granulocytes), and TER119 (Ly-76; erythroid cells), followed by phycoerythrin (PE)-labeled streptavidin. Then, the percentages of cells of each lineage were determined by flow cytometry analysis (Table 1). Lineage-negative cells were not detected with the mixture of lineage antibodies. Values are expressed as the %mean ± standard deviation (SD) of 3 independent experiments. Essentially, UCBCs did not contain mature T cells (before T-cell depletion: 0.9 ± 0.4; after T-cell depletion: 0.8 ± 0.4). The percentage of T cells among BM cells decreased to a level similar that of UCBCs after T-cell depletion (before T-cell depletion: 3.8 ± 1.7; after T-cell depletion: 0.8 ± 0.2). In addition, UCBCs contained approximately 4 times as many lineage-negative cells as compared to BM cells.

Survival rate after transplantation

RAG2(-/-) BALB/c recipients were lethally irradiated at 8 Gy and then given a transplant containing a high dose (1×10^6 cells), medium dose (0.5×10^6 cells), or low dose (0.1×10^6 cells) of UCBCs obtained from (GFP.B6 × B6) F1 fetuses or BM cells obtained from GFP.B6 mice (Figure 1). There were 5 recipients in each dose group. As a control, 9 mice were irradiated but not given a transplant. All mice given a low dose of UCBCs died within 2 weeks (Figure 1A). On the other hand, 40% of mice given a low dose of BM cells were still alive at 16 weeks after transplantation (Figure 1B). However, the survival rates of both mice given medium or high doses of UCBCs and medium or high doses of BM cells were essentially similar. These results indicate that the number of UCBCs with the capacity for both self-renewal and the ability to differentiate into every hematopoietic lineage is lower than in BM cells.

Reconstitution of the hematopoietic system

Eight-gray-irradiated RAG2 (-/-) BALB/c

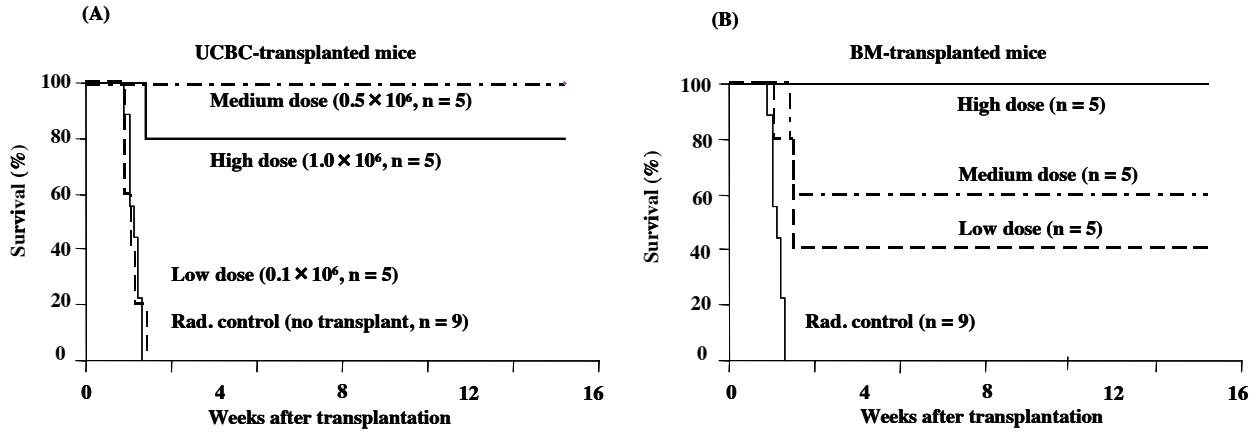


Figure 1. Effect of cell-dose on survival after transplantation. Survival curves of recipients receiving UCBCs (A) or BM cells (B) at a high dose (1.0×10^6 cells, n = 5), medium dose (0.5×10^6 cells, n = 5), and low dose (0.1×10^6 cells, n = 5) up to 16 weeks after transplantation are shown. As a negative control, 9 X-ray-irradiated RAG2 (-/-) BALB/c were prepared without any transplantation.

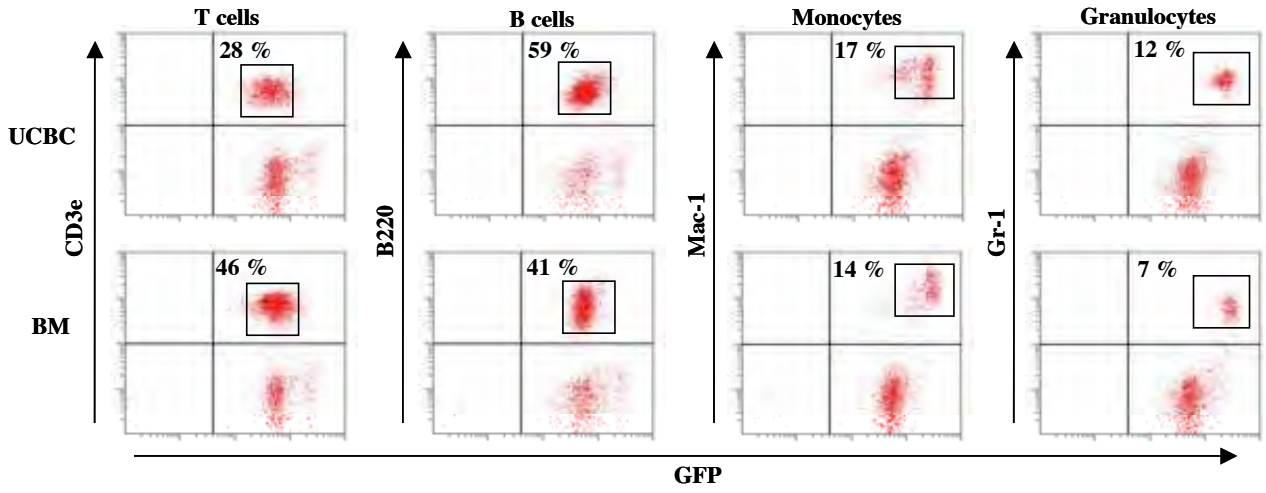


Figure 2. Reconstitution of the hematopoietic system by donor cells in allogeneic recipients.At 16 weeks after transplantation, peripheral blood cells of RAG2(-/-)BALB/c recipients were analyzed by flow cytometry by using biotin-labeled antibodies such as anti-CD3 (T cells), anti-B220 (B cells), anti-CD11b (monocytes) and anti-Gr-1 (granulocytes), followed by the addition of streptavidin-PE. All GFP fluorescent lineage cells of donor origin including T cells, B cells, monocytes and granulocytes were observed in each representative UCBC- (upper 4 panels) or BM cell-transplanted (lower 4 panels) recipient.

mice that had received 0.5×10^6 GFP transgenic UCBCs or BM cells were analyzed 16 weeks after the cell transfer (Figure 2). Engrafted cells in the recipients' peripheral blood were detected by staining with a biotinylated antibody against a lineage marker such as the T cell receptor (CD3e; T cells), B220 (CD45R; B cells), Mac-1 (CD11b; monocytes), or Gr-1 (Ly-6G; granulocytes), followed by PE-labeled streptavidin. Flow cytometric analysis revealed that the transferred cells had developed into T cells, B cells, Mac-1^+ cells, and granulocytes in allogeneic UCBC-transplanted and BM-transplanted mice. Every lineage consisted only of GFP^+ cells, indicating that the cells were of donor origin.

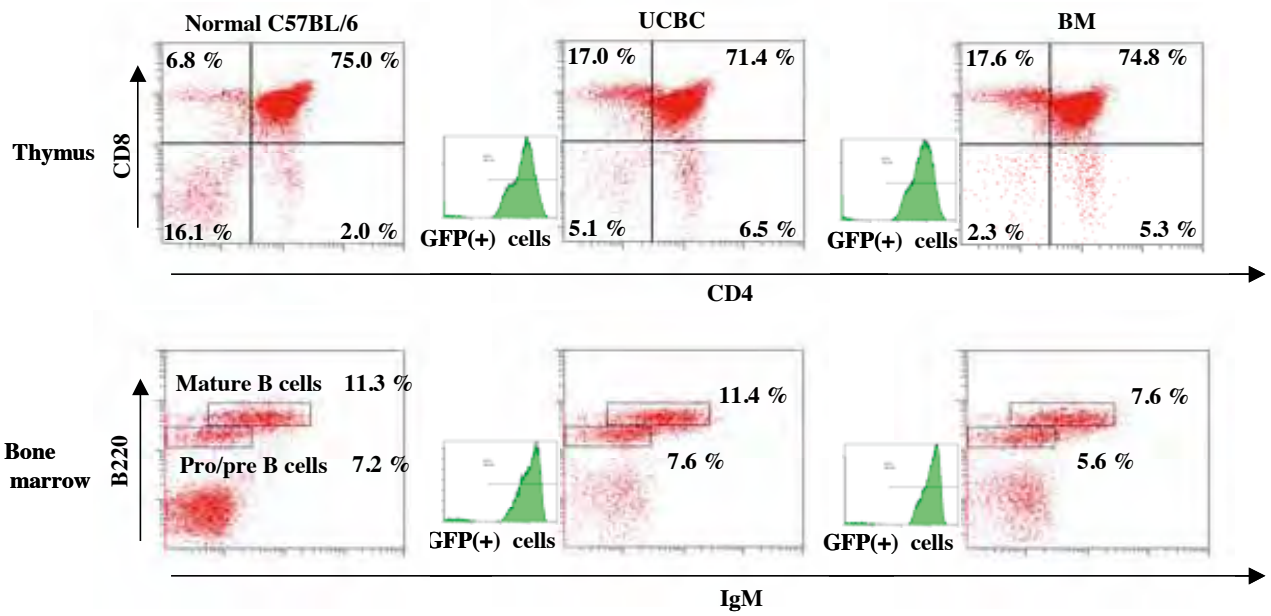


Figure 3. Developmental processes of T cells and B cells in lymphoid organs..Both thymocytes and BM cells were harvested from UCBC- and BM-transplanted recipients at 16 weeks after transplantation and stained with PE-labeled anti-CD4 and PerCP-Cy5.5-labeled anti-CD8, and with PE-labeled anti-B220 and PerCP-Cy5.5-labeled anti-IgM antibodies, respectively. All developmental processes of T cells in the thymus (upper 3 panels) and B cells in bone marrow (lower 3 panels) were detected in a representative UCBC- and BM-transplanted recipient similar to a normal B6 mouse.

Similar results were obtained for recipients that had received medium or high doses of UCBCs and BM cells.

Developmental processes of T cells in the thymus and B cells in bone marrow

The thymus and BM were harvested from chimeric RAG2 (-/-) BALB/c mice that survived for more than 16 weeks after transplantation of allogeneic UCBCs or BM cells (Figure 3). As a control, normal B6 mice were used for comparison. Within the thymus, GFP^+ cells included all 4 populations, including double-negative, double-positive, and CD4 and CD8 single-positive. This observation confirms the normal intrathymic development of T-lineage cells derived from UCBCs and BM cells. When bone marrow cells were stained with anti-B220 antibody and anti-IgM antibody, 2 distinct B-cell lineages ($B220^+IgM^-$ and $B220^+IgM^+$) were observed among GFP cells, corresponding to pro/pre B cells and newly matured B cells, respectively which indicate that normal development of UCBC- and BM-derived B lineage cells ocurred in the recipients' bone marrow. Similar results were observed in other recipients (data not shown). These results suggest that lymphocytes developing from UCBCs are able to achieve normal maturation in lymphoid organs even after fully MHC-mismatched transplantation.

Function of UCBC-derived B cells

The functional maturity of allogeneic UCBC- or BM-derived B cells in RAG2 (-/-) BALB/c mice was examined (Figure 4). There were 7 recipients in each group. Over 16 weeks after transplantation, the chimeric recipients were immunized with 2 biweekly intraperitoneal injections of 50μg of 2,4,6-trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH), initially with complete Freund's adjuvant and the second time without the adjuvant. The immunized mice were bled from the tail vein 2 weeks after the second immunization. The production of anti-TNP antibody was examined by performing an enzyme immunosorbent assay in TNP-bovine serum albumin-coated plates. Bound TNP-specific immunoglobulin (Ig)M and IgG were detected using horseradish peroxidase-conjugated

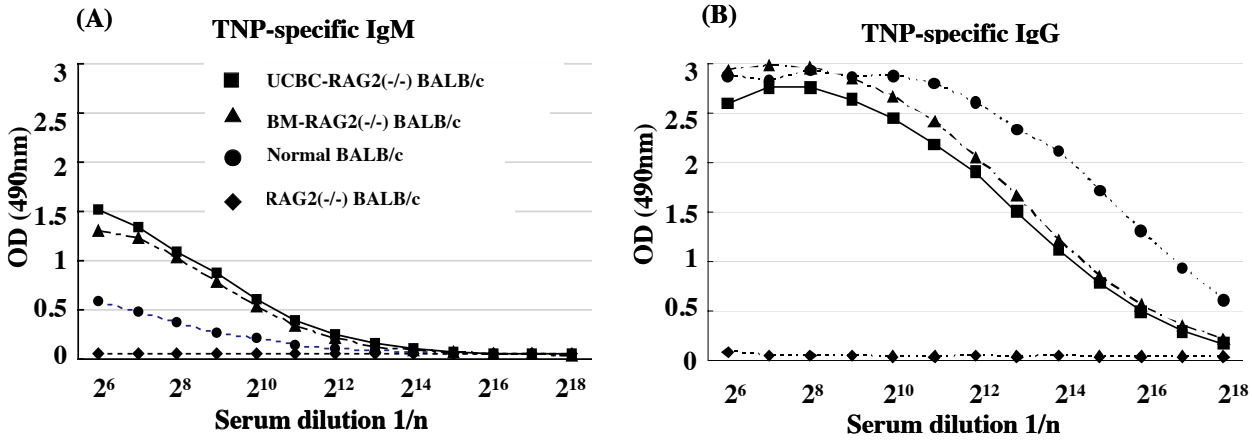


Figure 4. B cell function in transplanted recipients. Similar IgM (A) and IgG (B) antibody production against TNP dependant on titration was observed among UCBC- and BM- transplanted mice, non-transplanted RAG2(-/-)BALB/c mice and normal BALB/c mice, indicating B cells reconstituted from allogeneic UCBC were functionally competent. Mean OD values obtained from 7 mice in each group are plotted.

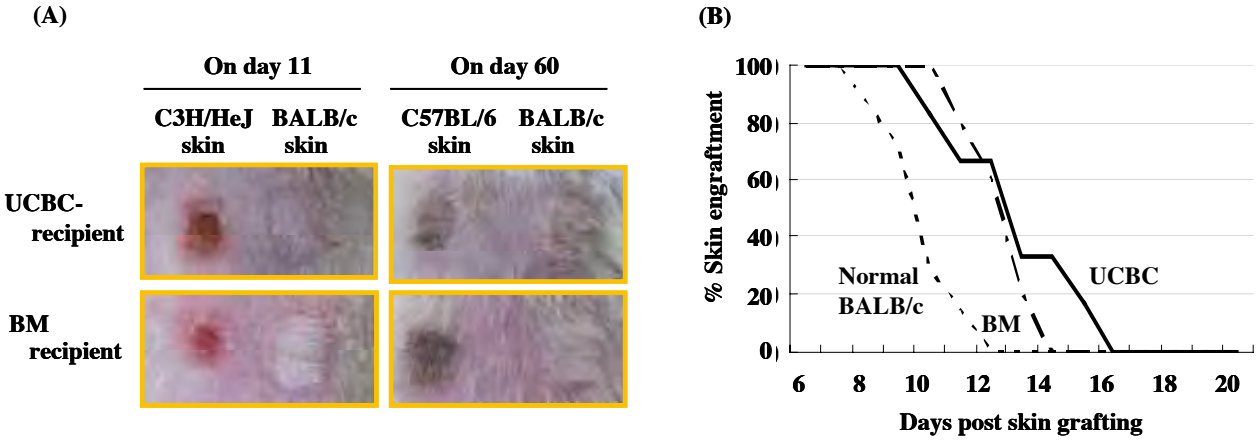


Figure 5. T cell function in transplanted recipients**.** (A) Over 16 weeks after allogeneic UCBC or BM transplantation, RAG2(-/-)BALB/c recipients were simultaneously grafted with a piece of C3H/HeJ and BALB/c skin on their backs. A representative photograph shows skingraft rejection of C3H/HeJ skin but not of BALB/c skin in UCBC- and BM- transplanted recipients, indicating that the T cells reconstituted from allogeneic UCBCs were functionally competent. (B) Rejection time for C3H/HeJ skin was compared with 6 UCBC- and 5 BM-transplanted recipients, and 7 normal BALB/c mice. Essentially, rejection time for C3H/HeJ skin in both recipients was slower than rejection time in normal BALB/c mice.

anti-mouse IgM and anti-mouse IgG antibodies, respectively, followed by the addition of O-phenylenediamine dihydrochloride. Optical density (OD) was measured at 490 nm. Seven non-transplanted RAG2(-/-)BALB/c and normal BALB/c mice were used as controls. Both IgM (Figure 4A) and IgG (Figure 4B) antibody responses to TNP-KLH were successfully induced in both UCBC-transplanted mice and BM-transplanted mice. These results indicate that recipient mice have the ability to induce antibody responses to T-dependent antigens such as TNP-KLH, and Ig class switching confirmed that both B cells and helper T cells derived from allogeneic UCBCs were immunologically competent.

Function of UCBC-derived T cells

The T-cell response to skin grafts was examined in chimeric RAG2 (-/-) BALB/c mice that had received allogeneic UCBCs and BM cells (Figure 5). Dermis for skin grafting was harvested from the tails of BALB/c, B6 and C3H/HeJ mice and placed on the shaved back of chimeric RAG2 (-/-) BALB/c mice that had survived for more than 16 weeks after transplantation. The grafts were protected immediately by wrapping them with a bandage. After 7 days, the bandage was removed to allow observation. Rejection time was determined as the day when the grafted skin became completely detached. On day 11 after skin grafting, UCBC- or BM-reconstituted RAG2 (-/-) BALB/c mice rejected third-party skin grafts (C3H/HeJ, $H\text{-}2^K$), indicating the presence of functional $CD8^+$ cells as well as $CD4^+$ cells (Figure 5A). In contrast, skin grafts from BALB/c or B6 mice were still maintained in these chimeric mice on day 60. In chimeric RAG2 (-/-) BALB/c mice, T-cell function was restricted by both MHC from B6 and BALB/c mice. Essentially the same observations were obtained after skin grafting in other chimeric mice. In addition, rejection time for C3H/HeJ skin was compared with 6 UCBC- and 5 BM-transplanted recipients and 7 normal BALB/c mice (Figure 5B). Interestingly, rejection time for C3H/HeJ skin in both types of transplant recipients was slightly delayed compared with normal BALB/c mice.

Conclusions

Hematopoietic stem cells among allogeneic UCBCs differentiate in RAG2 (-/-) BALB/c mice into phenotypically mature T cells, B cells, $Mac\text{-}1^+$ cells, and $Gr\text{-}1^+$ cells. Furthermore, the chimeric recipients reconstituted with UCBC generate specific antibody responses to T-dependent antigen and also reject third-party skin grafts. These abilities are quite similar to those of BM chimeras. Our observations indicate that T and B cells derived from allogeneic UCBCs are immunologically fully competent.

In an allogeneic environment, low-dose UCBC transplantation is unable to rescue X-ray-irradiated recipients. However, transplantation of higher doses of UCBCs results in a high survival rate comparable to that of BM transplantation, indicating that UCBC-HSC and BM-HSC have essentially the same ability to reconstitute the hematopoietic system. It is still unclear whether UCBC-HSC and BM-HSC are completely the same or whether the former is an immediate precursor of the latter.

Since the quantity of individual cord blood samples is limited, the use of pooled cord blood might be required in a clinical situation. In mixed UCBC transplantation [13,14], MHC restriction can be more complex. As shown in Fig.5A, chimeric RAG2 (-/-) BALB/c mice reconstituted with UCBCs from B6 mice accepted skin grafts from both B6 and BALB/c mice. Thus, it is necessary to have a clear understanding of the mechanisms driving MHC restriction in such cases. These problems can be solved only by *in vivo* experiments. Hopefully, murine UCBC transplantation will allow us to explore these issues in future studies.

Acknowledgements

The authors would like to thank Dr. Nobukata Shinohara (Kitasato University) for providing helpful suggesions on the experimental design.

References

[1] Gluckman E, Broxmeyer HE, Auerbach AD, 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, Rocha V, boyer-Chammand A, 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, et al. 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, Misawa K, Tanaka 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, 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, et al. 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, et al. Does cord blood transplantation result in lower graft-versus host disease? *Hum Immunol*.56: 1-5 (1997).

[8] Tomonari A, Iseki T, Ooi J, et al. Cytomegalovirus infection following unrelated cord blood transplantation for adult patients: a single institute experience in Japan. *Br J Haematol*. 121: 304-311 (2003).

[9] Migishima F, Oikawa A, Kondo S, et al. Full reconstitution of hematopoietic system by murine cord blood. *Transplantation*. 75: 1820-1826 (2003).

[10] Oikawa A, Ito K, seguchi H, et al. Development of immunocompetent lymphocytes in vivo from murine umbilical cord blood cells. *Transplantation*. 84: 23-30 (2007).

[11] Okabe M, Ikawa M, Kominami K, et al. 'Green Mice' as a source of ubiquitous green cells. *FEBS Lett*. 407: 313-319 (1997).

[12] Park SY, Kojima M, Suzuki H, Shinohara N. Effective blocking of natural cytotoxicity of young rabbit serum on murine thymocytes by high concentration of glucose in complement-dependent cytotoxicity method. *J Immunol methods*. 154: 109-119 (1992).

[13] Barker JN, Weisdolf DJ, DeFor TE, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood*. 105: 1343-1347 (2005).

[14] Nauta AJ, kruisselbrink AB, Lurvink E, et al. Enhanced engraftment of umbilical cord blood-derived stem cells in NOD/SCID mice by cotransplantation of a second unrelated cord blood unit. *Exp Hematol*. 33: 1249-1256 (2005).

Correlations of cell surface antigens with the individual differences of radio-sensitivity in human hematopoietic stem/progenitor cells

Satoru Monzen, Naoki Hayashi, Kenji Takahashi and Ikuo Kashiwakura

Department of Radiological Life Sciences, Hirosaki University Graduate School of Health Sciences, 66-1 Hon-cho, Hirosaki, Aomori, Japan

Abstract. In order to characterize the individual differences of radio-sensitivity in human hematopoietic stem/progenitor cells (HSPCs), we examined the relationship among cell surface antigens, clonogenic potential and radiation survival. The expressions of CD34, CD38, CD45RA, CD110 and Tie-2, early differentiation pathway-related antigens in hematopoiesis, were analyzed on the surface of HSPCs enriched by CD34 antigen prepared from human placental/umbilical cord blood. The significant positive relationship was observed between CD38 antigen and CD45RA, CD110 and Tie-2, respectively. No significant relationship was observed in almost all cases among the antigens and the number of colony-forming cells CFC; however, the number of megakaryocytic progenitor cells correlated negatively with the rate of Tie-2^+ cells. With respect to the radio-sensitivities, the expression of Tie-2 antigens correlated significantly with the surviving fraction of CFC, suggesting that the individual radio-sensitivity of CFC is predictable with respect to the Tie-2 rate of HSPCs to some extent. In addition, the number of progenitor cells correlated strongly with its surviving fraction. These results suggest that the individual radio-sensitivity of HSPCs is predictable with respect to the number of progenitor cells to some extent, especially, its dependency on the presence of immature HSPCs such as Tie-2^+ cells.

Key Words: radio-sensitivity, hematopoietic stem/progenitor cells, individual differences, CD34, Tie-2

Introduction

The highly glycosylated transmembrane protein CD34 is strongly expressed on hematopoietic stem/progenitor cells (HSPCs). Therefore, human HSPCs enriched based on this antigen have thus far been used in the studies regarding in vitro hematopoiesis. $CD34^+$ cells are heterogenous populations that contain various functional cells such as lineage-committed progenitors, early progenitors (1, 2) and some stromal cells (3). In addition, normal human $CD34^+$ cells secrete numerous growth factors, cytokines, and chemokines that contribute to the intercellular cross-talk networks and regulate various stages of hematopoiesis (4), thus indicating a diversity of $CD34^+$ cells.

Recent studies have reported the relationship between hematopoiesis and the cell surface antigens such as CD19, CD34, CD38, CD45RA, CD110 (thrombopoietin receptor), interleukin-3 receptor and Tie-2 (tyrosine kinase with immunoglobulin and the epidermal growth factor homology domains 2) (5-7). However, although the relationship between these antigens and hematopoiesis has become clear to some extent, little information has been reported to date with respect to the relationship between the heterogeneity of HSPCs and the individual differences of their radio-sensitivities. This understanding can predict the hematopoietic recovery from radiation exposure as well as the extent of radiation damage in hemaopoiesis. In addition, a diagnosis of the specific radio-sensitivity in patients who are suffering from

malignant diseases allows radiation therapy and/or chemotherapy can thus be performed more effectively. Therefore, an understanding of the individual radio-sensitivity of HSPCs is very important (8-11).

In the present study, in order to characterize the individual differences of radio-sensitivity of HSPCs, we examined the relationship among the expressions of the early differentiation pathway-related antigens in hematopoiesis, their clonogenic potential, and radiation survival.

Materials and Methods

Growth factors and fluorescence antibodies

The recombinant human interleukin-3 (IL-3) and the recombinant human stem cell factor (SCF) were purchased from Biosource (Tokyo, Japan). The recombinant human granulocyte-colony stimulating factor (G-CSF), the recombinant human granulocyte/ macrophage-colony stimulating factor (GM-CSF), and erythropoietin (Epo) were purchased from Kirin Pharma Co. Ltd. (Tokyo, Japan), from PeproTech (Rocky Hill, NJ, USA) and from Sankyo Co. Ltd. (Tokyo, Japan), respectively. Recombinant human angiopoietin-1 (Ang-1) was purchased from R&D Systems, Inc. (Minneapolis, MN, USA). These factors were administered at the following concentrations: Ang-1, 200 ng/ml; IL-3 and SCF, 100 ng/ml; G-CSF and GM-CSF, 10 ng/ml; and Epo, 4 U/ml medium. The fluorescence-labeled fluorescein isothiocyanate (FITC)-conjugated anti-human CD34 monoclonal antibodies (mAbs) were purchased from Beckman Coulter Immunotech (Marseille, France). Phycoerythrin (PE)-cyanin-5-forochrome tandem (PC5)-conjugated anti-human CD45RA and PE-conjugated anti-human CD110 were purchased from Becton Dickinson Biosciences (San Jose, CA, USA). PE-conjugated anti-human Tie-2 were purchased from R&D Systems. Mouse IgG1-FITC, -PC5 and -PE (Beckman Coulter Immunotech) were used as the isotype controls.

Culture of CD34$^+$ cells and in vitro irradiation

Each experimental method with respect to collection and purification of placental/umbilical cord blood CD34$^+$ cells, flow cytometry analysis, in vitro irradiation, methylcellurose culture, plasma clot culture and identification of megakaryocyte colonies by immunofluorescence was performed according to the previous report (12, 13).

Statistical analysis

The data were analyzed by a univariate analysis using Student's t-test, Welch's t-test, Tukey-Kramer test, Mann-Whitney's U-test and the Pearson's correlation coefficient. The statistical analysis was performed using the software program Origin (OriginLab®, Northampton, MA, USA) for Windows. A value of $p<0.05$ was considered to be statistically significant.

Results

Flow cytograms of each cell surface antigen in HSPCs

HSPCs were enriched from human CB by using the CD34-positive cell-sorting kit. The expression of the cell surface antigens, including CD34, CD38, CD45RA, CD110 and Tie-2 in the sorted cells, were analyzed by flow cytometry. The positive rate of CD34 antigen ranged from approximately 80% to 98%, thus indicating 88.6% was the average value (data not shown). The typical cytograms between each antigen are shown in Figure 1. Although there was no significant correlation for almost all the antigen combinations, a significant positive relation was found between CD38 and CD45RA ($r=0.455$, $p<0.01$), CD110 and Tie-2 ($r=0.534$, $p<0.001$) , respectively (Figure 2).

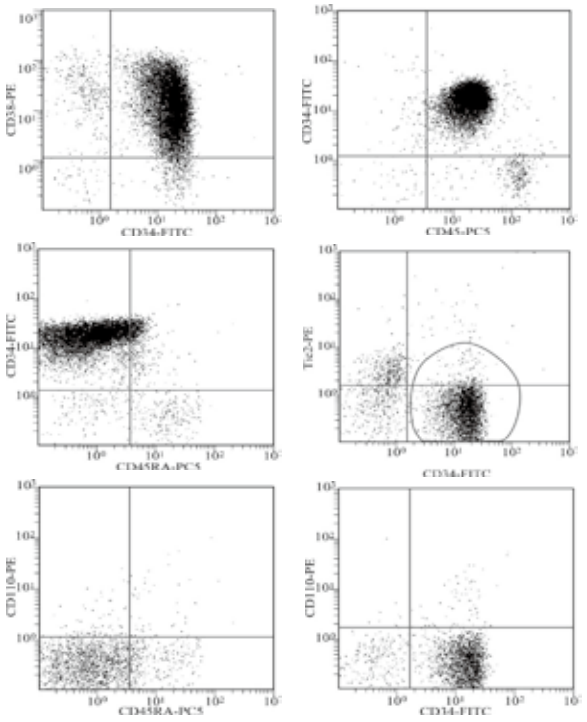


Figure 1. Representative flow cytograms showing the relationships between cell surface antigens

analyzed in the current study. The expressions of CD34, CD38, CD45RA, CD110 and Tie-2 were analyzed on the surface of $CD34^+$-enriched HSPCs prepared from human CB.

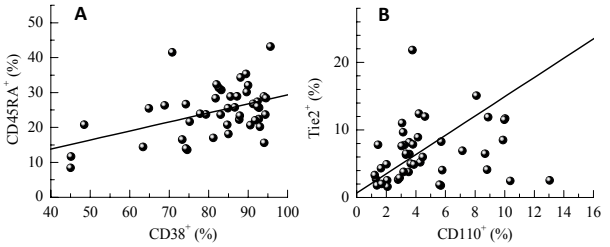


Figure 2. The relationship between each cell surface antigen. The expression of cell surface antigens including CD34, CD38, CD45RA, CD110 and Tie-2 in the freshly prepared CB $CD34^+$ cells was analyzed by flow cytometry. [**A**] The correlation between the expression of CD38 and CD45RA on the surface of $CD34^+$ cells (n=30), [**B**] The correlation between the expression of CD110 and Tie-2 (n=30). * $P < 0.05$. ** $P < 0.01$.

Relation of cell surface antigens with the clonogenic potential of HSPCs

The number of myeloid progenitor cells was measured using a methylcellulose culture supplemented with a combination of SCF, IL-3, G-CSF, GM-CSF and Epo. This combination supports maximum colony formation, and each concentration has a saturated amount. In the case of CFU-Meg, the plasma clot culture was applied for the measurement. The distributions of the number of each progenitor cell, including CFU-GM, BFU-E, CFU-Mix, and CFU-Meg, detected in 1×10^3 cells are shown in Figure 3. The mean number of CFCs, composed of CFU-GM, CFU-E and CFU-Mix, was 77.6 ± 47.2 (data not shown) and the range was approximately 70 to 500. In addition, CFU-GM was a major population (70%) in CFCs (data not shown). At this time, the number of CFU-Meg distributed with an approximately eight-fold discrepancy and its average was significantly higher in comparison to CFU-GM, BFU-E and CFU-Mix, respectively. In addition, a significant difference was also observed between CFU-GM and CFU-Mix.

An analysis of the relationship between the expression of the cell surface antigens and the number of each progenitor cell was performed (Figure 4). No significant relationship was observed in all of the cases, with respect to the expression rate of each cell surface antigen and the number of progenitor cells (Figures 4-A, B, C). However, the number of CFU-Meg negatively correlated with the rate of the $Tie\text{-}2^+$ cells (Figure 4-D).

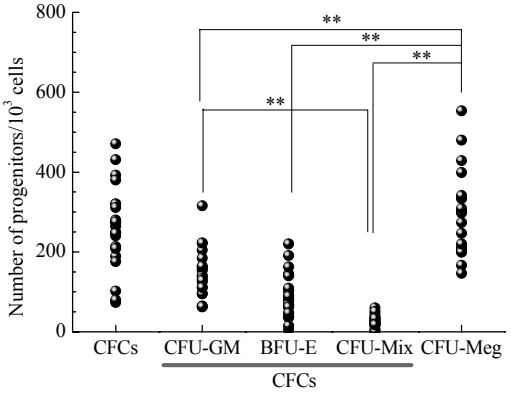


Figure 3. The distribution of the surviving fractions of each hematopoietic progenitor cell (n=20). $CD34^+$ cells were assayed for the number of CFU-GM, BFU-E and CFU-Mix using a methylcellulose culture. The number of CFU-Meg was assessed by a plasma clot culture technique.

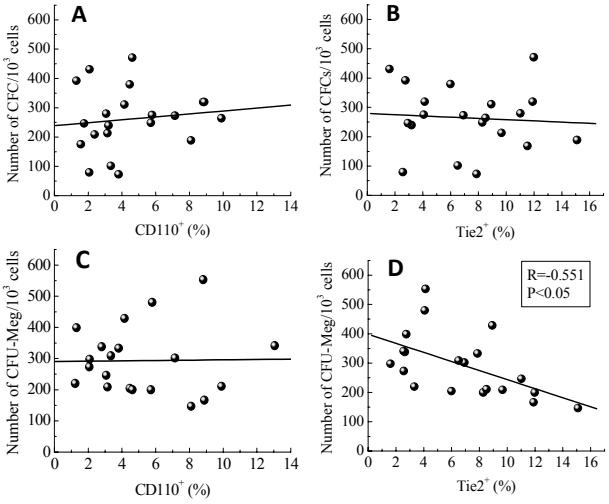


Figure 4. The relationship between the cell surface antigens and the number of hematopoietic progenitor cells (n=20). [**A**] the expression of CD110 and the CFC number contained in 1×103 cells, [**B**] the expression of Tie-2 and the CFC number , [**C**] the expression of Tie-2 and the CFU-Meg number, [**D**] the expression of CD110 and the CFU-Meg number.

Relation of cell surface antigens with the individual differences of radio-sensitivity

HSPCs were exposed with 2 Gy X-irradiation and were then assayed for their surviving fraction.

The distributions of each value containing CFC and each progenitor cell is shown in Figure 5. The surviving fraction of CFCs ranged from approximately 0.1 to 0.4. In contrast, the value of CFU-Mix was widely distributed such as 0.1 – 0.8 despite their small distribution in the number of progenitor cells (Figure 3), rendering a significant difference with CFU-Meg. Whereas, the distribution of CFU-Meg value is small raging from approximately 0.05 to 0.3 despite their large distribution in the number of progenitor cells, it showed a significant difference with BFU-E.

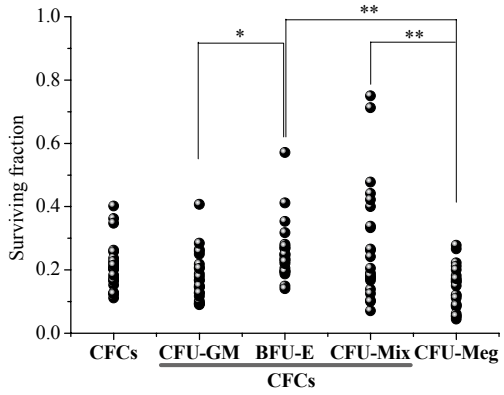


Figure 5. The distribution of surviving fraction of each hematopoietic progenitor cell (n=20). $CD34^+$ cells exposed to 2 Gy X-irradiation was assayed for the number of CFU-GM, BFU-E and CFU-Mix using a methylcellulose culture. The number of CFU-Meg was assessed using a plasma clot culture technique. * $P < 0.05$. ** $P < 0.01$.

A correlation coefficient was estimated to assess any correlations between the cell surface antigens of HSPCs with each surviving fraction. As shown in Figure 6, no significant relation was observed in almost all of the combinations; however, a statistically significant correlation was found only between the rate of Tie-2^+ cells and the surviving fraction on CFCs ($r=0.591$, $p<0.01$), suggesting that the individual radio-sensitivity of CFC is predictable with respect to theTie-2 rate of HSPCs to some extent. At this time, the gating for the analysis of Tie-2 rate changed to the rate of Tie-2^+ cells in the $CD34^+$-enriched population consistent with the study of Yuasa and co-workers and as shown in Figure 1-E (14). As a result, a significant correlation disappeared (data not shown), thus suggesting that these cells do not strongly express CD34 antigen.

In order to assess the correlation between the individual differences of each progenitor number with the surviving fraction of each progenitor in more detail, a correlation coefficient was estimated (Figure 7). The number of progenitor cells correlated strongly with its surviving fraction, suggesting that the individual radio-sensitivity of hematopoietic progenitor cells depends on its number.

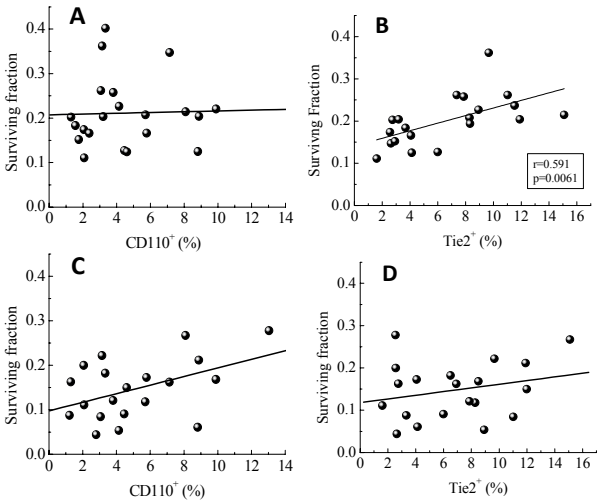


Figure 6. The relationship between the cell surface antigens and the surviving fraction of each hematopoietic progenitor cell (n=20). [**A**] the expression of CD110 and the CFC surviving fraction, [**B**] the expression of Tie-2 and the CFC surviving fraction, [**C**] the expression of CD110 and the CFU-Meg surviving fraction, [**D**] the expression of Tie-2 and the CFU-Meg surviving fraction.

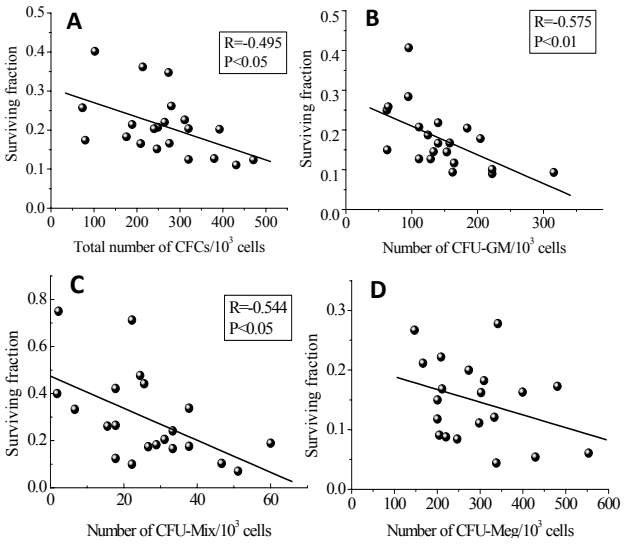


Figure 7. The relationship between the number of hematopoietic progenitor cells and the surviving fraction of each hematopoietic progenitor cell (n=20). [**A**] the CFCs numbers contained in 1×10^3 cells and the CFCs surviving fractions, [**B**] the CFU-GM numbers contained in 1×10^3 cells and the CFU-GM surviving fractions, [**C**] the CFU-Mix numbers and the CFU-Mix surviving fractions, [**D**] the CFU-Meg numbers and the CFU-Meg surviving fractions.

Effects of Ang-1 on the clonogenic potential and radio-sensitivity of HSPCs

Tie-2 is known to be a receptor that acts in early hematopoiesis and its ligand is Ang-1 (15-18). In order to show whether Tie-2-activation protects X-irradiated HSPCs, the effect of Ang-1 on X-irradiated cells was examined. As shown in Figure 8, the addition of Ang-1 to the non-irradiated or X-irradiated cells did not affect the clonogenic potential of HSPCs. i.e., there was no significant difference observed.

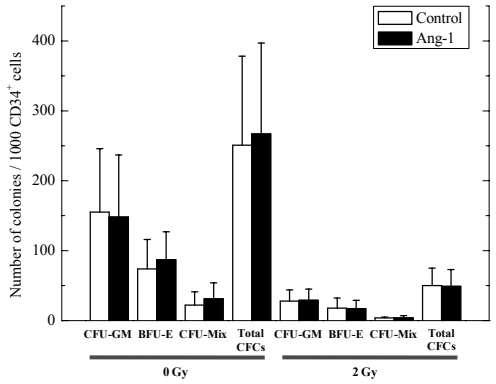


Figure 8. The effect of Ang-1 on non-irradiated and X-irradiated hematopoietic stem/progenitor cells. Progenitor cells were assayed using a methylcellulose culture described in Materials and Methods. The treatment with Ang-1 (200 ng/ml) carried out after X irradiation at 0 or 2 Gy. The values are the means + SD of nine separate experiments. There were no significant differences observed in any of the data.

Discussion

In the present study, we focused on the expression of early hematopoiesis-related antigens, CD38, CD45RA, CD110 and Tie-2 antigens including CD34 in HSPCs based on previous studies (15-21). CD38 is a novel multifunctional ectoenzyme widely expressed in cells and tissues, most notably in leukocytes (19). In particular, it is well known that $CD34^+CD38^-$ cells are more primitive cells than $CD34^+CD38^+$ cells. CD45RA antigen, a member of the CD45 antigen family, is expressed in all cells of hematopoietic origin except for erythrocytes (20). Furthermore, CD110 is the receptor for thrombopoietin (TpoR) and is expressed on HSPCs and on the cells of the megakaryocytic lineage and platelets (21). Therefore, it was reported that TpoR is expressed on bipotent and unipotent progenitors of the erythroid/megakaryocyte pathway and is an important tool for isolation of the myeloid progenitors (6). In addition, Tie-2^+ HSPCs are known to be more primitive cells (7, 14, 22). In the present study, the results showed that a significant positive correlation was observed between CD38 and CD45RA, CD110 and Tie-2, respectively (Figure 2), thus indicating that the former populations are mature cells. In contrast, the latter populations are primitive cells as determined from previous studies.

With respect to the relationship among the cell surface antigens, the number of progenitor cells and their radio-sensitivities, the surviving fractions of CFU-GM and CFU-Meg were lower than that of the CFU-Mix (Figure. 5) despite the fact that its concentration in 1×10^3 cells were significantly higher than the other progenitors (Figure 3). These results are consistent with our previous reports that CFU-Meg is a higher radio-sensitive progenitor cells than the other myeloid progenitor cells (23). In addition, significant negative correlations were observed between the number of progenitor cells and the surviving fractions (Figure 7), thus suggesting that the radio-sensitivity of individuals having many HSPCs is higher than that of individuals having few HSPCs. In order to investigate whether these phenomenon depend on the rate of immature cells that co-exist in HSPCs, the relationship between the $CD34^+CD38^-$ populations and the surviving fraction of each progenitor cell was examined. Although the clonogenic progenitor cells content of the $CD34^+CD38^-$ fraction was lower than that of $CD34^+CD38^+$ fraction (24), no significant correlation was observed with the surviving fractions (data not shown). In contrast, the present results suggest that the individual radio-sensitivity of CFC is predictable with respect to the Tie-2 rate of HSPCs to some extent. Tyrosine receptors such as Tie-2 mediate various signals from extra-cellular

to intra-cellular through the interaction between a receptor and its ligand (15-18). Ang-1 induces angiogenesis, cell migration and cell survival due to Tie-2-activation (7, 15-18, 25-27). A soluble, stable and potent Ang-1 variant protected against radiation-induced apoptosis in microcapillary endothelial cells and prolonged survival in irradiated mice (27). Therefore, we hypothesized that Tie-2-expressed HSPCs also acquired the radio-resistant ability by the treatment with Ang-1. However, the Tie-2/Ang-1 interaction showed no activity on proliferation and radiation survival (Figure 7). These data showed that Tie-2-expressed HSPCs have other specific radio-protective molecules. Woodward et al. demonstrated that Wnt/β-catenin mediates radiation resistance of mouse mammary progenitor cells (28). Wnt-signal is important for maintaining hematopoietic stem cells (29). Cancer stem cells have been reported to be radio-resistant because they maintain their quiescent status, such as the cell cycle of G_0, and also have surviving signals such as Wnt/β-catenin (28, 30). Taken together, some molecules including Wnt/β-catenin expressed on Tie-2^+ cells may be involved in the individual radio-sensitivity of HSPCs.

In conclusion, the present results suggest that the individual radio-sensitivity of HSPCs is predictable with respect to the number of progenitor cells to some extent. It is dependent on the presence of immature HSPCs such as Tie-2^+ cells. These findings may be a predictor not only of the radio-sensitivity of hematopoiesis in normal tissues but also of the effects of radiation therapy and DNA damage-inducing chemotherapy on malignant patients. Additional studies to resolve the above issues are presently being conducted in our laboratory.

References

[1] Simmons PJ and Torok-Storb B. CD34 expression by stromal precursors in normal human adult bone marrow. *Blood* 78: 2848-2853 (1991).

[2] Simmons PJ and Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO 1. *Blood* 78: 55-62 (1991).

[3] Torok-Storb B, Cellular interactions. *Blood* 72: 373-385 (1988).

[4] Majka M, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzkowski Z, Kowalska MA, Gewirtz AM, Emerson S. G and Ratajczak M Z. Numerous growth factors, cytokines, and chemokines are secreted by human $CD34^+$ cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. *Blood* 97: 3075-3085 (2001).

[5] Manz MG, Miyamoto T, Akashi K and Weissman IL. Prospective isolation of human clonogenic common myeloid progenitors. *Proc. Natl. Acad. Sci. USA* 99: 11872-11877 (2002).

[6] Edvardsson L, Dykes J and Olofsson Tor. Isolation and characterization of human myeloid progenitor populations TpoR as discriminator between common myeloid and megakaryocyte/erythroid progenitors. *Exp. Hematol*. 34: 599-609 (2006).

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

[8] Schnarr K, Dayes I, Sathya J and Boreham D. Individual radiosensitivity and its relevance to health physics. *Dose-Respose* 5: 333-348 (2007).

[9] Azria DA, Rosenstein BS and OZsahin M. Radiation-induced sede effects with or without systemic therapies: prime time for prediction of individual radiosensitivity. *Int. J. Radiat. Oncol. Biol. Phys*. 71: 1293-1294 (2008).

[10] Gabelova A, Farkasova T, Gurska S, Machackova Z, Lukacko P and Witkovsky V. Radiosensitivity of peripheral blood lymphocytes from healthy donors and cervical cancer patients; the correspondence of in vitro data with the clinical outcome. *Neoplasma* 55: 182-191 (2008).

[11] Oriya A, Tahakashi K, Inanami O, Miura T, Abe Y, Kuwabara M and Kashiwakura I. Individual differences in the radiosensitivity of hematopoietic progenitor cells detected in steady-state human peripheral blood. *J. Radiat. Res*. 49: 113-121 (2008).

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

[13] Hagiwara T, Kodama I, Horie K, Kato T and Miyazaki H. Proliferative properties of human umbilical cord blood megakaryocyte progenitor cells to human thrombopoietin. *Exp. Hematol*. 26: 228 235 (1998).
[14] Yuasa H, Takakura N, Shimomura T, Suenobu S, Yamada T, Nagayama H, Oike Y and Suda T. Analysis of human TIE2 function on hematopoietic stem cells in umbilical cord blood. *Biochem. Biophys. Res. Commun*. 298: 731-737 (2002).
[15] Dumont DJ, Gradwohl G, Fong GH, Puri MC, Gertsenstein M, Auerbach A and Breitman M L. Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo. *Genes Dev*. 8: 1897-1909 (1994).
[16] Puri MC, Partanen J, Rossant J and Bernstein A. Interaction of the TEK and TIE receptor tyrosine kinases during cardiovascular development. *Development* 126: 4569-4580 (1999).
[17] Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ and Holash J. Vascular-specific growth factors and blood vessel formation. *Nature* 407: 242-248 (2000).
[18] Jones N, Iljin K, Dumont DJ and Alitalo K. Tie receptors: new modulatiors of angiogenic and lymphangiogenic responses. *Nat. Rev. Mol. Cell Biol*. 2: 257-267 (2001).
[19] Petzer AL, Hogge DE, Landsdorp PM, Reid DS, Eaves CJ. Self-renewal of primitive human hematopoietic cells (long-term-culture-initiating cells) in vitro and their expansion in defined medium. *Proc. Natl. Acad. Sci. USA* 93: 1470-1474 (1996).
[20] Gratama JW, Sutherland DR, Keeney M, Papa S. Flow cytometric enumeration and immunophenotyping of hematopoietic stem and progenitor cells. *J. Biol. Regul. Homeost. Agents.* 15: 14-22 (2001).
[21] Borge OJ, Ramsfjell V, Cui L, Jacobsen SE. Ability of early acting cytokines to directly promote survival and suppress apoptosis of human primitive $CD34^{+}CD38^{-}$ bone marrow cells with multilineage potential at the single-cell level: key role of thrombopoietin. *Blood* 90: 2282-2292 (1997).
[22] Arai F and Suda T. Maintenance of quiescent hematopoietic stem cells in the osteoblastic niche. *Ann. N.Y. Acad. Sci.* 1106: 41-53 (2007).
[23] Kashiwakura I, Kuwabara M, Inanami O, Murakami M, Hayase Y, Takahashi T.A, Takagi Y. Radiation sensitivity of megakaryocyte colony forming cells in human placental and umbilical cord blood. *Radiat. Res*. 153: 144-152 (2000).
[24] Issaad C, Croisille L, Katz A, Vainchenker W, Coulombel L. A murine stromal cell line allows the proliferation of very primitive human $CD34^{++}/CD38^{-}$ progenitor cells in long-term cultures and semisolid assays. *Blood* 81: 2916-2924 (1993).
[25] Martin V, Liu D, Fueyo J and Gomez-Manzano C. Tie2: a journey from normal angiogenesis to cancer and beyond. *Histol. Histopathol*. 23: 773-780 (2008).
[26] Papapetropoulos A, Fulton D, Mahboubi K, Kalb RG, O'Connor DS, Li F, Altieri DC and Sessa WC. Angiopoietin-1 inhibits endothelial cell apoptosis via the Akt/survivin pathway. *J. Biol. Chem*. 275: 9102-9105 (2000).
[27] Cho C-H, Kammerer RA, Lee HJ, Yasunaga K, Kim K-T, Choi H-H, Kim W, Kim SH, Park SK, Lee GM and Koh GY. Designed angiopoietin-1 variant, COMP-Ang1, protects against radiation-induced endothelial cell apoptosis. *Proc. Natl. Acad. Sci. USA* 101: 5553-5558 (2004).
[28] Woodward WA, Chen MS, Behbod F, Alfaro MP, Buchholz TA and Rosen JM. WNT/b-catenin mediates radiation resistance of mouse mammary progenitor cells. *Proc. Natl. Acad. Sci. USA* 104: 618-623 (2007).
[29] Nemeth MJ and Bodine DM. Regulation of hematopoiesis and the hematopoietic stem cell niche by Wnt signaling pathways. *Cell Res*. 17: 746-758 (2007).
[30] Baumann M, Krause M and Hill R. Exploring the role of cancer stem cells in radioresistance. *Nat. Rev. Cancer* 8:545-554 (2008).

Acknowledgments

This work was supported by KAKENHI, Grant-in-Aid for Young Scientists (B) (No. 20790874 KT), Grant-in-Aid for Scientific Research (B) (No. 18690327 IK) and a Grant for Co-medical Education Program in Radiation Emergency Medicine by the Ministry of Education, Culture, Sports, Science and Technology, Japan (2008). This work was also supported by a Grant for Priority Research on Issues of Great Urgency Designated by the President of Hirosaki University

(2007) and Grant for Hirosaki University Institutional Research (2008). The part of this paper has already been presented in the Radiation Research Volume 173, Number 2, 184-190 (2010). I would like to thank the Society of Radiation Research for permission to quote from copyright source.

Radiation Emergency Medical Preparedness in Japan and a Criticality Accident at Tokai-mura

Makoto Akashi

Research Center for Radiation Emergency Medicine,
National Institute of Radiological Sciences (NIRS)
Address: 4-9-1 Anagawa, Inage-ku, Chiba-shi, Chiba, 263-8555, Japan
e-mail: akashi@nirs.go.jp

Abstract. Although radiation exposure accidents fortunately occur only rarely, potential sources for exposure accidents can be found anywhere. When persons are accidentally exposed to radiation, physicians may be involved in their assessment and care; of course, their early diagnosis and dose assessment are crucial. After a criticality accident in 1999, with three persons heavily exposed to neutrons and also γ-rays, the system of radiation emergency medical preparedness has been further strengthened for nuclear facilities such as power plants, reprocessing facilities and research reactors in Japan. In the revised system, hospitals involved in this system were classified into three levels, depending on their locations and capabilities. When there is whole-body exposure to radiation, however, there is damage to not only a single organ but also, naturally, to multiple organs. Therefore, treatment by experts in various medical fields is required. The National Institute of Radiological Sciences (NIRS) has constructed collaborative systems with extramural specialists: the Medical Network Council for Radiation Emergency, the Network Councils for Chromosome Analysis for dose assessment, and the Network Councils for Physical Dosimetry. In this article, the Japanese system for radiation emergency medical preparedness and also lessons learned from the Tokai-mura accident will be introduced.

Keywords: radiation emergency, medical preparedness, criticality accident

Introduction

Radiation cannot be seen by the human eye, smelled, heard, or otherwise detected by our normal senses, nor do symptoms/signs appear soon after radiation exposure. Moreover, these symptoms and signs are not specific for radiation exposure. Therefore, it is not easy for us to realize that a radiation accident has occurred. Thus, radiation exposure leads to a highly emotional problem and causes widespread public concern, and the psychological aspects of radiation accidents also require attention, besides which, we are also exposed to natural radiation in daily life. As compared to B and C among nuclear, radiological, biological and chemical (NRBC) materials, R cannot be neutralized or stabilized chemically, and vaccine or antibody against R cannot be produced. In contrast, radiation can be relatively easily detected or measured, usually by devises, compared to B and C; we see radiation monitoring stations showing the present radiation levels around nuclear facilities.

A radiation accident is defined as an unintentional exposure to ionizing radiation or contamination with radionuclides, resulting in

possible deleterious effects for the exposed and/or contaminated individuals [1]. Since the discovery of X-rays in 1885 by Roentgen and of radioactivity by Becquerel in 1886, society has been exposed to radiation accidents. As early as 1886, Becquerel observed erythema on his abdomen and ascribed it to radioactive materials. Today, on the other hand, devices and locations whereby an individual could be exposed to radioactive materials are anything but rare. These potential sources of exposure accidents include industrial radiography, therapeutic devices, sterilizers, transportation accidents, and nuclear power plants; devices used for industrial radiography and accelerators are frequent sources of external exposure accidents.

Radiation accidents especially requiring treatment are rare. Medical response to a radiological emergency means providing first-aid treatment as well as taking appropriate actions to protect yourself and others from radiation. However, there are few medical professionals with any experience with radiation accidents, for the simple reason that they are not common. Nonetheless, it is on the basis of past experiences that medical care by medical professionals and first response by ambulance and police staff can be carried out. In order to respond to radiation accidents, knowledge on radiation and lessons learned from past accidents are essential. In this article, I would like to introduce our system for radiation emergency medical preparedness in Japan, which has been intensively revised after the criticality accident in 1999 [2]. Moreover, the medical response and the response to the public in this criticality accident will be described.

Medical Response System for Radiation Emergency in Japan

After the criticality accident, the system of radiation emergency medical preparedness was further strengthened for nuclear facilities such as power plants, reprocessing facilities and research reactors in Japan [3]. In the present system, treatment for radiation exposure is performed at 3 levels: primary level in hospitals near nuclear facilities; secondary level in local general hospitals; tertiary level by more equipped and advanced hospitals. Hospitals at the primary level provide first-aid treatment, primary assessment of contamination with radionuclides, and removal of contamination on the body surface. They may also administer stable iodine if its contamination in patients is predicted or suspected. Therefore, these hospitals have to be equipped with radiation detectors such as survey meters, the minimum requirement for decontamination, and stable iodine tablets. Secondary-level hospitals provide medical and radiological triage, decontamination, and treatment of local radiation injuries and whole body exposure, and also start treatment for internal contamination. Thus, most medical facilities at the secondary level are general hospitals that are equipped with inverse-isolation rooms and a decontamination facility with a water drainage/storage tank, and a whole-body counter. Tertiary hospitals are expected to receive patients heavily exposed to radiation and/or contaminated and to provide diagnostic and prognostic assessments of radiation-induced injuries and biological and radiological dose estimation. Thus, these hospitals with high-level dose-assessment function including biological and physical dosimetry, radiation measurement, and capability of stem cell transplantation, intensive care, and plastic surgery for burn-treatment have been designated as tertiary hospitals. In Japan, 51 and 32 hospitals have been designated as primary and secondary facilities, respectively, by the local governments where nuclear facilities are located. In addition, NIRS and Hiroshima University Hospital have been designated as tertiary hospitals in the east and west blocks of Japan, respectively, by the national government.

Role of NIRS as a National Center for Radiation Emergency

As well as being a tertiary hospital, NIRS has been also designated as the national center of radiation emergency medical preparedness in Japan, and it provides direct or consultative services to local governments and hospitals as well as to Hiroshima University in case an actual radiation incident occurs, facilitates the establishment of a network of available equipment and staff specializing in radiation exposure, contamination, dose assessment, and radiation protection, and assists in the preparation of relevant documents and guidelines. Whole-body exposure to radiation causes damage to not only a single organ but, naturally, also to multiple organs [4]. This means that treatment by experts in various medical fields will be required. Moreover, it is difficult for a

hospital to treat two or more victims heavily exposed at the same time. NIRS has constructed a collaborative system with extramural specialists. This is the medical network council for radiation emergency established in July of 1999. This council consists of experts from more than ten general hospitals and institutes, who will cooperate to conduct treatment, especially treatment for radiation burns, severe bone marrow failure, and gastrointestinal injuries. In the criticality accident at Tokai-mura, treatment of three victims was carried out at NIRS and hospitals of this network. We have also established the Network Councils for Chromosome Analysis for dose assessment. The Network Councils for Physical Dosimetry has been built to provide quick and precise dose estimation by radiation measurement and/or re-construction of the accident.

JCO Criticality Accident

1. Outline of the Accident

Tokai-mura is located about 130 km northeast of Tokyo and faces the Pacific Ocean (Fig. 1). This "Nuclear Village" has a population of 34,000, and contains many nuclear facilities including the Japan Atomic Energy Agency (JAEA). Thus, Tokai-mura is a place of advanced nuclear science research known to the world. On 30 September 1999, at 10:35, a criticality accident occurred at the uranium conversion facility in Tokai-mura, Ibaraki prefecture, Japan. The criticality event occurred at a nuclear fuel company named as JCO when a worker was pouring a solution of enriched (^{235}U) into a precipitation tank directly. He bypassed a dissolution tank and buffer column supposed to be used in order to avoid criticality; the amount of uranium was several times more than the specified mass limit. At the accident, three workers were severely exposed to γ-rays and neutron irradiation. The estimated doses were 24.5 GyEq, 8.3 GyEq, and 3.0 GyEq. Despite all medical efforts including stem cell transplantation, two workers died 83 and 211 days after the accident, respectively. A number of workers and members of the public also received low doses of radiation. Around 150 people who lived or stayed within about 350 m of the facility were evacuated, and 310,000 people within a 10-km radius were advised to stay indoors for 18 hours.

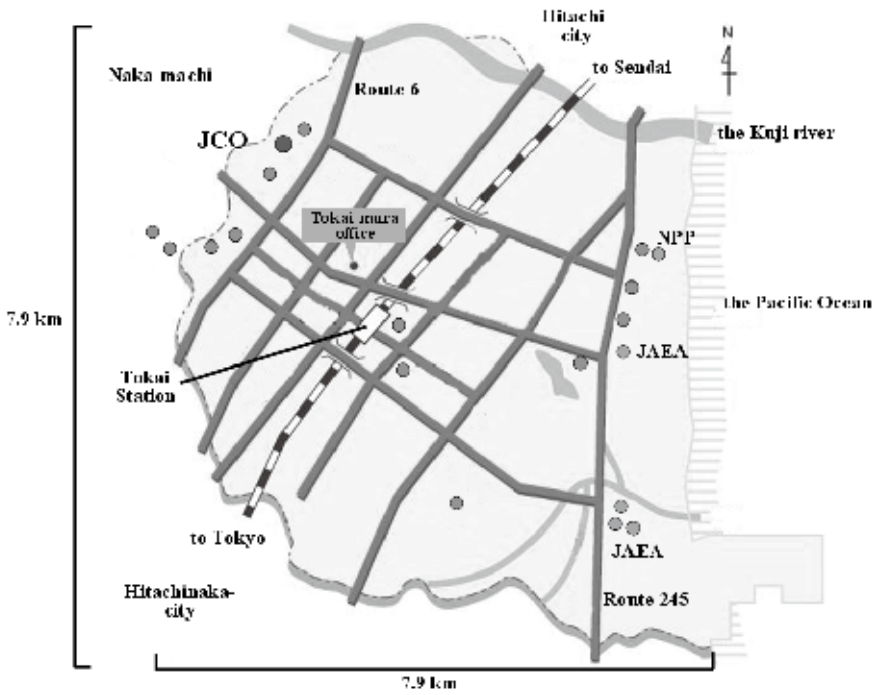


Figure 1. Nuclear facilities at Tokai-mura*
*Adapted and modified from HP of Tokaimura at http://www.vill.tokai.ibaraki.jp/as-tokai/01jigyosyo/jigyosyoichi.htm
○: nuclear facilities, NPP : nuclear power plants, JAEA: the Japanese Atomic Energy Agency

2. Dose Assessment

After the exposure, the workers were transferred to the National Mito Hospital to receive first aid treatment, and then about 5 hours after the accident were transferred to NIRS. A dose estimation team was established at NIRS, and they made efforts to measure a lot of specimens: blood, vomitus, clothes and so on [5]. Average whole-body doses were estimated from prodromal symptoms, lymphocyte counts, chromosome analysis, and measurement of specific activity of ^{24}Na in blood samples.

Table 1. Estimated doses

Methods	Workers		
	A	B	C
[1)]Preliminarily estimated doses for making treatment decisions	16-20 or more	6-10	1~4.5
Prodromal symptoms	over 8	4~6 or over 6	less than 4
Blood cell counts (mainly lymphocyte counts)	16~23	6~8	1~5
Chromosome analysis	16~ over 20	6.9~10	2.8~3.2
Specific activity of Na-24 in the blood (neutron and γ-ray: Gy)	(5.4, 9.9)	(2.9, 4.1)	(0.81, 1.5)
Total dose (assuming RBE=1.7)	19	9.0	2.9
Whole-body counter (neutron and γ-ray: Gy)	-	-	(0.62, 1.1)
[2)]Finally estimated doses	16~25	6~9	2~3

[1)] "Preliminarily estimated doses" were urgently evaluated within 7 days after admission of the three workers to NIRS in order to predict their prognosis and to make decisions for treatment strategies. The values were primarily determined by measuring the activity concentration of ^{24}Na in the blood and by using Sarov's conversion coefficient [6].

[2)] Estimated doses of all results of dose reconstruction analyses [7].

3. Treatment of Heavily Exposed Patients

3.1. Worker A

Worker A developed nausea, vomiting and a transient 20-30 second loss of consciousness, and diarrhea within 1 h. On admission to NIRS, he was febrile without any evidence of infection, slightly drowsy and systolic blood pressure of 70 mmHg. He also had diffuse erythema on his body surface, facial edema, injection of the conjunctiva bulbi, and painful bilateral parotid swelling. He complained of diffuse tenderness of the abdominal wall by palpation, and difficulty in voiding. These findings indicated that he was exposed to high-dose radiation, comparable with the victims of prior accidents with fatal outcome. Granulocyte colony-stimulating factor (G-CSF) was administered intravenously on the evening of day 1. Shortly after its administration, the patient complained of mild dyspnea and systemic rash. The symptoms resolved after inhaled oxygen concentration was increased to 50%. The patient's facial edema slightly improved on day 2. However, he complained of painful right forearm swelling on day 2, which subsequently became more severe. Although the patient continued to have watery diarrhea and complained of diffuse abdominal tenderness, he was apparently well on days 1 and 2, suggesting that he was at the latent phase of acute radiation syndrome. His white blood cells (WBC) increased until day 3, then rapidly decreased and almost reached zero by day 7. Lymphocytes disappeared on day 3. The number of platelets also decreased steeply, necessitating

platelet transfusion starting from day 5. Hemoglobin concentration was rather elevated initially, possibly reflecting concentration of blood, but then rather decreased by day 7. From the preliminary dose estimation based on his symptoms and signs, recovery of bone marrow was considered to be quite unlikely. The patient was transferred to the University of Tokyo Hospital on day 3 [8]. On days 7 and 8, peripheral blood stem cells (PBSCT) were transplanted from a family member with identical HLA. On day 17, marrow biopsy showed that the transplanted cells had been engrafted. However, he needed transfusions of over 4,000 ml per day. Hypoxemia attributable to pulmonary edema advanced, and on day 10, endotrachea1 intubation and artificial ventilation were introduced. For the first 3 weeks, the major problems were bone marrow suppression and respiratory complications. After 3 weeks, progressive and generalized skin loss and gastrointestinal injuries manifested themselves, causing massive body fluid and blood loss. Infection prophylaxis was carried out. Additionally, hematopoietic growth factors such as G-CSF, erythropoietin (EPO), and thrombopoietin (TPO) and blood components were administered as needed. Respiratory failure caused by pulmonary edema progressed. Pentoxifylline and vitamin E were administered intravenously throughout the course to prevent radiation lung injury. The skin of highly irradiated areas, such as right forearm and anterior chest, was swollen and started to form blisters within 2 weeks. Skin damage was serious overall the body. Body fluid lost from skin ranged from 2000 to 4500 mL/day. Repeated upper and 1ower gastrointestinal tract endoscopies demonstrated mucosal integrity loss similar to the skin loss. Despite the repeated use of cultured allogenic skin grafts and pharmacological intervention, skin effusion and gastrointestinal bleeding continued to increase. On day 58, cardiopulmonary arrest caused by hypoxia occurred, from which the patient was resuscitated. Following cardiac arrest, his course became quite turbulent. The patient died of multiple organ failure on day 83. The major autopsy findings included marked atrophy and degeneration of striated muscles of the extremities and torso, marked hypoplasia of bone marrow with predominant immature cells, loss of intestinal, esophageal, and tracheal epithelium, and lung edema.

3.2. Worker B

Worker B also experienced nausea and vomiting within 1 h of exposure, but had no early diarrhea. Although his blood pressure was normal on the day of the accident, it was rather low for the next few days. The patient was slightly drowsy, febrile, had erythema on his body surface and salivary gland swelling, and complained of epigastralgia on admission, indicating that he would also undergo a severe form of acute radiation syndrome (ARS), although to a lesser magnitude than Worker A. G-CSF was started on day 1. The number of leucocytes slightly increased on day 2, almost plateaued on day 3 and then rapidly decreased, reaching almost zero by day 7. Lymphocytes also rapidly decreased; they numbered zero on day 7. His platelet number and hemoglobin concentration decreased rather gradually. Because he was expected to exhibit severe skin and gastrointestinal injuries soon, the medical staffs reached the conclusion that it would be more beneficial than detrimental to support his leucopenic period with hematopoietic stem cell transplantation. He was transferred to the Institute of Medical Science, University of Tokyo, on day 5 to receive umbilical cord blood transplantation. The graft initially took, and then was gradually replaced by his own hematopoietic cells. The patient also had edema of the right forearm in the first several days, and later evolved severe skin lesions involving a large part of his body surface, in particular the face and extremities. His skin injury was treated extensively with grafts. The staff of Kyorin University Hospital provided radiation burn treatment and intensive care throughout the duration of the treatments. Radiation burns slowly worsened during the subsequent two months, causing exfoliation of almost 70% of the skin by day 70, which corresponded to a class II burn. Skin graft was performed on the lesions that were thought to be unlikely to cure by themselves: forearms and lower legs. Allograft was performed on the forearm lesions of a class IId burn (around 15%). On day 88, moreover, his own autograft, which had been cultured and provided by Tokai University Hospital, was taken on the lower leg sections of a class IId burn (20%). Both the allografts and autografts engrafted successfully (over 90%), and notably improved his general status. On day 120, autograft was successfully transplanted on the face, which almost entirely covered the wound in one month. However, his

skin developed progressive fibrosis, beginning about 3 months after exposure; strong fibrosis and sclerosis appeared throughout the body during the subsequent sub-acute period.

He also devolved gastrointestinal bleeding and infectious complications. The patient received cord-blood stem cell transplantation. On day 7, the number of peripheral lymphocytes reached zero, and cord-blood stem cells were transplanted into the patient on day 10. Cytokines were also applied, such as G-CSF, GM-CSF (granulocyte and macrophage colony stimulating factor), TPO, and EPO. The transplanted stem cells were engrafted, but the residual marrow of the patient was still functioning. Cyclosporin-A and methylprednisolone were used for GVHD prophylaxis. His own bone marrow eventually recovered about 2 months later. During this period, there existed stable mixed chimerism between donor cells and recipient cells. Despite recovery of his bone marrow function, T-cell subset abnormality was observed; there were increased numbers of naive T cells and helper T-cell subtype 1, but mitogenic responses of T cells and the allogeneic mixed leukocyte reaction were severely suppressed [9, 10]. Moreover, endogenous immunoglobulin production remained low until 120 days after the accident. Thus, he was immunologically deficient and needed a sterile environment. The patient suffered pneumonia by methicillin-resistant staphylococcus aureus (MRSA), causing respiratory insufficiency and leading to acute respiratory distress syndrome (ARDS). Secondary to radiation-induced oropharyngeal mucosal damage, he developed obstructive sleep apnea syndrome. His infectious complications included cytomegalovirus (CMV) infection. Bleeding from the GI tract also started on day 145 and did not stop until his death. He eventually developed refractory respiratory fai1ure and died of multi-organ failure on day 2l1. The autopsy findings showed marked generation of collagen fibers of the dermis, marked atrophy of striated muscles of extremities and torso, hypocellular bone marrow, segmental distribution of multiple erosions of the gastrointestina1 tract, and various presentations of organized pneumonia with bleeding and neutrophil infiltration.

3. 3 Worker C

When the accident occurred, Worker C was sitting in the corridor behind a thin wall screening the precipitation tank [11]. After Workers A and B were evacuated, Worker C remained at the site for approximately five minutes trying to make emergency calls, and he looked into the precipitation room several times. Since he was walking around, he was likely to have been relatively uniformly exposed. When he arrived at NIRS, he had no prodromal symptoms except a little nausea while he was in the helicopter. Dose estimation at NIRS based on initial symptoms and signs of Worker C suggested that his bone marrow might be able to recover. Therefore, he remained at the NIRS hospital and was treated without hematopoietic stem cell transplantation. Bone marrow aspirates from the sternum and iliac crest on day 1 showed decreased erythroid series and well preserved myeloid series. The patient's leucocyte count was normal on day 1, then increased in response to G-CSF, which was started in the evening of day 2. Numbers of neutrophils then started to decrease, reaching a nadir on day 20. The patient was kept under reverse isolation during his neutropenia. Following recovery of the neutrophil count, G-CSF was reduced and eventually discontinued on day 28. The decrease in platelet numbers was slower than in the other two patients, but still necessitated platelet transfusion on days 17, 20 and 23. The number of lymphocytes was lowest on day 2 and then also made a slow recovery. The concentration of hemoglobin slowly decreased without any evidence of bleeding. He did not show any complications such as serious infection. During admission he showed spotty epilation as well as marked diminution of beard growth. In addition, he had localized painless defect of oral mucosa without awareness, which was pointed out on day 19. These symptoms were presumably caused by irradiation, and they improved gradually. He left the hospital on day 82. Cooperation of an ophthalmologist, dermatologist, and circulatory system and diabetes specialists from Chiba University, and a dentist from Tokyo Dental University was provided. The patient received periodic counseling by a psychiatrist from Chiba University.

4. Dose Assessment for Residents

The criticality accident affected the residents around the JCO facility. The Health Management Inspection Committee of the Nuclear Safety Commission (NSC) recommended that dose estimation for the area be performed at the

evacuated zone of 350 m around the JCO facility. The concerned people were the residents in the evacuated zone and employees whose companies were located in this area. Persons who stayed in the evacuated zone temporarily during the criticality period were also included. Sodium-24 activity was measured for only seven of them by whole-body counter. Dose estimation of the residents was carried out based on (1) interview of residents who stayed in the zone during the criticality period, (2) spatial distributions of the dose rates of neutrons and γ-rays evaluated from radiation monitoring data, and (3) calculation of shielding effects of buildings. A personal behavior survey was performed jointly by the Science and Technology Agency (STA), NIRS, Ibaraki prefecture, Tokai-mura, and Naka-machi. The data of the dose-rate distribution in the environment and the shielding effect of buildings were analyzed by the Japan Atomic Energy Research Institute (JAERI) and were then used for the estimation [12,13, 14, 15, 16].

Table 2. Radiation Doses of JCO Employees, Residents, and Rescue Personnel for Nuclear Emergency

Dose (mSv)	JCO Employees	Rescue Personnel	Residents	Total
0≦, 5＜	123	253	207	583
(1≦)	(82)	(51)	(103)	(236)
5≦, 10＜	15	7	18	40
10≦, 15＜	6	0	6	12
15≦, 20＜	10	0	2	12
20≦, 25＜	8	0	1	9
25≦, 30＜	1	0	0	1
30≦, 35＜	2	0	0	2
35≦, 40＜	0	0	0	0
40≦, 45＜	1	0	0	1
45≦, 50＜	3	0	0	3
Total	169	260	234	663
(1≦)	(128)	(58)	(130)	(316)

5. Dose Assessment for JCO Employees and Persons Involved in Emergency Response

In total, 169 employees of JCO and its related companies were working at the site, besides the three heavily exposed workers. Among them, 18 persons were engaged in drainage work of cooling water from the precipitation tank, and 6 persons were involved in the work of pouring boric acid into the tank. Personnel from the national and local governments and related nuclear organizations were engaged in disaster prevention-related work, such as radiation monitoring, construction of a radiation shield, and consulting about countermeasures. They totaled 234 people, including three members of the emergency service staff involved in rescue work for the three heavily exposed workers. Some media organizations dispatched 26 employees to the area in question. Dose evaluation to these people was performed based on whole-body measurement of ^{24}Na activity and area monitoring [12].

6. Evacuation and Establishment of Fist-aid Care Centers

This accident affected not only JCO employees, but also residents of the surrounding area. A low level of radiation was released from the nuclear facility, resulting in members of the public being exposed. According to the NSC Health Management Inspection Committee Report, the effects of this radiation were as follows [17]:

1) The radiation level was not high enough to cause any noticeable radiation effect on health.

2) Possibility of radiation effects on health was extremely low, and no effects were detectable. Nevertheless, residents were concerned, since they believed that they had been “unnecessarily” exposed to radiation.

Ibaraki prefecture set up 5 first-aid and care centers during 22 days, and conducted radiation survey for 14,236 persons. Tokai-mura, Naka-machi, Hitachinaka city, Hitachi city, Hitachiota city, Kanasagou-machi, and Urizura-machi also performed radiation survey for 62,026 persons. No

radiation other than a background level was detected. A total of 788 persons were involved: 55 medical doctors, 220 public health nurses, 39 nurses, 144 radiological technologists, and 330 others.

The Ibaraki prefectural government had received the 1st report (possibility of a criticality accident but no description of γ-rays or neutrons) of the accident from JCO almost an hour later. Thus, no information on the nature of the accident was provided. Tokai-mura established the headquarters for the nuclear emergency almost an hour and 45 minutes after the accident and asked Ibaraki prefecture what measurements should be undertaken to provide information to the public. Almost 2 hours after the accident, Ibaraki prefecture revealed to the mass media that the possibility of a criticality accident was high. Tokai-mura asked residents to stay at home and listen to the disaster broadcast on the radio. Ibaraki prefecture informed the Hitachinaka and Mito health centers about the accident and asked them to prepare for distribution of stable iodine. Hitachi city also established headquarters for a nuclear emergency. Moreover, the Hitachi city government requested the residents within 350 m of the facility (about 150 persons) to evacuate to the nearby community center based on its own decision 4 1/2 hours later, since no further information of the accident had been provided. Nine and a half hours passed before set-up of a first-aid and care station was decided. In the Hitachinaka health center, a team for radiation survey consisting of the JAERI staff and the health center personnel, a first-aid diagnostic/decontamination team consisting of personnel from the Ibaraki Prefectural Central Hospital, and a relief team from the Japanese Red Cross Society, Ibaraki branch, were organized. Ibaraki prefecture set up the health consultation office in the health service disease control division. The headquarters for nuclear emergency of Ibaraki prefecture asked residents within a 10-km radius of JCO to stay indoors for 12 hours after the accident, and the director of the department of health and social services of Ibaraki prefecture decided to move the first-aid and care station at the Hitachinaka health center to the nursing school at the Mito Red Cross Hospital, since the Hitachinaka health center was located within 10 km of JCO. Ibaraki prefecture asked NHK, a national broadcaster, to announce about the movement of a first-aid and care station 14 hours after the accident.

Thus, the first-aid and care station was set up and opened at the nursing school of the Mito Red Cross Hospital almost 22 hours after the accident occurred, and radiation survey for body-surface contamination, medical examination, and health consultation were started. Since many people visited the station, more radiation measuring devices were needed and radiation technologists were called from the Mito Red Cross Hospital and the Ibaraki Prefectural Central Hospital. Ibaraki prefecture decided to establish other first-aid and care stations at the Hitachi, Mito, and Omiya health centers. Ibaraki prefecture lifted the indoors-sheltering recommendation to the 10-km-radius residents almost 30 hours after the accident. Thus, full-scale activity was started in the first-aid and care stations. The first-aid and care station of the Mito Red Cross Hospital was closed 4 days after the accident, and on the following day the last station was closed.

7. Explanation of Health Effects to Residents in the Accident Area

The mayor of Tokai-mura requested NIRS to send experts to provide a better understanding of radiation effects to the public. Upon this request, NIRS sent two experts to Tokai-mura. Explanations of the health effects on residents were given at the Tokai-mura Culture Center, in Naka-machi, and in Tokai-mura. Types of radiation and their qualities, and the effects of radiation on human health were explained by experts from NIRS. The results of radiation assessment and the actions planned thereafter were also explained. For those who could not attend, lectures were recorded on video. In addition, the outline was published in local bulletins.

7.1 Health Consultation Office and Telephone Consultations

The results of the dose evaluation for local residents were shown to the mass media by the Science and Technology Agency. Tokai-mura set up a health consultation office, and NIRS supported the health consultations. The health consultation office dealt with large numbers of residents at the early stages. However, after the initial rush, the number of residents asking for consultation was less than expected. Most of the consultations were about uneasiness and distrust, but these residents were unable to express their uneasiness in the presence of large audiences. Rather than medical

issues, psychological problems were frequently dealt with. Many journalists also came to the consultation office and took photographs. However, the response to mass media had not been appropriately planned by local governments. Thus, several problems were encountered during the initial stages.

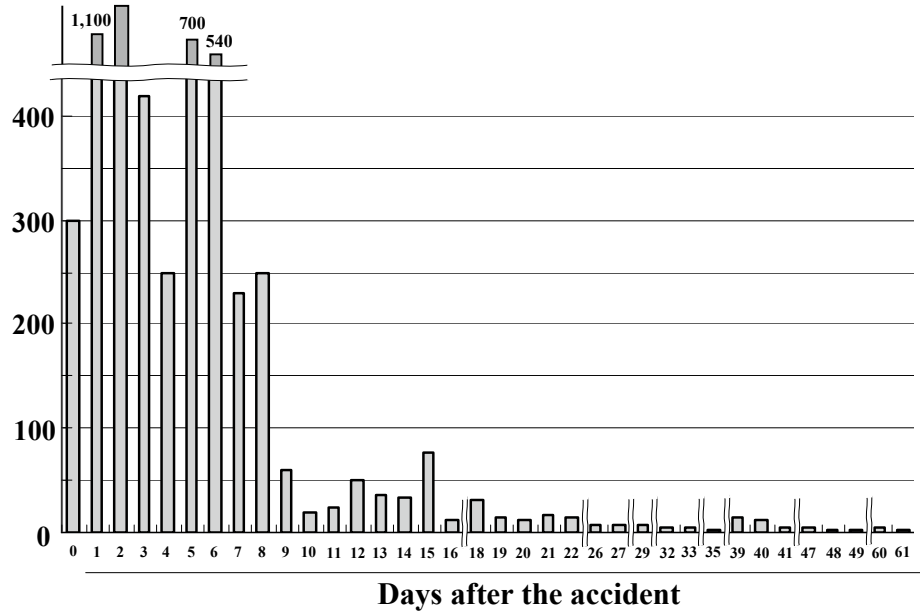


Figure 2. Number of people attending health consultations

Tokai-mura also established a telephone consultation office at the Tokai-mura civic center. STA requested NIRS to send a medical doctor and a researcher with knowledge of the effects of radiation on the human body as consultants.

8. Requests for Measurement of Body-surface Radioactivity

NIRS received requests for measurement of radioactivity on the body surface from 42 people: 11 mass media, 7 transportation workers, 13 construction workers at Tokai-mura, 9 people who passed through the areas surrounding the accident site, and 2 others. None of them were considered to be significantly contaminated. In order to make them feel easy, however, radiation surveys were performed for them. Using survey meters for alpha, beta, and gamma rays, measurements were taken of the individual's body surface and of clothing. Before performing the test, these individuals were interviewed concerning their location at the time of, or after the accident. Of the 42 subjects, none showed higher than background level readings. In addition, 13 of them were checked for internal radioactivity; none showed higher than background levels.

Discussion

Although radiation accidents requiring treatment rarely occur, medical preparedness for such accidents is vital, since radiation is essential to modern life. Accidents of external exposure are different from contamination with radionuclides; exposed patients do not carry radioactive materials after the radioactive materials have been removed. However, there are a lot of misunderstandings concerning radiation exposure, contamination, and effects, as even medical professionals have few opportunities to learn about radiation.

Radiation exposure causes biological or medical effects on human health. Moreover, radiation accidents can trigger psychological and socio-economical effects even when no biological or medical effects are detectable. The current system for the annual exchange and sharing of information on radiation accidents should result in a

smooth medical response. In this respect, NIRS has introduced training courses and meetings for medical professionals in Asia. We believe that efforts should be made toward the establishment of a medical network for radiation emergency in the Asian region.

Acknowledgments

I thank Ms. Aki Yamamoto for her excellent secretarial assistance.

References

[1] Akashi M. International Cooperation in Radiation Emergency Medical Preparedness: Establishement of a Medical Network in Asia. In Nakashima M, Takamura N, Tsukasaki K, Nagayama Y, Yamashita S (eds): Radiation Health Risk Sciences .Proceeding of the First International Symposium of the Nagasaki University Global COE Program "Global Strategic Center for Radiation Health Risk Control":254-259 (2008)

[2] Akashi M. Initial symptoms of Victims in the Tokaimura Criticality Accident. In Ricks RC, Berger ME, O'hara FM(eds): The Medical Basis for Radiation-Accident Preparedness : Proceeding of the Fourth International REAC/TS Conference on the Medical Basis for Radiation-Accident Preparedness March 2001 Orlando, Florida:303-312 (2001)

[3] Park KD, Jang M, Akashi M. Training Programs for radio-nuclear emergency response in Asian region. *Health Phys*. in press

[4] Akashi M. Role of infection and bleeding in multiple organ involvement and failure. *Br J Radiol*. Suppl 27 : 69-74(2005)

[5] Akashi M, Hirama T, Tanosaki S, Kuroiwa N, Nakagawa K, Tuji H, Kato H, Yamada S, Kamata T, Kinugasa T, Ariga H, Maekawa K, Suzuki G,Tujii H. Initial symptoms of acute radiation syndrome in the JCO criticality accident in Tokai-mura. *J Radiat Res* 42 Suppl:S 157-166 (2001)

[6] International Atomic Energy Agency (IAEA), "The Criticality Accident in Sarov", STI/PUB/1106, IAEA, Vienna (2001).

[6] Murata H and Akashi M (Eds). The Report of the Criticality Accident in a Uranium Conversion Test Plant in Tokaimura. *NIRS-M-154* (2002).

[7] Ishii T, Futami S, Nishida M, Suzuki T, Sakamoto T, Suzuki N, Maekawa K. Brief note and evaluation of acute-radiation syndrome and treatment of a Tokai-mura criticality accident patient. *J Radiat Res* 42 Suppl: S167-182(2001)

[8] Nagayama H, Ooi J, Tomonari A, Iseki T, Tojo A, Tani K, Takahashi TA, Yamashita N, Shigetaka A. Severe immune dysfunction after lethal neutron irradiation in a JCO nuclear facility accident victim. *Int J Hematol* 76: 157-164 (2002)

[9] 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)

[10] Hirama T, Tanosaki S, Kandatsu S, Kuroiwa N, Kamada T, Tsuji H, Yamada S, Katoh H, Yamamoto N, Tsujii H, Suzuki G, Akashi M. Initial medical Management of patients severely irradiated in the Tokaimura criticality accident. *Br. J. Radio*l. 76: 246-253 (2003)

[12] Mizuniwa H, Kurihara O, Yoshida T, Isaka K, Tsujimura N, Momose T, Kobayashi H,Hayashi N, Miyabe K, Noda K, Kanamori M, Shinohara K. Dose Evaluation to Workers at JCO Criticality Accident Based on Whole Body Measurement of Sodium-24 Activity and Area Monitoring. *Nippon Genshiryoku Gakkai-Shi*. 43:56-66 (2000). (in Japanese)

[13] The Criticality Accident Investigation Committee of Nuclear Safety Commission. The Report of the Criticality Accident Investigation Committee (1999). (in Japanese)

[14] Endo A, Yamaguchi Y, Sakamoto Y, Yoshizawa M, Tsuda S. External Doses in the Environment from the Tokaimura Criticality Accident. *Radiat. Prot. Dosim*.93:207-214 (2001).

[15] Endo A, Yamaguchi Y, Sumita K. Analysis of Time Evolution of Neutron Intensity Measured with a High-sensitive Neutron Collar during the JCO Criticality Accident. *J. Nucl. Sci. Technol*. 40:628-630 (2003).

[16] Fujimoto K, Yonehara H, Yamaguchi Y, Endo A. Dose Estimation Based on a Behavior Survey of Residents Around the JCP Facility. *J. Radiat. Res*. 42: Suppl. S85-S93 (2001).

[17] Health Management Committee of Nuclear Safety Commission. The Report of the Health Management Committee (2000). (in Japanese)

Overview of NIRS educational programs on radiation emergency medical preparedness

Hideo Tatsuzaki*

Diagnosis Section, Department of Radiation Emergency Medicine,
Research Center for Radiation Emergency Medicine,
National Institute of Radiological Sciences (NIRS),
Chiba, Japan

Abstract. The National Institute of Radiological Sciences (NIRS) has a function of education and training for professionals in the field of radiation emergency medicine. It has training facilities and related equipment. From 2001 to 2009, NIRS has organized 12 international training courses or workshops. About 260 overseas professionals, mainly from Asian countries, have been participated in these meetings. The institute also organized many courses for Japanese professionals from prefectures with nuclear facilities until last year. In this year, we are planning to start new courses with wider perspectives. The new courses are focused on radiation accidents and radiation terrorisms in addition to accidents in nuclear power plants. We plan two types of courses: one for medical staffs (hospital management) and another for first responders (pre-hospital management). Accidents can happen anywhere and a threat of nuclear or radiological terrorism is emerging in the world. Thus, these new educational courses are highly expected.

Key Words: radiation emergency medicine, education, training, first responder, medical staff

Introduction

Radiation accidents are rare events and a few professionals have experience on them. For that reason, education and training for radiation emergency medical preparedness are important. The International Atomic Energy Agency (IAEA) and the World Health Organization (WHO) also stated that "Arrangements should be made for all medical and paramedical staff to be trained in the principles of radiation protection, including the health effects of radiation and the methods for dealing with patients who have been accidentally irradiated or contaminated, with or without complications." [1]

Function of NIRS

The National Institute of Radiological Sciences (NIRS) is an independent administrative institution, which was originally established in 1957 as a national research laboratory. It has been conducting comprehensive scientific research for radiation and health with various fields of specialities. The two scientific fields in its current mid-term plan are radiation-related life science research and radiation safety and emergency medical services for radiation exposure. Radiation emergency medical preparedness is an important part in its mission. Additionally, the institute is designated as a tertiary level referral hospital in the national radiation emergency medical system.

The NIRS has a function of education and training for professionals. It has a designated

* Corresponding to: Hideo Tatsuzaki, Section Head, Diagnosis Section, Department of Radiation Emergency Medicine, Research Center for Radiation Emergency Medicine, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba-shi, Chiba, 263-8555, Japan.
E-mail: tatsuza@nirs.go.jp

training facility (figure 1) and accommodations for participants. The training facility contains two lecture rooms and three exercise rooms located in a radiation controlled area. As a tertiary level referral hospital, NIRS has special facilities and equipment for radiation emergency medicine such as a treatment room for contaminated patient or whole body counters. These facilities and equipment are also used for training purposes. The institute has organized many training courses in the field of radiation emergency medicine and related subjects both for Japanese and foreigners.

Figure 1. Training school building of NIRS.

International training courses

NIRS organized many courses for foreigners, sometimes in cooperation with other organizations, such as the IAEA, the WHO, or the Japanese Nuclear Safety Commission. From 2001 to 2009, NIRS has organized 12 international training courses or workshops as indicated in Table 1. Most of the workshops also have some training components. About 260 overseas professionals, mainly from Asian countries, participated in these meetings. They were mainly medical staffs, such as medical doctors or nurses, health physicists, or administrators who were responsible for national system of radiation emergency medicine. A photography at a typical decontamination exercise is shown in figure 2.

Figure 2. Exercise at international training course at NIRS.

Training courses for Japanese

Until last year, the institute also organized many courses for Japanese professionals from prefectures with nuclear facilities, in order to prepare accidents in nuclear facilities. Previous courses were introduced in another paper in Japanese [2]. In this year, we are planning to start new courses with wider perspectives. The new courses are focused on radiation accidents and radiation terrorisms in addition to accidents in nuclear power plants. We plan two types of courses: one for medical staffs (hospital management) and another for first responders (pre-hospital management). The Hospital course is planned to include a desktop drill to study various simulated cases and an exercise in which participants are expected to perform each role in a situation accepting a patient. The Pre-hospital course is also planned to include a desktop drill to study accident cases and an exercise to study proper management at an accident site including zoning and covering of contaminated patients. Accident during transportation of RI will be also included.

Radiation is used in many places other than nuclear power plants, consequently, accidents can happen anywhere. More over, a threat of nuclear or radiological terrorism is emerging in the world. Thus, these new educational courses are highly expected.

References

[1] International Atomic Energy Agency and World Health Organization. Planning the medical response to radiological accidents. Safety Reports Series No. 4. Vienna, International Atomic Energy Agency. (1998)

[2] Tatsuzaki H. Radiation Emergency Medicine Pre-hospital Seminar, Radiation Emergency Hospital Seminar (in Japanese). ER Magazine 5(2):277-280 (2008).

Table 1. International training courses and workshops organized by NIRS from 2001 to 2009.

year & month	Title	cooperation	Number of partici-pating countries*	number of participants*
2001 Aug	IAEA/RCA-NIRS Regional Training Course on " Medical Preparedness and Medical Response to Radiation Accidents"	IAEA/RCA	12	25
2004 Mar	IAEA/RCA Regional Training Course on " Medical Management for Radiation Accident"	IAEA/RCA	10	16
2005 Jan	KIRAMS/NIRS Seminar of "Radiation Emergency Medical Preparedness"		1	24
2005 Sep	Seminar on "Medical Treatment of Patients Contaminated with α Emitters"		1	15
2006 Nov	International Workshop on Radiation Emergency Medical Preparedness within the Framework of the Asian Nuclear Safety Network	IAEA	12	19
2007 Feb-Mar	NIRS Seminar for trainers on Radiation Emergency Medicine in Asia Region		7	16
2007 Dec	NIRS Training Course for Taiwanese Medical Professionals on Radiation Emergency Medical Preparedness		1	26
2007 Dec	NIRS Training Course for Korean Medical Professionals on Radiation Emergency Medical Preparedness		1	23
2008 Jan-Feb	NIRS/ NSC/ IAEA Workshop on Medical Response to Nuclear Accidents in Asia	NSC, IAEA	10	21
2008 Nov	NIRS Training Course for Korean Medical Professionals on Radiation Emergency Medical Preparedness		1	25
2008 Nov	NIRS Workshop on Cytogenetic Biodosimetry for Asia and NIRS-ISTC Workshop on Cytogenetic Biodosimetry	ISTC	13	30
2009 Feb	NSC/NIRS Workshop on Medical Response to Nuclear Accidents in Asia	NSC	14	21

*excluding Japan

Chromosome abnormality as a genetic indicator for dose estimation and carcinogenesis

Mitsuaki A. Yoshida

Biodosimetry Section, Department of Radiation Dose Assessment,
Research Center for Radiation Emergency Medicine,
National Institute of Radiological Sciences,
4-9-1 Anagawa, Inage-ku 263-8555, Japan. myoshi@nirs.go.jp

Abstract. Many types of chromosome abnormality are induced in cells by ionizing radiation. In particular, dicentric (Dic) and centric ring (Rc) are considered to be relatively specific to radiation and the frequency of these abnormalities increases depending on the irradiation dose. Therefore, Dic and Rc are being used as a significant biological marker for dose estimation in an exposed individual. In general, the dose estimation is very important process for the exposed person.

On the other hand, characteristic chromosome abnormality has also been identified in almost of human cancer. Therefore, such specific chromosome abnormality can be used for a cancer diagnosis. In fact, the chromosome change such as Philadelphia chromosome; the translocation between chromosomes 9 and 22 is a significant genetic marker to determine a kind of leukemia; chronic myelocytic leukemia (CML). Moreover, identification of such non-random chromosome alterations specific to each human cancer has been contributed to the isolation of oncogenes and tumor suppressor genes associated with the tumor development. Thus, the chromosome abnormality gives more important genetic information to the field of life science, especially radiation dose assessment and cancer development.

Key Words: chromosome aberration, dose assessment, cancer

1. Introduction

Dose estimation is considered to be very important as the first step in the medical treatment of persons exposed to radiation [1]. It was well known that many types of chromosome abnormality are induced in cells by irradiation and the frequency of these abnormalities increases depending on the irradiation dose. Therefore, such chromosome aberrations induced by radiation, in particular, dicentric and centric ring which are specific to radiation are being used as a significant biological marker for dose estimation in an exposed individual [2]. Also, characteristic chromosome abnormalities were specifically identified in almost human malignancy [3] and being used for the diagnosis of disease. In the present study, I will introduce the process for the dose estimation due to dicentric scoring in peripheral blood lymphocytes and the role of specific chromosome abnormality in carcinogenesis.

2. Dose Estimation by Chromosome analysis

2.1. Chromosome abnormality induced by radiation exposure

As mentioned above, many types of chromosome aberrations are identified in cells

Corresponding to: Mitsuaki Yoshida, Section Head, Biodosimetry Section, Department of Radiation Dosimetry, Research Centre for Radiation Emergency Medicine, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan.

by radiation exposure. In particular, dicentric (Dic), fragment (Fr), translocation (Tr) and centric ring (Rc) are produced as a result of failure in DNA repair (Fig. 1, 2). Dicentric is an exchange between the fragments with centromere from two broken chromosomes. Also, aberrations caused by two DNA strand breaks in two different chromosomes produce translocation as well as Dic + Fr. The ratio of these Dic +Fr versus Tr should theoretically be 1:1. As Dic and Rc are unstable chromosome aberrations, the cells having these aberrations will be eliminated through repeating the cell division.

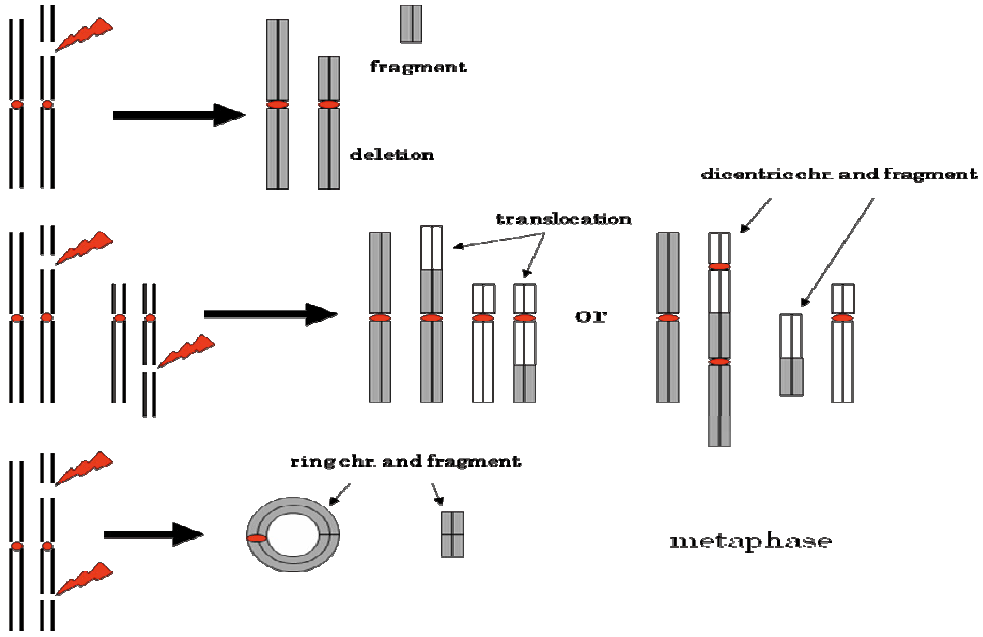


Fig. 1 Schematic figure of chromosome aberrations induced by irradiation.

2.2. General feature of human lymphocytes

The general features of human lymphocytes are summarized in IAEA Technical Report Series No. 405 [2]. In brief, the total number of human lymphocytes in a healthy young adult is estimated to be approximately 500 x 10^9 and about 2 % out of these are present in the peripheral blood, the remaining (98%) in other tissues, such as thymus, lymph nodes, spleen, bone marrow and so on. The lymphocytes are classified into two main types, i.e. T and B cells. Moreover, on the basis of the type of surface antigen, T cells are divided into CD4 and CD8 subtypes. These T cells are stimulated in vivo by phytohaemagglutinin (PHA) and used for biodosimetry.

2.3. Protocol for the culture of peripheral blood lymphocytes

In general, peripheral blood lymphocytes (PBL) are used for dicentric scoring in dose estimation. In this section, the outline of the protocol for PBL culture will be introduced. The method to obtain a lot of metaphases for Dic scoring was developed by Hayata *et al.* [4].

1. Peripheral blood (3-10ml) should be obtained from the exposed person by using heparin as an anticoagulant.
2. Lymphocytes are separated from whole blood (3ml) by centrifugation using Ficoll Hypaque column and so on.
3. Separated lymphocytes are washed by culture medium without serum.
4. The lymphocytes collected by centrifugation are cultured in the RPMI1640 medium supplemented with 20% fetal bovine serum, phytohaemagglutinin (PHA) as a mitogen and Colcemid to stop the cell cycle at M-stage.
5. After 48 hours culture, the lymphocytes are treated with hypotonic solution, 0.075M KCl and then fixed with methanol/acetic acid (3:1).
6. Slide are prepared either immediately or the

next day and stained with Giemsa solution.

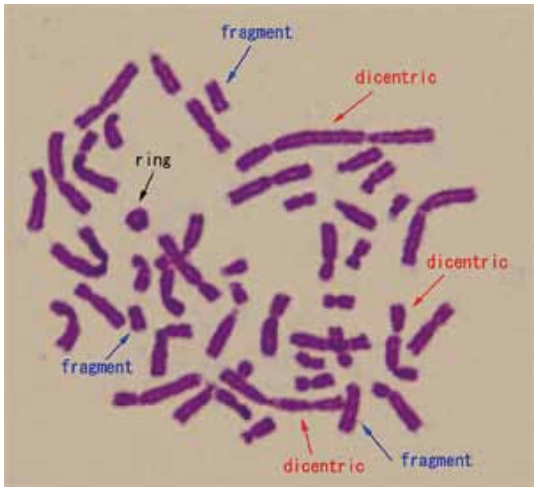


Fig. 2 Chromosome aberrations (dicentric, ring and fragment) in human lymphocytes irradiated with 5Gy gamma-ray.

2.4. Dose estimation by Dic scoring

Unstable chromosome aberrations, such as Dic and Rc are easily identified under a microscope. Scoring of Dic should be performed in 1,000 metaphases or aim at about 100 Dic. The yield of Dic (Y) is related to the dose (D) by the equation:

$$Y = A + \alpha D + \beta D^2 \quad \text{------ (1)}$$

where A is the background yield and, α and β are coefficients obtained by experimental study using irradiated blood. The frequency of Dic is depending on types of radiation [5]. In low linear energy transfer (LET) radiation, such as X-rays or gamma-rays, the dose-response relationship of Dic shows linear at low doses and quadratic at high dose. In high-LET radiation, such as neutron, proton and alpha-rays, Dic are mostly induced by a single track and the dose-relationship of Dic becomes linear. Thus, the dose response curve obtained from the above equation shows the specificity depending on the type of radiation.

The dose estimation by Dic scoring should be performed on the cases of acute external exposure on whole body. The blood sampling should be performed within the duration from 24 hours to about 40 days after the radiation exposure, because the lymphocytes with unstable chromosome aberrations will be eliminated.

2.5. Dose estimation by translocation analysis

It has been recognized that analysis of stable translocation is needed to address biodosimetry for long term exposure. Although translocation is detected by the karyotype analysis using banding methods, it is too laborious to use in biodosimetry. However, in recent years, chromosome painting, which is one of FISH method (Fig. 3), is used to detect translocations routinely for biodosimetry in the cases of long term exposure. The chromosome painting method to stain only limited chromosome pairs was proposed by Lucas *et al.* [6]. Dose by chromosome painting will be estimated by the following equation:

$$F_G = F_p / 2.05 f_p (1-f_p) \text{ ---- (2)}$$

where F_G is the full genome aberration frequency, F_p is the translocation frequency detected by chromosome painting and f_p is the fraction of genome hybridized. After F_G was determined from above equation (2), the dose will be estimated using equation (1).

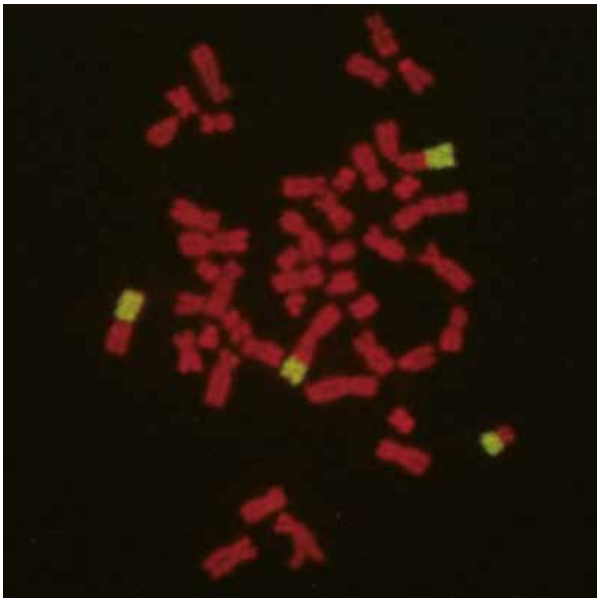


Fig. 3 An example of chromosome painting. The painting probes for chromosome 8 was hybridized in human cancer cells.

2.6. Dose estimation by PCC-ring method

Generally, when the cycling cells enter into M-stage, chromatin condenses into chromosome. However, when the cells in M-stage are fused with the cells in interphase, chromatin of the cells in interphase condenses prematually. This is termed premature chromosome condensation (PCC). After exposure to higher dose radiation, the cell cycle of lymphocytes after stimulation with PHA will be arrested at G2 phase or cell death is induced in such lymphocytes. Therefore, in such cells, the Dic assay is not available for the dose estimation. It is useful for biodosimetry to induce premature chromosome condensation (PCC) in irradiated lymphocytes by treatment with a specific inhibitor of protein phosphatase, such as Okadaic Acid and Calyculin A [7].

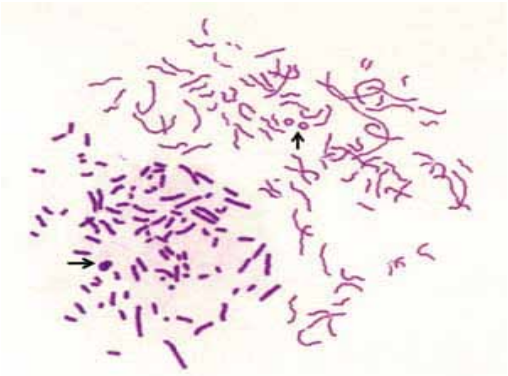


Fig. 4 Premature chromosome condensation (PCC) in irradiated lymphocytes. Arrows indicates ring chromosomes.

In lymphocytes showing PCC, it is easier to score ring chromosome than Dic, as shown in Fig. 4. The frequency of ring chromosome also shows dose dependency and it will be estimated by scoring of ring chromosome.

2.7. Chromosome Network for dose estimation in Japan

The National Institute of Radiological Sciences (NIRS) is playing a core role as a designated radiation emergency hospital at the national level as well as the regional level. The Chromosome Network in Japan was established just after the critical Tokai-mura accident in 2001. The purposes of this network are integrating work to be ready for a large-scale radiation accident, establishing standard methods for cytogenetic dose estimation, creating a standard dose response curve, preparing a training program for cytogenetic dose estimation and training successors for biodosimetry work. The Chromosome Network consists of the following nine organizations (Fig. 5): the National Institute of Radiological Sciences in Chiba, the Asahikawa Medical College in Hokkaido, the Institute for Environmental Sciences in Aomori, the Tokyo Medical and Dental University in Tokyo, the Human Service Center in Aichi, the Kyoto University in Kyoto, the Osaka Prefecture

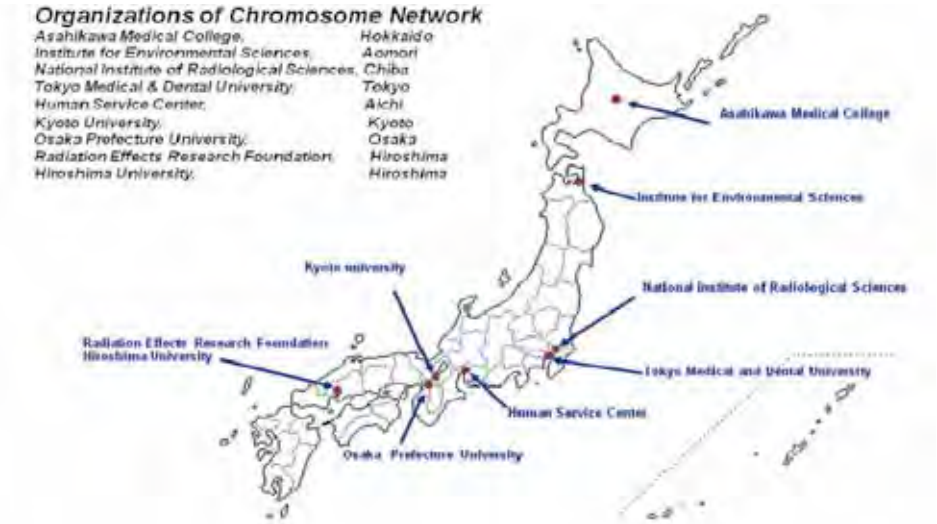


Fig. 5 Chromosome Network in Japan is composed with nine organizations.

University in Osaka, the Radiation Effect Research Foundation and the Hiroshima University in Hiroshima [8]. In order to analyze the chromosome aberrations in irradiated cells as correct as possible and estimate the irradiated dose, the congruous criteria was decided and also standardization of cytogenetic methodology from the blood culture to the cell fixation was conducted in the network. When we have a radiological accident, basically, the members in NIRS will act from blood sampling to the dose estimation and the Network council will confirm the results. However, we have mass casualty accident, the all members in Network will act cooperatively in order to assess the irradiated dose.

3. Chromosome abnormality and Cancer

Nonrandom chromosome changes have been identified in a variety of human cancers and leukemias [3, 9]. The results of cytogenetic analyses in haematological diseases have suggested that such specific chromosome rearrangements may be one of the significant steps associated with the pathogenesis of human malignancies. The involvement of oncogene and tumor suppressor genes in the development of malignancy has been suggested by the finding of such disease specific chromosome alterations, which are indicated in Tables 1 and 2. In recent years, the specific chromosome abnormalities are used for the diagnosis and prognosis of diseases.

3.1. Chromosome changes in haematological disorder

Most typical chromosome change is a reciprocal translocation between chromosomes 9 and 22 in chronic myelocytic leukemia (CML) [3]. This translocation was identified in almost 90% of CML and creates the fused protein of *abl* and *bcr* gene product [10]. Another characteristic abnormality is the reciprocal translocation between chromosomes 8 and 2, 14, 21 which was observed in Burkitt's lymphoma [3]. The *c-myc* oncogene on chromosome 8 is activated by fusion with immunoglobulin genes on chromosomes 2, 14 and 22 [11]. It has been suggested that reciprocal translocations may contribute to the activation of oncogene and the production of oncogenic protein. In recent years, these disease specific chromosome changes are being used for the diagnosis of haematological disorder.

3.2. Chromosome changes in carcinoma

On the other hand, the chromosome abnormality with deletion type is remarkably identified in human carcinoma derived from epithelial cells. In particular, loss of chromosome 13 was frequently found in retinoblastoma developed hereditally and non-hereditally in child. Cancer associated gene was identified by finding of the deletion of chromosome 13 and named as *Rb* gene as a candidate tumor suppressor gene. Hypothesis that inactivation of two tumor suppressor

Table 1. Examples of representative haematological disorders with characteristic chromosome

haematological disorders	chromosome translocations	cancer associated genes
chronic myelocytic leukemia (CML)	t(9;22)(q34;q11)	BCR-ABL
acute myelocytic leukemia (AML)	t(8;21)(q22;q22)	AML1-MTG8
acute pro-myelocytic leukemia (APL)	t(15;17)(q22;q21)	PML-RARA
Burkitt's lymphoma	t(8;14)(q24;q32)	MYC- IGH
	t(2;8)(p11;q24)	IGLK-MYC
	t(8;22)(q24;q11)	MYC- IGLL

Table 2. Candidate of tumor suppressor genes in human malignancy

TSG	chromosome	diseases
RB1	13q14	retinoblastoma, osteosarcoma
P53	17p13	colon cancer., lung cancer., Li-Fraumeni syndrome
WT1	11p13	Wilm's tumor
APC	5q21	Familial Polyposis Coli, colon cancer
MCC	5q21	colon cancer
DCC	18q21	colon cancer
NB	1p36	neuroblastoma
VHL	3p25	von Hippel-Lindau syndrome, renal cell carcinoma
RCC	3p21	renal cell carcinoma
HRCA1	3p14	familial renal cell carcinoma
MTS1	9p21	malignant melanoma, lung cancer, breast cancer, bladder cancer
BRCA1	17q21	familial breast cancer
NF1	17q11	neurofibomatosis type 1
NF2	22q12	neurofibromatosis type2
MEN1	11q13	thyroid medullary carcinoma
MEN2	10q11	thyroid medullary carcinoma

*TSG: tumor suppressor gene

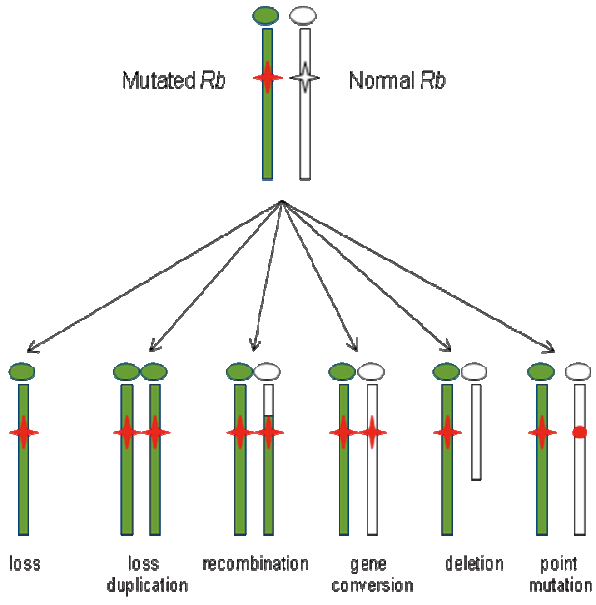


Fig. 6 Six ways for loss of normal TSG allele. Red star indicates mutant allele, white star normal allele and red circle allele with point mutation.

genes on homologous chromosomes may be involved in carcinogenesis was proposed based on analysis of retinoblastoma [12]. Loss of normal allele is occurred through some changes of DNA sequence; loss of one chromosome with normal allele, loss of normal chromosome and duplication of chromosome with mutant allele, somatic recombination resulting in two mutant allele, conversion of mutant gene, partial deletion of the region involving normal allele and point mutation in normal allele (Fig. 6).

Previously, the author has analyzed the chromosome abnormality in human renal cell carcinoma (RCC) from Japanese patients [13]. In that study, deletion on a short arm of chromosome 3 (3p) was frequently identified in RCC showing histologically non-papillary type (Fig.7). In particular, unbalanced translocation between chromosome 3 and 5 resulting in both deletion of 3p and partial trisomy of 5q was found in the specimens with 3p deletion. Such unbalanced translocation may be produced by somatic recombination between chromosomes 3 and 5 (Fig. 8). The results in a series of chromosome analysis suggested that putative tumor suppressor gene may be located on commonly deleted region

on 3p. The existence of tumor suppressor gene associated with RCC development was testified by the introduction of chromosome 3 derived from normal cell into RCC cells using microcell fusion technique [14].

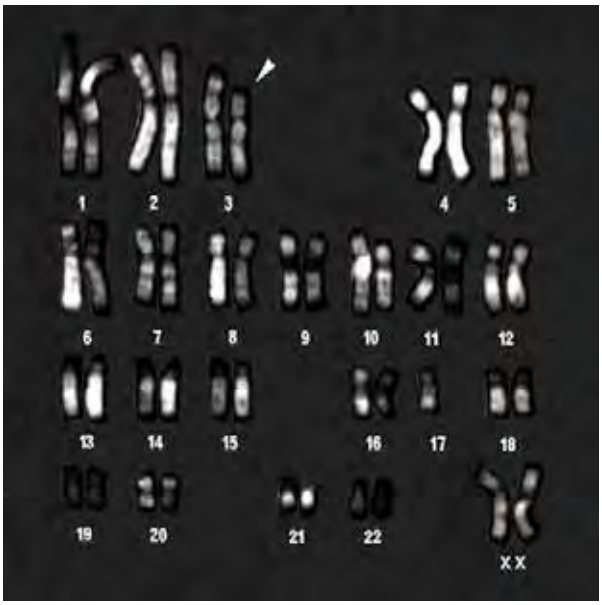


Fig. 7 Karyotype of RCC with non-papillary type. Arrow head indicates deletion of a short arm of chromosome 3. Loss of chromosome 3 is specific to human RCC.

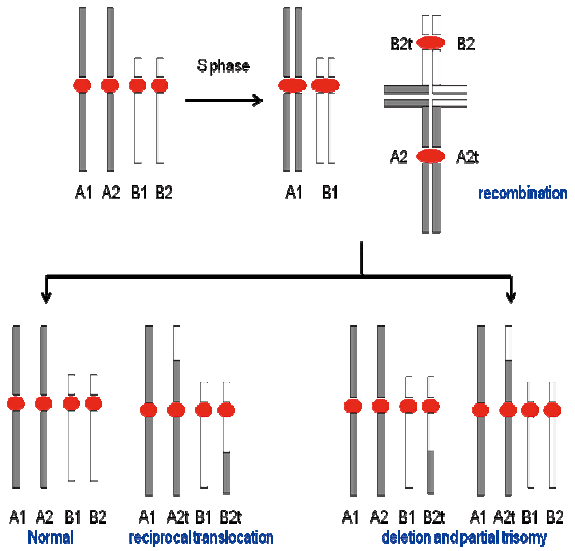


Fig. 8 Somatic recombination resulting in loss and partial trisomy after equal chromosome segregation. A1 and A2, B1 and B2 are homologous chromosomes, respectively.

4. Conclusion

Common phenomenon in irradiated cells and cancer cells is that these cells have the chromosome abnormality, which is significant genetic marker for the dose assessment in exposed persons and for the characterization of cancer. However, it is not elucidated whether the cells having chromosome abnormalities induced by irradiation will be malignant or not. It is the fact that the specific chromosome alterations are playing significant roles in the course of cancer development and also the frequency of cancer developed in exposed persons is much higher than that in normal population. Depending on the investigation of exposed person in previous accidents, if the DNA damaged is formed in the chromosome region containing the cancer associated genes, the possibility that such cells will be malignant is not neglected completely. Most important is the chromosomal region and genes receiving the DNA damage by irradiation.

References

[1] Lloyd DC, Edwards AA, Moquet JE, Guerrero-Carbajal YC. The reole of cytogenetics in early triage of radiation casualties. Appl. Radiat. Isot. 52: 1107-1112 (2000).

[2] IAEA 2001. Cytogenetic analysis for radiation dose assessment. A manual, Technical Report Series 405 IAEA Vienna.

[3] Sandberg AA. The Chromosomes in Human Cancer and Leukemia. Elsevier, New-York (1990).

[4] Hayata I, Tabuti H, Furukawa A, Okabe N. Robot system for preparing lymphocyte chromosome. J. Radiat. Re. 33(Suppl) : 231-241 (1992).

[5] Dufrain R. In vitro human cytogenetic dose-response system. The Medical Basis for Radiation Accident Preparedness, Hubner KF and Fry SA eds., Elsevier, North Holland Inc., pp. 357-374 (1980).

[6] Lucas JN, Awa A, Straume T, Poggensee M, Kodama Y, Nakano M, Ohtaki K, Weiser H-U, Pinkel D, Gray J,

Littlefield G. Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation. Int. J. Radiat. Biol. 62 : 53-63 (1992).

[7] Kanda R, Eguchi-Kasai K, Hayata I. Phosphatase inhibitors and premature chromosome condensation in human peripheral lymphocytes at different cell-cycle phases. Somatic cell and Mol. Genet. 24:1-8 (1999).

[8] Yoshida MA, Hayata I, Tateno H, Tanaka K, Sonta S, Kodama S, Kodama Y, Sasaki MS. The chromosome network for biodosimetry in Japan. Radiat. Measurement 42:1125-1127 (2007).

[9] Mitelman F. Catalog of chromosome aberrations in cancer. Wiley-Liss, Inc. (1998).

[10] Shtivelman E, Lifshitz B, Gale RP, Canaani E. Fused transcript of abl and bcr genes in chronic myelocytic leukemia. Nature 315: 550-554 (1985).

[11] Tsujimot Y, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 226: 1097-1099 (1984).

[12] Knudson AG. Two genetic hits (more or less) to cancer. Nature Rev. Cancer 1:157-162 (2001)

[13] Yoshida MA, Ikeuchi T, Tachibana Y, Takagi K, Moriyama M, TonomuraA. Rearrangements of chromosome 3 in nonfamilial renal cell carcinomas from Japanese patients. Jpn. J. Can. Res, (Gann) 79:600-607 (1988).

[14] Yoshida MA, Shimizu M, Ikeuchi T, Tonomura A, Yokota J, Oshimura M. In vitro growth suppression and morphological change in a human renal cell carcinoma cell line by the introduction of normal chromosome 3 via microcell fusion. Mol. Carcinogen. 9:114-121 (1994).

Experimental Studies on Biological Effects of Continuous Exposure of Mice to Low-Dose-Rate Gamma-Rays in a Special Reference to Transgenerational Effects and Biological Defense System

Yoichi Oghiso *, Satoshi Tanaka, Ignacia Braga Tanaka, III, Daisaku Takai, and Shingo Nakamura

Department of Radiobiology,
Institute for Environmental Sciences,
Rokkasho, Aomori, Japan

Abstract. We demonstrated in a large-scale life-span study that a significant life-shortening due to early neoplastic death is observed in mice after continuous γ-irradiation for approximately 400 days with a high dose of 8000 mGy at a low-dose-rate of 20 mGy/day, while no significant difference in life span was found in mice irradiated with a low dose of 20 mGy at a low-dose-rate of 0.05 mGy/day. Among experimental research projects on biological effects of continuous low-dose-rate radiation exposures in mice, study on transgenerational effects is under investigation to determine whether or not the effects of long-term, paternal γ-irradiation at low-dose-rates could be inherited by the progeny mice. The other study on biological defense system shows some significant changes in immune system and metabolisms related to body weights increase of mice continuously irradiated with low-dose-rate and high dose γ-rays, both of which could be relevant to early neoplastic death observed in the life-span study.

Key Words: low-dose-rate radiation, continuous exposure, biological effects, animal studies

* Corresponding to: Yoichi Oghiso, Department of Radiobiology, Institute for Environmental Sciences, 1-7 Ienomae, Obuchi, Rokkasho, Kamikita, Aomori, 039-3212, Japan.
E-mail: oghiso@ies.or.jp

Introduction

Cancers as well as genetic effects are believed to be stochastically induced even by low-dose and low-dose-rate radiations [1]. Irrespectively, recent reports on the biological effects of low-dose radiations indicate different mechanisms from high-doses, although much remains to be elucidated on low-dose radiation effects.

The Institute for Environmental Sciences (IES) studies biological effects of low-dose-rate and low-dose radiation exposures in mice to evaluate human risks based on dose levels attributable to radioactive substances released into the environment from the nuclear fuel recycling facility.

In this paper, the large-scale life-span animal study on biological effects of long-term, low-dose-rate radiation exposures are briefly reviewed, and the current research topics on transgenerational effects and biological defense system in mice continuously exposed to low-dose-rate γ-rays are provided as one of the most important but unresolved subjects concerning low-dose radiation effects as described in the recent UNSCEAR report [2].

Life-Span Studies on Biological Effects of Continuous Low-Dose-Rate Radiation Exposure

The previous life-span study using a total of 4000 specific pathogen-free (SPF) B6C3F1 mice continuously exposed to γ-rays at three different low-dose-rates (0.05 mGy/day, 1 mGy/day, 20 mGy/day) and different total accumulated doses (20 mGy, 400 mGy, 8000 mGy) for approximately 400 days, indicate a significant life-shortening mainly due to early neoplastic death in both male and female mice after exposure to high-dose (8000 mGy), and in females alone exposed to middle-dose (400 mGy), while no significant difference was found in life span of both male and female mice exposed to low-dose (20 mGy) as shown in table 1 [3].

Among the causes of death, malignant lymphomas were one of the most frequent tumors related to early neoplastic death, although their incidences were not different between irradiated and non-irradiated groups of mice [4].

In addition, the incidences of myeloid leukemias in males, lung and ovary tumors in females, and hemangiosarcomas and liver tumors in both sexes were significantly increased after exposure to 8000 mGy, compared to non-irradiated controls [4]. It is, however, unknown why and how such biological consequences occur following continuous low-dose-rate irradiations.

Current Experimental Research on Biological Effects of Continuous Low-Dose-Rate Radiation Exposure

Based on the results obtained from the life-span study as described above, we currently study four research projects on biological effects of continuous low-dose-rate radiation exposure in mice. The first one is on the transgenerational effects to determine whether or not the effects of long-term, paternal γ-irradiation at low-dose-rates could be inherited by the progeny mice. The second one is on the biological defense system to clarify whether or not changes in the immune system and metabolisms related to increase of body weights observed in the life-span study [3] could lead to development and progression of tumors after continuous γ-irradiation at low-dose-rates. The third one is on the tumor-related genes to clarify whether or not alterations of tumor-related genes and their expression could lead to generation and development of malignant lymphomas and leukemias observed in mice continuously exposed to γ-rays at low-dose-rates. The fourth one is on the biological dosimetry for low-dose-rate and low-dose radiation exposures to establish the methodology for accurate and quick estimation of exposure doses with chromosomal aberrations of peripheral lymphocytes upon an urgent radiation exposure by animal model using mice continuously irradiated with low-dose-rate γ-rays. Out of these research projects, current experimental results

Table 1. Effects of continuous low-dose-rate gamma-irradiation on survival of SPF B6C3F1 mice.

Dose rate (mGy/day)	Total accumulated dose (mGy)	Mean life span ± SE (days)	Mean life-shortening ± SE (days)
Males			
0	0	912.7 ± 8.2	
0.05	20	905.8 ± 8.3	6.9 ± 11.7
1.0	400	895.2 ± 8.2	17.5 ± 11.6
20	8000	812.0 ± 7.6 **	100.7 ± 11.2 **
Females			
0	0	860.5 ± 6.3	
0.05	20	851.8 ± 6.7	8.7 ± 9.2
1.0	400	839.8 ± 7.5 *	20.7 ± 9.8 *
20	8000	740.8 ± 6.8 **	119.6 ± 9.3 **

* Statistically significant ($p < 0.05$).
** Statistically significant ($p < 0.0001$)

obtained from the first and second research are briefly shown in the next section, while the third and fourth research topics are described in this issue by Tanaka et al.

Transgenerational Effects of Continuous Low-Dose-Rate Radiation Exposures of Male Mice

To study transgenerational effects of continuous low-dose-rate γ-rays, male C57BL/6J mice (sires) were continuously irradiated for approximately 400 days with ^{137}Cs γ-rays at low-dose-rates of 20 mGy/day, 1 mGy/day, and 0.05 mGy/day with total accumulated doses equivalent to 8000 mGy, 400 mGy and 20 mGy, respectively, and kept together with age-matched, non-irradiated control mice under a SPF condition. Immediately after completion of irradiation, male mice are mated with non-irradiated females to produce F1 mice. Randomly selected F1 males and females are mated to produce F2 mice. All mice are kept until their natural death and subjected to pathological examinations upon death. Several parameters including the life span, cause of death, tumor incidence as well as number of offspring will be investigated to evaluate biological effects of low-dose-rate irradiation. All the experiments are divided into 6 batches, and continuous irradiation of the first 4 batches was completed by 2008. Preliminary results show that there is no significant difference in the pregnancy rate and weaning rate for parent and F1 progeny mice between experimental groups. There is, however, a slight but significant decrease in the mean litter size and mean number of weaned pups per female mated with male mice exposed to 8000 mGy at 20 mGy/day as compared to non-irradiated controls. No significant difference was so far found in life span, cause of death and tumor incidence of F1 and F2 progeny mice between experimental groups.

Effects of Low-Dose-Rate Radiation Exposures on Biological Defense System

To investigate changes in the immune system following long-term and low-dose-rate radiation exposure, the proportions of T-helper (Th1 and Th2) cells and proliferative responses of T cells were examined in spleens from female SPF mice of three strains (C57BL/6J, C3H/HeN and B6C3F1) after continuous γ-irradiation at low-dose-rate of 20 mGy/day for 50 ~ 400 days with total accumulated doses of 1000 mGy ~ 8000 mGy. Although strain differences were observed both in the proportions of Th cell subsets and T cell proliferation, significant increase in the proportion of Th2 cells and significant decrease in mitogen (Con A)-induced and allo-antigen-induced (MLR) T cell proliferation were prominently found in B6C3F1 mice received at least total doses of 1000 mGy to 2000 mGy, compared to age-matched, non-irradiated mice. These results indicate that low-dose-rate and high dose irradiation could induce changes in innate immune system, which might be related to impaired tumor immunity leading to early neoplastic death of irradiated B6C3F1 mice observed in the life-span study.

To elucidate changes in lipid metabolisms related to the increase in body weights of irradiated female SPF B6C3F1 mice observed in the life-span study, the body weights and feed consumption of mice were measured during continuous γ-irradiation at low-dose-rate of 20 mGy/day, and all mice were autopsied at 40-weeks of age for histochemical and biochemical analyses on factors related to lipid metabolisms. The body weights of irradiated mice were significantly increased during the period of continuous irradiation compared to those of age-matched, non-irradiated mice, although feed consumption was not different between irradiated and non-irradiated control mice. The weights of visceral adipose tissues were significantly increased in irradiated mice, and significantly larger sizes and fewer numbers of adipocytes were noted in adipose tissues of irradiated mice by morphometry of histological sections. The lipid contents in hepatocytes were also found to be more intensely stained by oil red O on the liver sections from irradiated mice. As well, serum contents of triglyceride and total cholesterol were increased, and serum leptin levels were significantly higher in irradiated mice. These results indicate that the increase of body weights in mice continuously irradiated with low-dose-rate γ-rays is adiposity, closely associated with metabolic factors including leptin.

References

[1] United Nations Scientific Committee on the Effects of Atomic Radiation, Sources and Effects of Ionizing Radiation, Vol. II, Effects. Report to the General Assembly, with Scientific Annexes, United Nations, New York, 2000.
[2] United Nations Scientific Committee on the Effects of Atomic Radiation, Effects of Ionizing Radiation, Vol. I, Report to the General Assembly, with Scientific Annexes, United Nations, New York, 2006.
[3] Tanaka S, Tanaka III IB, Sasagawa S, Ichinohe K, Takabatake T, Matsushita S, Matsumoto T, Otsu H, Sato F. No lengthening of life span in mice continuously exposed to gamma rays at very low dose rates. Radiat. Res. 160: 376-379 (2003).
[4] Tanaka IB III, Tanaka S, Ichinohe K, Matsushita S, Matsumoto T, Otsu H, Oghiso Y, Sato F. Cause of death and neoplasia in mice continuously exposed to very low dose rates of gamma rays. Radiat. Res. 167: 417-437 (2007).

Acknowledgments

All the works are performed under contract with the Aomori Prefectural Government, Japan.

Chromosome aberration rates in splenocytes and genomic alterations in malignant lymphoma from mice long-term exposed to low-dose-rate gamma-rays

Kimio Tanaka[1*], Atsushi Kohda[1], Kenichi Satoh[2], Takashi Takabatake[1,3], Kazuaki Ichinohe[1] and Yoichi Oghiso[1]

[1] Department of Radiobiology, Institute for Environmental Sciences, Rokkasho, Aomori, Japan
[2] Department of Environmentrics and Biometrics, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan
[3] Experimental Radiobiology for Children's Health Research Group, National Institute for Radiobiological Sciences, Chiba, Japan

Abstract. We analyzed serially chromosome aberration rate in spleen cells of long-term gamma-irradiated C3H mice up to 400 days and 615 days, respectively, at the low-dose-rates (LDRs) of 20 mGy/22h/day and 1 mGy/22h/day, and compared with those induced by irradiation at 400 mGy/22h/day to evaluate dose and dose-rare effects. Dicentrics and translocations increased almost linear at the 20 mGy/22h/day irradiation and clear dose-rate effects were found in dicentric chromosomes among these dose rates. Clonal cells such as trisomy 15 increased rapidly more than 4000 mGy at the 20 mGy/22h/day, which might be associated with lymphomagenesis. Furthermore, the life span-shortening found in 20 mGy/22h/day-irradiated mice was attributable to earlier death due to all malignancies including malignant lymphomas (MLs) in irradiated mice. To elucidate the molecular mechanisms of murine lymphomagenesis by LDR irradiation, genomic copy changes were analyzed by CGH analysis. The genomic profile showed a high frequency of trisomy 15 and partial losses of chromosome 4 and 14 in MLs from irradiated mice, and partial gains on chromosomes 12 and 14 were in those from non-irradiated mice. These regions contained candidate genes for lymphomagenesis. These findings suggest that the long-term LDR irradiation could influence genomic changes and promote development of lymphoma.

Key Words: **low dose radiation, dose rate effects, biological dosimetry, chromosome aberrations, malignant lymphoma**

Introduction

It is essential to estimate individually exposure dose received by an accidental exposure to ionizing radiation. Methods for measuring biological effects of radiation are necessary to complement physical dosimetry to more accurate determination individual's biological effects. Dicentric and ring chromosomes of lymphocytes are used for currently the most reliable and sensitive biological markers to estimate individual's exposure dose. But they have still several technical limitations specially for a rapid or accurate estimation in the low dose exposure at high-dose-rate (HDR) irradiation and chronic low-dose rate (LDR) irradiation. The time needed for chromosome analysis is problematic in low-dose or high-dose irradiation accident involving many people. Then we established a precise and sensitive premature chromosome condensation- fluorescence *in situ* hybridization (PCC-FISH) method that combines PCC and FISH to detect dicentric chromosomes of lymphocytes from persons exposed to low dose as well as to high dose radiation.

Chronically exposed individuals such as nuclear workers, medical radiologists, residents in high-background radiation areas and residents inside the radio-contaminated buildings in Taiwan have higher incidences of chromosome aberrations than non-exposed individuals [1-5], however, the dose and dose-rate effects have not been well investigated, regarding chronological changes in chromosome aberration rates following continuous LDR radiation exposures. Epidemiological studies of human populations have uncertainties because of confounding factors influencing the analyses such as smoking, medical radiation exposure and so on. Thus, animal experiments are needed to complement risk assessment based on epidemiological studies. The dose rates of 1 mGy/22h/day (0.045 mGy/h) and 20 mGy/22h/day (0.91 mGy/h) are 400 and 8000 times higher than the natural background external radiation level, respectively. Using these facilities, we investigated biological effects of continuous LDR radiation exposures on life-span [6], cancer incidence [7], genomic and oncogene alterations in lymphoma and leukemia [8, 9, 10, 11], chromosome aberrations [12, 13], cellular response [14, 15] and so on in mice. Low-dose radiation exposure induces different biological responses from those by high-dose radiation exposure, including inverse dose-rate effects [16, 17, 18], adaptive response [19, 20] and low-dose hyper-radio-sensitivity [21]. These phenomena are explained by the ability of DNA repair and cellular response to radiation-induced DNA damage. The present study was designed o obtain dose response curve and to verify whether dose-rate effects are observed *in vivo* on chromosome aberration rates between LDR of 1 mGy/22h/day and medium dose-rate (MDR) of 400 mGy/22h/day radiations. These results will be important information to establish biodosimetry method for occupationally or accidentally exposed peoples to chronic LDR radiation.

Furthermore, we conducted array comparative genomic hybridization (CGH) to compare genomic loss or gain on whole mouse chromosomes in between malignant lymphomas (MLs) developed from chronic radiation at LDR (21 mGy/22h/day) and those from non-exposed MLs [8]. This comparative study will be applicable to identify radiation related- neoplasms developed after radiation accident as well as will be given informative results to evaluate the mechanism for radiation-induced neoplasms. We previously carried out a large-scale study using 4,000 specific-pathogen-free B6C3F1 mice to evaluate the effect of continuous low-dose-rate (LDR) radiation on life span and neoplasms [6]. In this experiment, irradiation was

administrated for approximately 400 days using ^{137}Cs γ-rays at dose rates of 21 mGy/22h/day, achieving total doses equivalent to 8,000 mGy. Comparisons of the mean life spans between the groups showed that irradiated groups had shorter life spans than the non-irradiated controls, and that the life-shortening observed in mice of both sexes irradiated with 21 mGy/22h/day was statistically significant [6]. Pathological examination indicated that the most frequent lethal neoplasm was ML in mice of both sexes and that life-shortening resulted primarily from earlier death due to MLs [7]. All MLs in our study were non-thymic lymphomas, and there was no significant increase in the incidence by irradiation. These observations suggested that the earlier development of the MLs might be triggered by continuous LDR irradiation via molecular pathogenesis.

Materials and Methods

1 Biological dosimetry for low dose irradiation by PCC-FISH method

Three ml of human peripheral blood obtained from adult volunteer was irradiated with 0.1, 0.3, 0.5, 1, 3 and 5 Gy of X-ray at the dose rate of 1.0 Gy/min. After irradiation, lymphocytes were separated using Vacutainer tube (Becton Dikinson) and then cultured for 48h at 37℃ with RPMI 1640 medium containing 20 % of bovine serum (PAA Laboratories) and 2% of phytohemaggulutinin（PHA）(HA15, Murex Biotech Ltd.). Calyculin A (WAKO; 50 nM) or okadaic acid (WAKO; 500 nM) was added 1 h before harvesting to obtain chromosomal metaphases showing PCC. Colcemide was not added for accumulation of metaphases in the experiment. Then metaphase preparations were made using automatic equipment (AD-STEC) and metaphases were hybridized with a centromere specific probe (Cambio) to examine dicentric chromosomes using FISH method. Metaphases were captured using a CCD camera and were analyzed under fluorescent microscope. For conventional method, metaphases were stained with Giemsa solution.

2 Biological diosimetry for chronic LDR irradiation

2.1 Radiation exposure and mice experiment

Continuous irradiation of the female SPF mice (C3H/HeN) with ^{137}Cs γ rays started from 8 weeks (56 days). Four to 13 mice were grouped for irradiation with each total dose together with age-matched, non-irradiated mice as controls. Groups of mice were irradiated with total doses of 100 to 8000 mGy at a LDR of 20 mGy/22h/day (0.91 mGy/h) for 5-400 days, and with total doses of 125 to 615 mGy at a LDR of 1 mGy/22h/day (0.045 mGy/h) for 125-615 days using a ^{137}Cs γ-rays irradiation device. Mice were not exposed daily between 10-12 a.m. in the morning for animal care. For comparison, MDR of ^{137}Cs γ-ray irradiation at 400 mGy/22h/day (18.2 mGy/h) and 200 mGy/22h/day (9.2 mGy/h) to achieve total doses of 400 to 8000 mGy for 1-20 days, and 200 mGy to 8000 mGy for 1-40 days was performed. Seven each mouse was irradiated by high-dose-rate (HDR) irradiation with total doses of 250 to 3000 mGy at a dose rate of 890 mGy/min, using ^{137}Cs γ-rays to obtain dose and dose-rate effectiveness factor (DDREF). Non-irradiated control mice were kept for the same period as irradiated mice.

Mice were sacrificed, and spleens were sterilely removed. For chromosome analysis, spleen cells were isolated and cultured in RPMI 1640 medium containing LPS (10 μg/ml), ConA (3 μg/ml) and 2-ME (50 μM) under a condition of 5 % CO_2 atmosphere with 95% humidity at 37℃. Colcemide (0.02μg/ml) was added for the last 2h of cultures to collect metaphase cells. Cells were treated by hypotonic solution with 0.075M KCl.

The supernatant was removed and the cells were fixed with Carnoy's solution. For conventional Giemsa staining method, FISH method using centromere probe, and multiplex fluorescence *in situ* hybridization (M-FISH), and cells were stained with Giemsa solution or fluorescence dyes. Dicentric chromosomes and centric ring chromosomes (Dic+Rc) by Giemsa staining method, dicentric chromosomes (Dic by FISH) by FISH method and translocations by M-FISH method were observed in 500-1000 metaphases per mouse in both irradiated and age-matched control groups.

2.2 Statistical analysis to evaluate dose and dose-rate effects

For statistical analysis, the 95% confidence interval (CI) for yields of chromosome aberrations was estimated by multiple linear regression analysis adjusted for age-related differences. Regression coefficient values in the linear regression lines or linear quadratic regression curves were estimated by multiple linear regression analysis. These values were compared with 95% CI among the four dose rates. The explanatory variables are 1) Intercept at age 56 days and 0 accumulated dose, 2) Age minus 56 days, 3) Accumulated dose for 1 mGy/22h/day group, 4) Accumulated dose for 20 mGy/22h/day group, 5) Accumulated dose for 200 mGy/22h/day group, 6) Accumulated dose for 400 mGy/22h/day group, 7) Accumulated dose for 890 mGy/min. The regression model for 6 groups can be expressed as non-exposed group: $y=b_1+ b_2xT$, 1 mGy/22h/day group: $y=b_1+ b_2xT+ b_3xD$, 20 mGy/22h/day group: $y=b_1+ b_2xT+ b_4xD$. 200 mGy/22h/day group: $y=b_1+ b_2xT+ b_5xD$, 400 mGy/22h/day group: $y=b_1+ b_2xT+ b_6xD$, 890 mGy/min group: $y=b_1+ b_2xT+ b_7xD +b_8xD^2$, where y is number of chromosome aberrations per 100 cells as response variable, b_j, j=1,…,8 are unknown regression coefficients and D is accumulated dose in mGy, and T is age-56. The linear quadratic regression curves model for 6 groups can be expressed as non-exposed group: $y=b_1+ b_2xT$, 1 mGy/22h/day group: $y=b_1+ b_2xT+ b_3xD+a_3xD^2$, 20 mGy/22h/day group: $y=b_1+ b_2xT+ b_4xD+a_4xD^2$. 200 mGy/22h/day group: $y=b_1+ b_2xT+ b_5xD+a_5xD^2$, 400 mGy/22h/day group: $y=b_1+ b_2xT+ b_6xD+a_6xD^2$, 890 mGy/min group: $y=b_1+ b_2xT+ b_7xD +a_7xD^2$, where y is number of chromosome aberrations per 100 cells as response variable, b_j, j=1,…,7 and aj, j=1,…..,7 are unknown regression coefficients and D is accumulated dose in mGy, and T is age-56. In both models, values of b_1+b_2xT were common among different dose rates in each type chromosome aberration (Dic+Rc and Dic by FISH). We estimated the unknown regression coefficients using the weighted least square estimator with respect to the number of observed cells using SPSS version 15 software.

3 Array CGH analyses on genomic alterations in malignant lymphomas (MLs)

In order to elucidate the effects of irradiation on the molecular pathogenesis of these murine MLs, we analyzed genomic alterations in the MLs by array-CGH method. In present study, genomic aberrations in 41 each ML from mice irradiated with a dose rate of 21 mGy/22h/day and from non-irradiated mice of B6C3F1 strain were precisely compared. These stored samples had been used for life-span study [6, 7]. The array carried 667 mouse BAC clones densely selected for the genomic regions not only of lymphoma-related loci but also of surface antigen receptors such as immunoglobulin genes and T cell receptor genes, enabling to classify B or T cell origin of MLs [8]. The DNA and control DNA were digested with *Bam*H1, followed purification. DNA (500 ng) was labeled with Cy3- or Cy5-dCTP and purified using a Bioprime Array CGH labeling system (Invitrogen). Test and control DNA were combined, precipitated with 62.5 μg of mouse *Cot1* DNA, and

re-suspended in 30 μl of hybridization buffer, and 3 μl of yeast tRNA for 3h with 10 μl of denatured hybridization buffer containing 50 μg herring sperm DNA and 10 μg of mouse *Cot1* DNA. Hybridization was performed for 45-48h at 37℃ using a MAUI hybridization dual chamber lid. After hybridization, the slides were washed for 15 min twice at 45℃ in washing solution. Hybridization array were scanned using the Axion 40008B scanner (Axion Instruments). Two-side Fisher's test for probability was used to compare the frequency of gains and losses between the irradiation and non-irradiated groups. A chromosome was scored as gained or lost if more than 90% of all clones on the chromosome had a $\log_2$ ratio greater or less than 0.1 or -0.1, respectively. As to the aberrations on partial chromosomal regions, the thresholds for copy number gain and loss were used at $\log_2$ ratios of 0.25 and – 0.25, respectively.

Results and Discussion

1 Application of PCC-FISH method for biological dosimetry in low dose irradiation

Detection rates of dicentric chromosomes were compared between PCC-FISH and conventional Giemsa staining methods (figure 1). PCC-FISH method could detect higher percentage of dicentric chromosomes than conventional Giemsa staining method. Numbers of dicentric chromosomes per a cell by PCC-FISH and conventional Giemsa staining method at each dose were 0.01 and 0.002 at 0.1 Gy, 0.06 and 0.029 at 0.5 Gy, and 0.19 and 0.115 at 1 Gy, respectively. Yields of dicentric chromosomes by PCC-FISH were about 1.2-5 times higher than those obtained using conventional Giemsa staining (table 1). Another alternative to metaphase FISH method, which requires 48h culture time for mitogen stimulation, is to score chromosome damage during interphase cells by using G1-chromosome premature condensation (PCC) [22, 23]. Analyzing painted interphase prematurely condensed chromosome can be minimized the examination time and can be useful for biodosimetry, although high quality technique is needed. The method is more sensitive than metaphase spreads especially at low dose range, and it was applied to detect cytogenetic abnormalities in CT scan subjecting patients [24]. On the other hand, G2-PCC ring method is much easier technique than G1-PCC method which requires 48h culture and 2h incubation with okadaic acid or calyculin A [25]. G2-PCC is useful and powerful method to allow analysis of damaged cells with condensed interphase chromosomes, which are arrested at G2 phase of cell cycle after irradiation. Okadaic acid and calyculin A, specific inhibitors of type 1 and type 2A protein phosphatases, can directly induce PCC at any phase of cell cycle. It has also been applied for biodosimetry for the victims exposed to very high dose radiation [25]. Also, G2-PCC-FISH method can accurately detect dicentric chromosomes, and it will be applicable for biodosimetry for victims exposed to low dose radiation less than 100 -500 mGy with HDR.

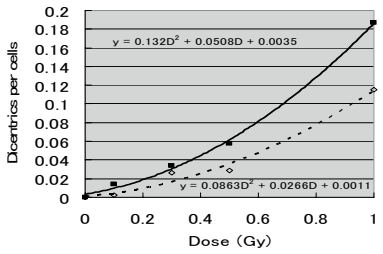


Figure 1 Comparison of conventional Giemsa staining and PCC-FISH to detect dicentric chromosomes in human lymphocytes at the dose range less than 1000 mGy of X-ray irradiation. Upper line : PCC-FISH, Lower line: Conventional Giemsa staining method

Table 1 Detection rates of dicentric chromosomes were compared between two methods of PCC-FISH and conventional Giemsa staining

	PCC-FISH method			Conventional Giemsa staining method			Comparison of detection rate (A/B)
Dose (Gy)	Observed cells	Number of Dicentrics	Dicentrics per cell (A)	Observed cells	Number of Dicentrics	Dicentrics per cell (B)	
0	586	0	0	1210	0	0	0
0.1	589	6	0.01	1238	3	0.002	5
0.3	534	18	0.03	1148	30	0.026	1.2
0.5	552	32	0.06	1004	29	0.029	2.1
1.0	214	40	0.19	496	57	0.115	1.7
3.0	61	80	1.31	N.D.			
5.0	57	165	2.89	N.D.			

N.D.: not done

2 Biological dosimetry for chronic LDR irradiation

2.1 Incidences of dicentric and ring chromosomes and translocations

Dose-response relationships between the incidences of dicentric plus ring chromosomes (Dic+Rc) and total accumulated doses up to 3000 mGy, 4000 mGy, 8000 mGy and 615 mGy at different dose rates of 400 mGy/22h/day, 200 mGy/22h/day, 20 mGy/22h/day and 1 mGy/22h/day were obtained, respectively. Their dose-response relationships in the dose range of less than 8000 mGy are shown in figure 2. Dose-response curves for 890 mGy/min was linear-quadratic, and those for 400 mGy/22h/day, 200 mGy/22h/day, 20 mGy/22h/day and 1 mGy/22h/day increased almost linearly up to 8000 mGy and 615 mGy, respectively. Precise results on frequencies of chromosome aberrations and values of regression coefficient have been summarized in recently published manuscript [12]. Translocations detected by M-FISH, increased almost linearly up to a total accumulated dose of 8000 mGy following irradiation for about 400 days at a LDR of 20 mGy/22h/day (figure 3). These results will be important information to establish suitable biodosimetry method for occupationally or accidentally exposed peoples to chronic LDR radiation.

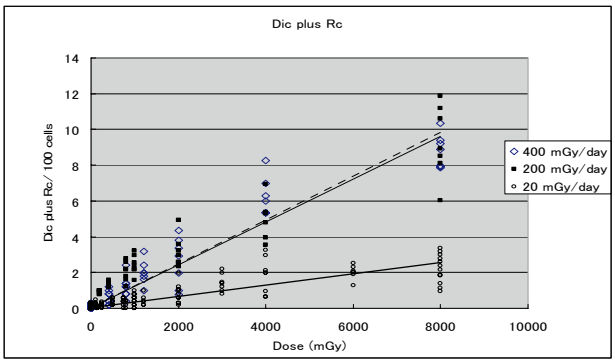


Figure 2 Frequencies of dicenric and centric ring chromosomes (Dic+Rc) per 100 lymphocytes from mice irradiated with MDRs (400 mGy/day; □, solid line and 200 mGy/day; ■, dashed line), and LDR (20 mGy/day; ○, solid line) radiation within the dose range of 8000 mGy. Each symbol indicates the value for an individual mouse. Dic plus Rc: Dic +Rc

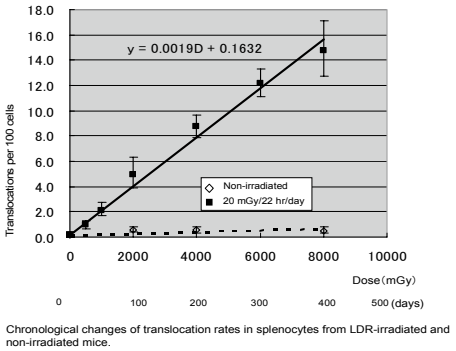


Figure 3 Chronological changes of translocation rates in splenocytes from LDR-irradiated and non-irradiated mice. *X axis is the total dose (mGy) or irradiation period in days, and Y axis is chromosome aberration rates (number of aberrations per 100 metapases). Horizontal bar around each value point shows 95% confidence intervals obtained from 3 mice.

2.2 Dose rate effects

The equations obtained and parameters for the regression fits were obtained by multiple regression analysis. Dose response relationship of chromosome aberration frequencies (Dic+Rc) was obtained in each dose rate using age-adjusted multiple liner regression analysis on the assumption that the relationship is shown by linear or linear quadratic model. Values of linear term, which are shown as slope, were significantly decreased with reduction of dose rates from 400 mGy/22h/day (18.2 mGy/h) to 20 mGy/22h/day (0.91 mGy/h)(figure 4, top). Statistical difference in 95% CI of the slope in the linear regression lines showing yields of aberrations of Dic by FISH was found between 1 mGy/22h/day and 20 mGy/22h/day less than the dose range of 1000 mGy on the assumption that the dose response relationship is shown by linear model (figure 4, bottom). These results are clearly indicating that dose-rate effects on chromosome aberration rates among 400-fold different dose rates from 400 mGy22h//day to 1 mGy/22h/day.

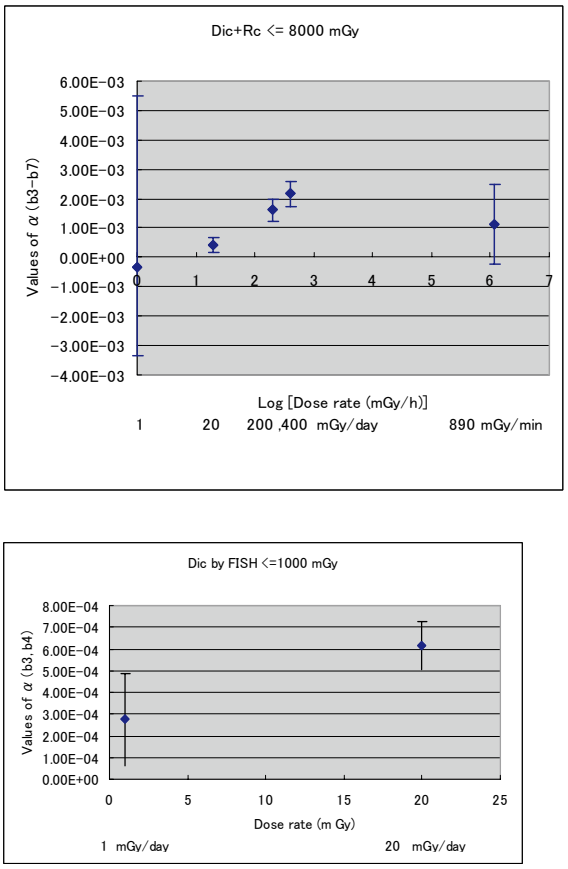


Figure 4 The values of linear term in chromosome aberration frequencies (number of aberrations per 100 metaphases) and dose (mGy) response curve for LDRs(400 mGy/22h/day and 200 mGy/22h/day) and LDRs (20 mGy/22h/day and 1 mGy/22h/day) γ-irradiations. Bars on each point show 95% confidence interval. Top figure shows values of dicentric and ring chromosomes detected by conventional Giemsa method. Bottom figure shows values of dicentric chromosomes detected by FISH method.

These present results imply that DNA damage of splenocytes from continuously irradiated mice can be repaired during MDR or LDR radiation exposure. According to the classical target theory, LDR irradiation has enough time to repair DNA damages

during a long irradiation period, so that dose-rate effects could be disappeared at less than a limited dose rate. However, if bystander effects might be occurred in some non-irradiated cells adjacent to irradiated target cells during continuous LDR irradiation, DNA repair undergoing after DNA damage might occur even in adjacent cells, locating within a distance influenced by cell to cell communication or by released clastogenic factor as well as in target cells. Thus, these concepts implicate that dose-rate effects might occur at lower dose rate. This finding also suggests that there is a discrepancy for the currently used formula to obtain dose-rate effectiveness factor (DDREF) recommended by ICRP, UNSCEARE and so on [26-29]. DDREF of 4.5 for Dic by FISH was obtained by comparing chromosome aberration rates at the same total dose of 100 mGy, at HDR (890 mGy/min) and LDR (20 mGy/22h/day). DDREF was varied from 4.5 to 17.8 as accumulated doses were increased from 100 to 1000 mGy. Furthermore, ratio of values of linear terms of the LDR to HDR was 3.0, which might be used for DDREF.

2.3 Incidence of clone formations

Translocations in mice irradiated at 20 mGy/22h/day were approximately 20 times higher than those at the other two LDRs. Complex chromosome aberrations were found at total doses of more than 4000 mGy in mice irradiated at LDR (20 mGy/22h/day) and were saturated over 6000 mGy. The clone with the same chromosome aberrations in at least three cells, like trisomies of chromosome 15 and 7 was observed from doses of 4000 mGy, and was rapidly increased over 6000 mGy, but it was not found at 300 days in mice irradiated at 1 mGy/22h/day. The results indicate that there might be a threshold dose or dose-rate for formation of clones. These results on dose and dose-rate effects and clone formation are informative for evaluating the risk for cancer development by low dose radiation in human.

3 Array CGH analyses on genomic alterations in MLs developed from mice exposed to LDR irradiation

Array-CGH enabled immunogenotyping of murine MLs. Most of MLs (76 of 82) showed a loss within the *IgH* variable region and often accompanied losses within the *IgL*κ and/or *TCR*β regions. Since the rearrangement of *IgH* precedes that of *IgL*κ during normal B lymphocyte differentiation, the presence or absence of coincidental loss within the *IgL*κ region was likely to reflect differences in the developmental stage of B-cell origin. Comparing between the irradiation and non-irradiation groups, there seemed to be no significant difference between the groups in the developmental stages of origins. This suggested that continuous LDR irradiation did not significantly affect on the developmental stages of origins of MLs. Aneuploidy of each chromosome in all MLs was evaluated. The most notable change was a gain of chromosome 15, which was detected in the irradiation group with 1.6-fold higher frequency (44% *versus* 27%) than in the non-irradiation group (figure 5). Continuous LDR irradiation in the irradiation group might make some contribution to the occurrence of trisomy 15. Expression of oncogene *Myc* on chromosome 15 enforced in transgenic mice plays causal roles in both B-cell and T-cell lymphomagenesis with significantly short latent periods [30, 31]. A correlation between the high expression levels of *Myc* and trisomy 15 has been observed in murine T-cell lymphomas caused by genome hypomethylation [32]. Elevated *Myc* expression was observed in transgenic mice [33]. These are suggesting that trisomy 15 is closely linked to high expression levels of *Myc*. Recurrent gains and losses were also observed in many partial chromosomal regions. Figure 5 shows gain and loss frequencies in each BAC clone. No such predominant aberrations such as losses at genomic regions including *Ikaros* and *Bcl11b*, which are

predominantly found in HDR-radiation induced thymic lymphoma, could not be found in MLs in our life-span study (figures 5). Comparing the frequency of aberrations in MLs between irradiation and non-irradiation groups, we found that losses at regions including *Cdkn2a* (p=0.26) on chromosome 4 and *Rb1* (p=0.26) on chromosome 14 were significantly more frequent in the irradiation group (figure 5, indicated by arrowheads). In addition, losses at regions including *Trp73* (p=0.088) at distal chromosome 4 and *Rel* and *Bcl11a* (p=0.057) on chromosome 11 were preferentially associated with irradiation group (figure 5, indicated by arrowheads).

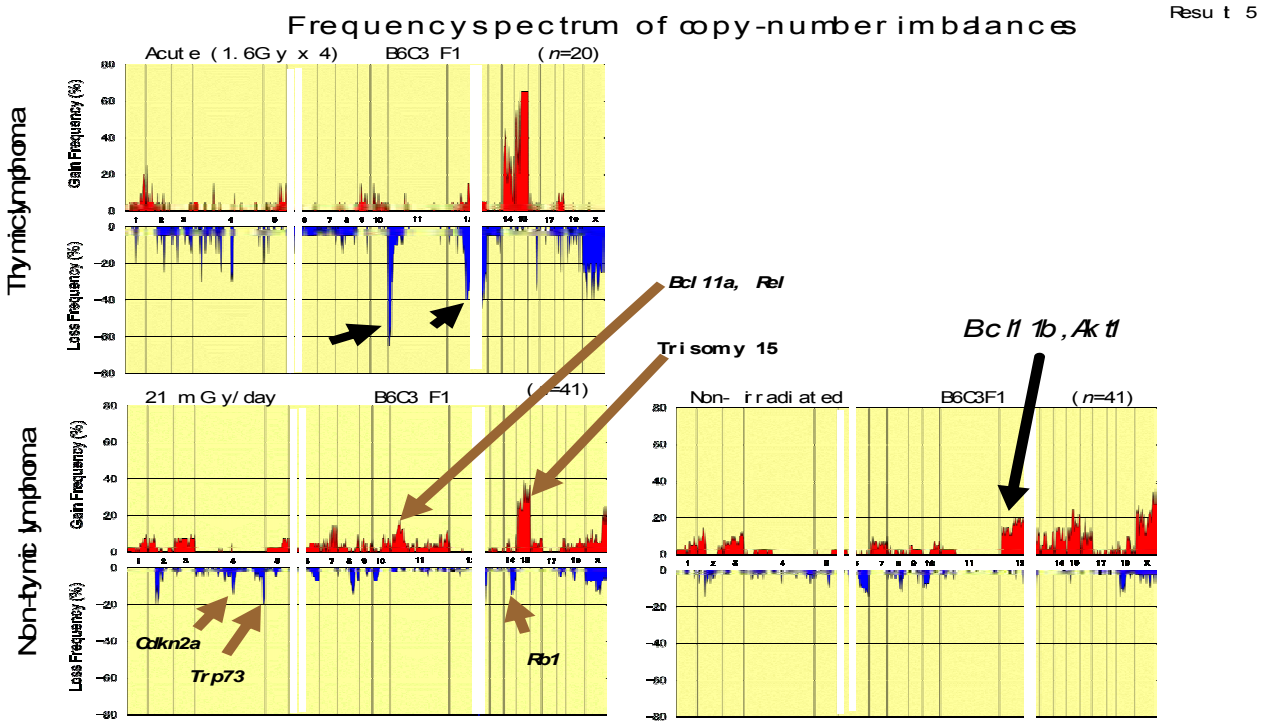


Figure 5 Summary of genome-wide gains and losses in 41 non-thymic lymphomas (MLs) of B6C3H mice irradiated with 21 mGy/22h/day and non-irradiated non-thymic lymphomas (MLs) of control mice (Bottom side). Genomic profile of HDR-X-ray induced thymic lymphoma is also shown for comparison [9] (Top side). Gains and losses are represented by the frequency of each BAC (y-axis) against its position along the chromosomes. Plus (pale) minus (dark) frequencies indicate gain and loss, respectively. Frequent genomic rearrangements at *IgL*κ and *IgH* are observed in non-thymic lymphomas (MLs), but frequent losses around *Ikaros* and *Bcl11b* on chromosomes 10 and 12, respectively, shown by thick small arrow are not observed, indicating thymic lymphoma and non-thymic lymphoma (ML) had quite different genomic alteration. Long arrowheads indicate the regions showing significantly or moderately more frequent (*Bcl11a* and *Rel* on chromosome 11, and *Myc* on trisomy 15) and more infrequent (*Cdkn2a* and *Trp73* on chromosome 4 , and *Rb1* on chromosome 14) , respectively, in the irradiation group than in the non-irradiation group. On the other hand, A1-F3 region on chromosome 12 shows more frequent (*Bcl11b and Akt1*) in the non-irradiation group.

Overall, genomic alterations preferentially detected in the irradiation group were gains on chromosomes 11 and 15, and losses on chromosomes 4 and 14. The candidate critical genes contained in these regions were *Bcl11a, c-myc, Cdkn2a*（alias *p16/Ink4*）, *Cdkn2b, Trp73* and *Rb1*. Similarly, a centromeric region on chromosome 11 is syntenic to a human chromosomal region of chromosome 2 (2p13), where *REL* and *BCL11a* genes are located, reported to be frequently amplified in human diffused type B cell lymphomas and B cell chronic lymphocytic leukemia [34, 35]. On the other hand, genomic alteration highly detected in non-irradiation group was partial loss of chromosome 12, which was consistent with our previous result obtained by loss of heterozygosity (LOH) analysis. Anti-apoptotic gene *Akt1* is a candidate oncogene in the deleted region of chromosome 12. These genomic alterations might implicate on the early death due to MLs in the irradiation group.

In summary, we found significant differences in the frequency spectrum of genomic alterations between irradiation and non-irradiation groups. Considering the above similarity in genomic alterations between human aggressive malignant lymphomas and murine malignant lymphomas developed in the irradiation group, the earlier development of MLs in the irradiation groups could be explained by the possibility that highly aggressive lymphomas develop preferentially by LDR irradiation. These findings suggest that lymphomagenesis under the effect of chronic LDR irradiation is accelerated by a mechanism different from spontaneous lymphomagenesis. Also, these results will be useful information for establishing method to identify radiation related-neoplasms developed long-term after radiation accident.

Acknowledgements

This study was performed under contract with the Aomori Prefectural Government, Japan. We thank Drs. Hayata I. and Yoshida M. of National Institute for Radiological Sciences, Japan for technical help of PCC method and useful suggestions.

References

[1] Evans HJ, Buckton KE, Hamilton G.E, Carothers A. Radiation-induced chromosome aberrations in nuclear-dockyard workers. *Nature* 277:531-534 (1979).

[2] Bauchinger M, Kolin-Jerrneim J, Schimid E, Dresp J. Chromosome analysis of nuclear-power plant workers. *Int. J. Radiat. Biol.* 38: 577-581 (1980).

[3] Lloyd DC, Purrot RJ, Reeder EJ. The incidence of unstable chromosome aberrations in peripheral blood lymphocytes from un-irradiated and occupationally exposed people. *Mutat. Res* 72: 523-532 (1980).

[4] Kumagai E, Tanaka R, Kumagai T, Onomichi M, Sawada S. Effects of long-term radiation exposure on chromosomal aberrations in radiological technologists. *J. Radiat. Res.* 31: 270-279 (1990).

[5] Hseh WA, Ni C, Hwang JJ, Fang JS, Lin SP, Lin YA, Huang TW, Chang WP. Evaluation of the frequencies of chromosome aberrations in a population exposed to prolonged low dose-rate ^{60}Co gamma-irradiation. *Int. J. Radiol. Biol.* 78: 625-633 (2002).

[6] Tanaka S, Tanaka IB, Sasagawa S, Ichinohe K, Takabatake T, Matsushita S, Matsumoto T, Otsu H, Sato F. No lengthening of life span in mice continuously exposed to gamma rays at very low dose rates. *Radiat. Res.* 160: 376-379

(2003).

[7] Tanaka IB Ⅲ, Tanaka S, Ichinohe K, Matsushita S, Matsumoto T, Out H, Oghiso Y, Sato F. Cause of death and neoplasia in mice continuously exposed to very low dose rates of gamma rays. *Radiat. Res.* 167: 417-437 (2007).

[8] Takabatake T, Fujikawa K, Tanaka S, Horiuchi T, Nakamura M, Nakamura S, Tanaka IB Ⅲ, Ichinohe K, Saitou M, Nishimura M, Shimada Y, Oghiso Y, Tanaka K. Array-CGH analysis of murine malignant lymphomas: Genomic clue to understanding the effects od chronic exposure to low-dose-rate gamma rays on lymphomagenesis. *Radiat. Res.* 166: 61-72 (2006).

[9] Takabatake T, Kakinuma S, Hirouchi T, Nakamura MM, Fujikawa K, Nishimura M, Oghiso Y, Shimada Y, Tanaka K. Analysis of changes in DNA copy number in radiation-induced thymic lymphomas of susceptible C57BL/6, resistant C3H and hybrid F_1 mice. *Radiat. Res.* 169: 426-436 (2008).

[10] Hirouchi T, Takabatake T, Yoshida K, Nitta Y, Nakamura M, Tanaka S, Ichinohe K, Oghiso Y, Tanaka K. Upregulation of *c-myc* gene accompanied by *PU.1* deficiency in radiation-induced acute myeloid leukemia in mice. *Experiment. Hematol.* 36: 871-885 (2008).

[11] Takabatake T, Ishihara H, Ohmachi Y, Tanaka I, Nakamura MM, Fujikawa K, Hirouchi T, Kakinuma S, Shimada Y, Oghiso Y, Tanaka K. Microarray-based global mapping of integration sites for the retrotransposon, intracisternal A-particle, in the mouse genome. *Nucleic Acid Res.* 36: (10) e59 (2008). doi:10.1093/nar/gkn235

[12] Tanaka K, Kohda A, Satoh K, Toyokawa T., Ichinohe K, Ohtaki M., Oghiso Y. Dose-rate effectiveness for unstable-type chromosome aberrations detected in mice after continuous irradiation with low-dose-rate γ-rays. *Radiat. Res.* 171: 290-301 (2009).

[13] Tanaka K, Kohda A, Toyokawa T, Ichinohe K, Oghiso Y. Chromosome aberration frequencies and chromosome instability in mice after long-term exposure to low-dose-rate γ-irradiation. *Mutat. Res.* 657: 19-25(2008).doi:10.10.16/j.mrgentox.2008.09 .006

[14] Sugihara T, Magae J, Wadha R, Kaul SC, Kawakami Y, Matumoto T, Tanaka K. Dose and dose-rate effects of low-dose ionizing radiation on activation of Trp53 in immortalized murine cells. *Radiat Res.* 162: 296-307 (2004).

[15] Sugihara T, Murano H, Tanaka K, Oghiso Y. Inverse dose-rate-effects on the expressions of extra-cellular matrix-related genes in low-dose-rate γ -ray irradiated murine cells. *J. Radiat. Res.* 49: 231-240 (2008).

[16] Vielenchik MM and Knudson AG. Jn. Inverse radiation dose-rate effects on somatic and germ-line mutations and DNA damage rates. *Proc. Natl. Acad. Sci. USA* 97:5381-5386 (2000).

[17] Vielenchik MM and KnudsonAG.. *Proc. Natl. Acad. Sci. USA* 100: 12871-12876 (2003).

[18] Vielenchik MM and Knudson AG.. Radiation dose-rate effects, endogenous DNA damage, and signaling resonance. *Proc. Natl. Acad. Sci. USA* 103: 17874-17879 (2006).

[19] Mitchel RE, Jacson JS, Morrison DP, Carlisle SM. Low dose of radiation increase the

latency of spontaneous lymphomas and spinal osteosarcomas in cancer-prone, radiation-sensitive *Trp53* heterozygous mice. *Radiat. Res.* 159: 320-327 (2003).

[20] Yonezawa M, Horie K, Kondo H, Kubo K. Increase in eodogenous spleen colonies without recovery of blood cell counts in radioadaptive survivial response in C57BL/6 mice. *Radiat. Res.* 161: 161-167 (2004).

[21] Krueger SA, Joiner MC, Weinfeld M, Piasentin E, Marples B. Role of apotosis in low-dose hyper- radiosensitivity. *Radiat. Res.* 167: 260-267 (2007).

[22] Durante M, Gerge K, Yang TC. Biological dosimetry by interphase chromosome painting. *Radiat. Res.* 145: 53-60. (1996).

[23] Durante M, Gerge K, Yang TC. Biodosimetry of ionizing radiation by selective painting of prematurely condensed chromosomes in human lymphocytes. *Radiat. Res.* 148: S45-S50 (1997).

[24] M'kacher R Violet D, Aubert b, Girinsky T, Dossou J, Béron-Gaillard N, Carde P. Parmentier C. Premature chromosome condensation associated with fluorescence in situ hybridization detects cytogenetic abnormalities after a CT scan: evaluation of the low-dose effects. *Radiat. Prot. Dosimetry* 103:35-40 (2003).

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

[26] ICRP (1991) Pub. 60, Annals of the ICRP, vol.21, no.1-3, Pregamon Press, Oxford.

[27] UNSCEARE (1993) Sources and Effects of Ionizing Radiation, with Scientific Annexes, United Nations, New York

[28] BEIR Ⅶ-2 (2006) National Research Council, Committee on Health Risks from Exposure to Low levels of Ionizing Radiation National Academic Press, Washington DC.

[29] NCRP (1980) Influence of Dose and its Relationships for Low-LET Radiations, pp.95-131, Report no.64, Bethesda, MD.

[30] Marinkovic D, Marinkovic T, Mahr B, Hess J and Wirth T. Reversible lymphomagenesis in conditionally c-MYC expressing mice. *Int. J. Cancer* 110: 336-342 (2004).

[31] Smith DP, Bath ML, Harris AW, Cory S. T-cell lymphomas mask slower developing B-lymphoid and myeloid tumors in transgenic mice with broad haemopoietic expression of MYC. *Oncogene* 24: 3544-3553(2005).

[32] Gaudet F, Hodgson JG, Eden A, Jacson-Grusby L, Dausman J, Gray JW, Leonhardt H, Jaenisch R. Induction of tumors in mice by genomic hypomethylation. *Science* 300: 489-492 (2003).

[33] Marinkovic D, Marinkovic T, Mahr B, Hess J, Wirth T. Reversible lymphomagenesis in conditionally c-MYC expressing mice. *Int. J. Cancer* 110: 336-342 (2004).

[34] Gilmore TD, Kalaitzidis D, Liang MC, Starczynowski DT. The c-Rel transcription factor and B-cell proliferation: a deal with the devil. *Oncogene* 23: 2275-2286 (2004).

[35] Nakamura T, Yamazaki Y, Saiki Y, Moriyama M, Laraespada DA, Jenkias NA, Copeland NG. Evi9 encode a novel zink finger protein that physcically interact withBCL6, a known human B-cell proto oncogene product. *Mol. Cell Biol.* 20: 3178-3186 (2000).

Radiation Detection and Measurement in Patients Contaminated with Alpha Emitters

Takumaro Momose[1*], Osamu Kurihara[1], Chie Takada[1], and Sadaaki Furuta[1]

[1] *Radiation Protection Department, Nuclear Fuel Cycle Engineering Laboratories, Japan Atomic Energy Agency, Tokai, Ibaraki, Japan*

Abstract. The basic principle of radiation control at plutonium facilities such as characteristics of plutonium and relevant radionuclides which operate in nuclear fuel cycle facilities is introduced and potential radioactive contamination in accidents is discussed. The decontamination procedure of skin at the Japan Atomic Energy Agency, Nuclear Fuel Cycle Engineering Laboratories (hereinafter referred to as JAEA-NCL) is introduced in practice for some of the operating principles and applications of various radiation detection and measurement instruments for contamination control and occupational monitoring for internal contamination. Special methods of measuring alpha emitters for performing radiological monitoring such as radio autography of plutonium contaminations on smear samples, nasal swab samples and measurement, in vivo and in vitro analysis for plutonium and uranium have been improved and proven. The internal dose calculation code was originally developed in order to estimate retrospectively in case of accidental intake of plutonium.

Key Words: radiation protection, radiation detection, monitoring instrument, plutonium, radiation contamination, nuclear emergency, internal exposure

Introduction

The medical treatment of patients contaminated with radioactive materials is one of the most important concerns in radiation emergency preparedness. Information on the level of external contamination and the subsequent radiation risk should be updated as soon as possible so that the proper medical treatment, as well as radiation control, can be initiated. The patients at nuclear fuel cycle related-facilities are possibly contaminated with alpha emitters, such as plutonium or uranium isotopes. Although skin contaminated with alpha emitters has no importance regarding external exposure, one should notice that internal contamination due to the intake of alpha emitters may result in serious exposure despite the amount taken in being small compared to typical beta emitters (e.g., ^{60}Co, ^{137}Cs). Several documents for a nuclide-specific safety guide have been published in the US [1] [2] . The International Atomic Energy Agency (IAEA) has also published reports on occupational radiation protection [3] [4].

This paper provides a brief review of practical techniques related to radiation measurements and internal dose assessments, especially for alpha emitters. The techniques shown in the present paper have been developed at JAEA-NCL in response to emergency situations with unexpected alpha emitter contamination.

Alpha emitters in nuclear fuel cycle facilities

Plutonium

Plutonium is one of the major elements, along with uranium, treated at nuclear fuel cycle facilities. The isotopic composition of plutonium mainly consists of ^{238}Pu, ^{239}Pu, ^{240}Pu and ^{241}Pu. The plutonium isotopes are produced as a result of neutron irradiation to uranium in thermal neutron reactors; ^{239}Pu is firstly produced through neutron

[*] Corresponding to: Takumaro Momose, Deputy Director, Radiation Protection Department, Nuclear Fuel Cycle Engineering Laboratories, Japan Atomic Energy Agency, 4-33 Muramatu, Tokai, Ibaraki, Japan
E-mail: momose.takumaro@jaea.go.jp

capture of ^{238}U to two sequential beta decays of ^{239}U (i.e., ^{239}U→^{239}Np→^{239}Pu) and higher mass numbered-plutonium isotopes are produced by a sequential neutron capture process, i.e. (n, gamma) reactions. The most abundant plutonium isotope in terms of the alpha activity is ^{238}Pu, in case of nuclear fuel graded plutonium.

One important characteristic of nuclear react or-grade plutonium is ^{241}Am in-growth from ^{241}Pu with relatively short physical half time (14.4 y). Thus, ^{241}Am is mostly accompanied with plutonium isotopes at nuclear facilities. Figure 1 shows the relative activity ratio of the plutonium isotopes and ^{241}Am as a function of time after plutonium has been extracted at a reprocessing plant. This figure also indicates the contribution of ^{241}Am to the total alpha activity in radiological samples (e.g. nasal swabs or excreta) vary depending on the process of concern at the facilities.

It is however important to know the activity ratio of ^{241}Am, especially for *in vivo* monitoring, such as lung monitoring or wound monitoring. Since ^{241}Am emits gamma rays (59.5 keV) that can be more easily detected than X-rays (13.6, 17.1 and 20.3 keV) from the plutonium isotopes, this results in a significant improvement of the detection sensitivity of plutonium compounds.

Concerning the chemical forms of plutonium in nuclear fuel cycle facilities, plutonium nitrate and oxide are commonly handled: the former exists in nitrate solutions prepared in the chemical process at a reprocessing plant and the latter exists in the process at mixed oxide uranium and plutonium (MOX) facilities.

The physicochemical form of the plutonium compounds vary depending on the process concerned. Note that it is of great importance to determine the chemical form and the activity median aerodynamic diameter (AMAD), because such properties greatly affect the dose coefficient. The international commission on radiological protection (ICRP) provides tables of the dose coefficients per unit intake (Sv Bq^{-1}) of various nuclides with typical physicochemical properties via inhalation or ingestion. The Annual Limit of Intake (ALI) corresponding to the annual dose limit of 20 mSv per a single year is calculated with the following equation.

$$\mathrm{ALI\ (Bq)} = 0.02\ \mathrm{(Sv)} / e(50)\ \mathrm{(Sv\ Bq^{-1})}$$

where, e(50) is the dose coefficient of the nuclide of concern. For example, the ALI of ^{239}Pu in the nitrate form is calculated as 630 Bq for when it is inhaled. This value is a few thousand times smaller than the corresponding value of ^{60}Co or ^{137}Cs. On the other hand, the ALI of ^{239}Pu in the same chemical form is 3.8E+05 Bq for ingestion. This is attributed to very poor uptake ratio of the plutonium compounds in the gastro-intestinal tract.

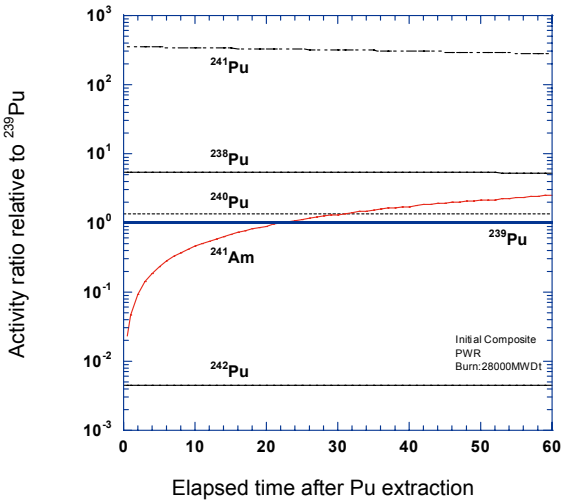


Figure 1 Variation of isotopic composition of plutonium with elapsed time. The initial isotopic composition of plutonium is calculated with ORIGEN code (burn-up: 28 000 MWDt).

Uranium

Uranium is relatively abundant in nature. Natural uranium consists of a mixture of ^{234}U, ^{235}U and ^{238}U isotopes, along with their decay products. The isotope ratio of ^{234}U, ^{235}U and ^{238}U in natural uranium are 0.0055%, 0.72% and 99.28%, respectively. The primary isotopes of uranium are long lived alpha-emitters with energies between 4.15 and 4.8 MeV. Their progeny include numerous other radio nuclides, some of which are radiologically significant. Major decay products of natural uranium are ^{234}Th, ^{234m}Pa and ^{231}Th.

Light water reactors for electric power generation are designed for use with enriched uranium of around 3-5% ^{235}U. The ^{235}U enrichment process also increases the concentration of ^{234}U. The isotope ratio of ^{234}U, ^{235}U and ^{238}U in typical commercial enriched uranium for light water reactors are 0.03%, 2.96% and 97.01%, respectively[1].

In nuclear fuel cycle facilities, the chemical properties of uranium change depending on the process. Typical chemical forms are ammonium diuranate ($(NH_4)_2U_2O_7$) known as yellow cake, UF_6 in which enrichment is performed and UO_2 which is used in the

reactor.

The limit for uranium intake is mainly concerned with the chemical toxicity to kidneys. A concentration of 3 μg of uranium per gram (μg U/g) of kidney tissue has traditionally been used as the guideline for controlling the chemical toxicity of uranium. This value corresponds to a total kidney burden of 1 mg for a control person with a kidney mass of 310 g.

Detection and measurement of alpha contamination in the work place

Measuring airborne radioactive concentration

Continuous airborne radioactive dust monitors (dust monitors) for alpha emitters are used extensively in plutonium and uranium facilities. Dust monitors should be placed beside the glove boxes and hoods in the work place. Dust monitors using surface barrier solid state Si detector have adequate detection capabilities for real-time monitoring at the DAC level. For ^{239}Pu, the dose coefficient is 3.2×10^{-2} mSv/Bq for absorption type M compounds based on the DAC of 7×10^{-7} Bq/cm^3, as given in the Japanese regulations for nuclear safety. JAEA dust monitors are capable of alarming at 0.5 DAC when averaged over 8hours (4 DAC-hours) under laboratory conditions. When monitoring for alpha emitters in areas with high radon concentrations an alarm set point may be necessary which is greater than 8 DAC-hours.

The conventional autoradiography using a ZnS(Ag) scintillator and Polaroid® film has been used for analysis of radioactivity on dust sample filters in order to visually discriminate between plutonium and Rn progenies.

In recent years, Uezu *et al* demonstrated that sequential decay events of radon progenies within a certain time interval (e.g., ^{214}Bi $\rightarrow$ ^{214}Po) could be discriminated by a time interval analysis (TIA) method [6]. Hashimoto *et al* and Sanada *et al*. developed a prototype dust monitor equipped with a background compensation circuit based on the TIA method [7][8]. This monitor successfully removed counts originated from the radon progenies, and would thus be effective for identifying only plutonium aerosols under conditions with high concentrations of radon progenies.

Koarashi et al.[9] and Takasaki et al. [10] developed an autoradiographical method using an imaging plate (IP) to identify plutonium particles on filter samples. Photostimulated luminescence (PSL) signals were obtained by exposing IPs to filter samples collecting Pu particles and naturally occurring radon decay products. The difference of specific activity between plutonium and Rn progenies induced different signals of the PSL intensity, which makes it possible to discriminate Pu particles from Rn progenies using an empirical Pu-discrimination level, and then to quantify the Pu activities on a particle-by-particle basis. The method is useful for a fast screening of filter samples for plutonium particles. Figure 2 shows plutonium particle imaging measured by IP.

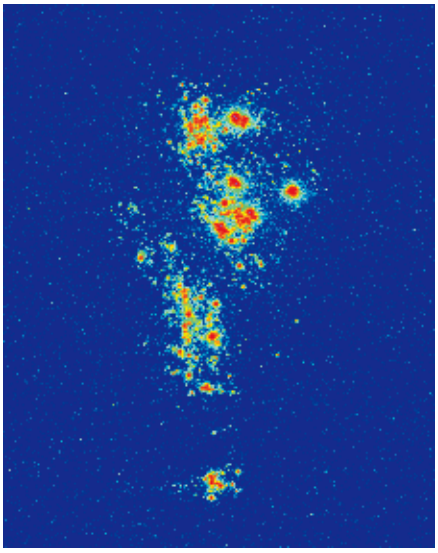


Figure 2 Plutonium particle imaging by IP[10].

IP:FUJIFILM BAS-MS2325 IP Reader:BAS-1800 II

Surface contamination

The detection and measurement of plutonium contamination is essential to ensure the measures for control. Typically, detection of plutonium contamination has been performed using check instruments that detect alpha activity. The ZnS alpha scintillation detector is usually used to detect plutonium contamination. The ZnS(Ag) scintillation detector had a detection limit of 2×10^{-2} Bq/cm^2 for gross alpha counting. Self-absorption of plutonium alpha particles within the source or in an irregular surface area may require the use of special X-ray and low energy photon detectors (e.g., thin NaI detector or a phoswich detector).

The smear method is also useful for measuring removable contamination.

Contamination check and decontamination for normal skin

External exposure is not caused by skin contaminated with alpha emitters because alpha particles cannot penetrate the layer insensitive to

radiation at the surface of the skin. But, internal exposure may be caused by skin contamination through inhalation, ingestion and/or absorption from the skin. Therefore decontamination should be started as soon as possible after skin contamination is discovered.

Skin checks and decontamination should be performed by radiation protection staff. The treatment and decontamination of wounds should be performed by medical staff.

Nonabrasive methods should be used for skin decontamination to protect tissue from further contamination. Surgical tape is effective for dry contamination to remove or avoid re-suspension. Wet decontamination should be used to remove residual contamination. The skin should be gently scrubbed with detergent and water.

The following procedure is recommended:

1. Get information about the contaminated patient from accompanying personnel (condition of health and details of the injury, situation of the accident, the area of skin contaminated, radionuclide and chemical forms of the contamination, etc.)
2. Remove contaminated clothing. Quickly check the patient to determine the contaminated skin areas. Have the medical staff treat and decontaminate breaks in the skin.
3. Wipe loose contamination with a gauze sponge or cotton applicators dipped in mild antiseptic detergent. Do not spread contamination to uncontaminated areas. Hair contamination can be wiping in the same way, but cutting the contaminated hair off is sometimes effective where necessary.
4. Use soft bristle scrub brushes for fingernails and other difficult-to-clean areas as long as the skin is kept intact. It may be difficult to decontaminate the cuticles and under the nails.
5. Dry the skin with cleansing tissue. After the skin is dry, check it for any remaining contamination.

The decontamination factor is defined as the ratio of the initial contamination level to the level after decontamination. Nonabrasive methods should be repeated until the decontamination factor between washes drops to below 2 or 3 with significant contamination still remaining.

Though there is a more abrasive decontamination detergent such as titanium dioxide paste, application should be considered carefully based on the effectiveness of decontamination. An abrasive detergent should be applied with a moist gauze sponge or soft hand brush to prevent damage to the skin surface. Liberal irrigation with a saline solution is suitable for eyes, nose, and mouth decontamination. Recently many detergents available as cosmetics and sanitation are applicable to radiological decontamination. Takasaki *et al.* examined ^{144}Ce, ^{137}Cs, ^{106}Ru and ^{60}Co removal rates of some commercial detergent experimentally on raw pig skin which imitates human skin well [11]. The averaged removal rates of typical detergents are listed in the reference. Neutral detergent, facial cleansers which include EDTA, orange oil cleaner, titanium dioxide which include dilute hydrochloric acid are provided for skin decontamination at the JAEA Tokai reprocessing plant. These detergents can also be practically applied to plutonium contamination.

Bioassay program

Indications of internal exposure include detection of facial contamination or nasal contamination, air monitoring that indicates significant contamination, or any wound in which contamination is detected or suspected.

Personal monitoring for intake of alpha emitters is performed using bioassay procedures. Bioassay monitoring includes both direct (*in vivo*) measurements of radioactivity in the body and indirect (*in vitro*) measurements of material excreted from the body.

Nasal swabs as an indicator of significant inhalation

In our experiments, the nasal swab method proved to be a good indicator of significant intake of alpha emitters via inhalation. Our proposed swab consists of a cotton swab and a filter paper wrapped around the top of the swab. The swab is inserted into each nostril and a portion of the alpha emitters are collected on the filter. The radioactivity of the filter is directly measured with a ZnS(Ag) scintillation counter. The minimum detectable activity (MDA) of this method is determined to be 0.07 Bq for a counting time of 5 minutes. The nasal swab method is sensitive enough to detect resulting internal exposure below the typical recording dose level (1 mSv), and is used as a trigger for deciding further individual monitoring protocols, such as *in vivo* or *in vitro* techniques.

Kurihara *et al*. investigated individual monitoring data obtained from past inhalation cases of plutonium compounds at our laboratories, and introduced the relationship between total alpha activity (almost all of ^{238}Pu, $^{239+240}$Pu and ^{241}Am) of the nasal swabs and that of the early faeces excreted for five successive days after inhalation [12]. It was found that the relationship was widely scattered as

shown in Figure 3, and the activity ratio between them was agreed with a log-normal distribution. The wide range of the activity ratio was perhaps due to differences in physiological conditions for each subject and/or physicochemical characteristics of inhaled materials. They proposed the activity of the nasal swab as use of practical action levels for initiating chelation therapy, using the activity ratio of the early faeces to the nasal swab. Assuming the activity ratio as 100 (the rounded 95% percentile value), the action levels of the nasal swab were determined to be 15 Bq and 4 Bq (corresponding to 10 ALI of committed effective dose, 200 mSv), for typical compounds of plutonium handled at MOX fuels fabrication facilities and the reprocessing plant in our laboratories.

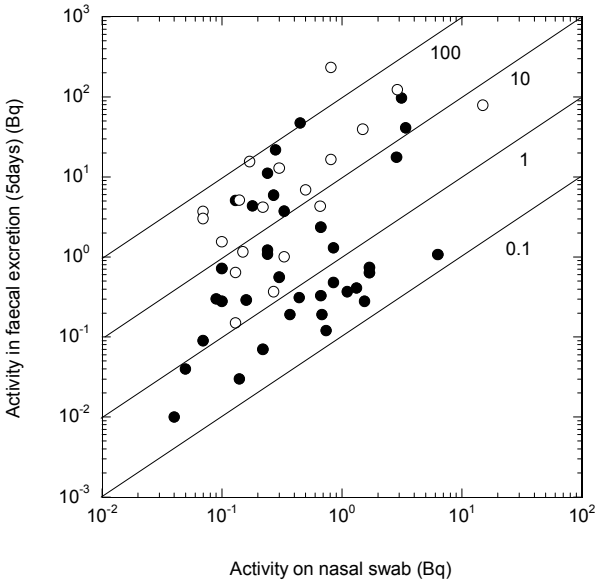


Figure 3 Relationship of activity between nasal swab and total faecal excretion for successive five days after intake.[12]
●PuO_2 ○$Pu(NO_3)_4$. Values below each line are the ratios of faeces to nasal swab.

In vivo monitoring

Lung counter

Some low-energy x-rays emitted by plutonium decay products are detectable with a photon counter outside the body. When direct measurement is used, the detection system should be calibrated for measuring radionuclides in the appropriate organs. A lung counter is a typical direct measurement system for plutonium and americium in the lungs. In general, the 17-keV L X-rays are detected for plutonium and the 60-keV gamma rays are detected for ^{241}Am. The counting efficiency of the JAEA Ge lung counter (Canberra, high-purity germanium detectors; ACT-II) was determined by using both torso phantom which developed by Lawrence Livermore National Laboratory and JAERI torso phantom. Collection factors based on chest wall thickness were also determined. The MDA of JAEA lung counting system for typical chest wall thickness is around 10 Bq of ^{241}Am and around 4 kBq of ^{239}Pu.

The americium-tracer method has been considered because of the advantage of having a small MDA of plutonium and being less affected by attenuation in the chest wall. However, estimation of the plutonium and americium ratio is not easy before measurement in general. Photon spectrometry of nasal swabs may indicate plutonium and americium ratio.

In 1996-1998, an international intercomparison study was performed by the IAEA using JAERI Asian torso phantoms which facilitated the calibration and comparison of lung counter or other in vivo counting systems [13]. In this program, using the germanium detector systems achieved reasonable agreement for normalized counting efficiency. This literature gave useful information on the lung counter calibration methodologies concerned to the correction factor for counting efficiency which depends on the chest wall thickness.

In case of internal contamination by mixtures of spent fuel material, whole body counting is also effective for internal dose assessment if the ratio of a readily detectable gamma-emitter, such as ^{137}Cs, to plutonium is known. Actually, when the fire and explosion occurred at the Bituminization Facility of Tokai Reprocessing Plant on March 11, 1997, personal dose of internal exposure caused by inhaling a mixture of ^{137}Cs and other radionuclides including plutonium were determined by this method. Isotopic ratios were determined by using a radio-chemical analysis of nasal swabs. In this case, only ^{137}Cs and ^{134}Cs could be detected by using a Ge whole body counter. As the ratios between ^{137}Cs and other nuclides such as ^{238}Pu, ^{239}Pu, ^{241}Am etc, were determined by radio chemical analysis of nose swab samples we could estimate all radionuclides which were taken in by this accident.

Development of ^{241}Am lung monitoring system using an imaging plate

Hirota *et al*. developed a new ^{241}Am lung monitoring system without shielding by using an imaging plate system [14]. The anthropomorphic torso phantom which was developed by the US Lawrence Livermore National Laboratory

containing a ^{241}Am lung was covered by imaging plates sealed in lightproof bags. The imaging plate system displayed ^{241}Am lung image characteristics of a lung shape in the torso phantom. The lower detection limit of the imaging plate systems is 14Bq for a 60 min exposure without background shielding. This system has the potential to be applied to mass personal monitoring in nuclear hazards.

In vitro analysis

Plutonium and americium in excreta are generally analyzed by the radiochemical separation method with anion-exchange using ^{236}Pu or ^{242}Pu and ^{243}Am as tracers by alpha spectrometry. The MDA of alpha emitters of plutonium isotopes ^{238}Pu and $^{239+240}$Pu including ^{241}Am are determined to be lower than 4 mBq/sample by alpha spectrometry with a surface barrier Si detector in a counting time of 80,000 sec at the 99.97 (3σ) confidence level. Beta emitter ^{241}Pu is measured by liquid scintillation counter [15].

Urinalysis

Urinalysis is an effective method for internal dose evaluation in cases of plutonium and uranium contamination. After chemical isolation, the plutonium and the uranium in urine samples are determined by alpha spectrometry and mass spectrometry. Uezu *et al.* developed systematic analytical methods for bioassay samples which included plutonium and reprocessed uranium by combination of ICP-MS and alpha spectrometry using a Si surface barrier detector [16]. Urine samples should be collected so as to minimize cross-contamination. Samples should be collected in contamination-free containers. For example it should be considered that uranium is occasionally kept in glass containers. Cleaning the surface of the container should also be considered for minimizing cross-contamination.

Fecal Analysis

Fecal analysis is an effective method for internal dose evaluation for plutonium and many other radioactive materials because more than half of the material deposited in the upper respiratory tract is cleared rapidly to the stomach and gastrointestinal tract. Both the total fecal and the urinary excretions should be collected for the first 3-5 days after intake. In cases of single dose intakes of plutonium, fecal analysis following intakes will give a more accurate result than urinalysis because of its high excretion rate. Our experience of intake cases of plutonium was summarized in another paper [12]. The relationship between activity on nose swab samples and that in early fecal excretion and the pattern of daily bowel motion were obtained from the cases. Early fecal excretion which were observed at JAEA facilities after PuO_2 inhalation have good agreement with predicted values obtained using ICRP 78 models as shown in figure 4.

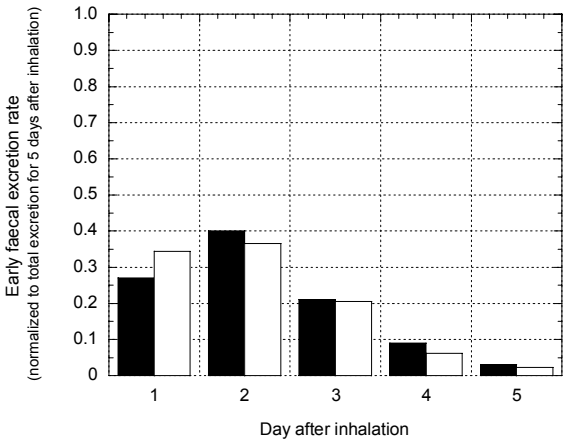


Figure 4 Early fecal excretion pattern after PuO_2 inhalation [12].
□: Observed values ■: Predicted values (ICRP78 Type S)

Internal dose calculation systems

The ICRP has developed biokinetic models that describe the behaviour of various nuclides in the human body. In the past few decades, these models have evolved to be more sophisticated so that realistic dose assessment can be conducted. The ICRP also assigns “typical” or “representative” numerical values to a wide range of parameters used in the models. Dosimetric quantities provided by the ICRP publications are calculated using these typical values in the models, but only those quantities do not fully cover realistic dose assessments in which we should take into account specific parameter values, such as the absorption rate, particle size, biokinetic characteristics of the subject and so on. For this purpose, we developed a new internal dose calculation system called the REtrospective Internal Dose Assessment Code (REIDAC) [17]. We designed the REIDAC so that the dosimetric quantities in the ICRP publications can be completely reproduced by original numerical solutions. Thus, specific dosimetric quantities can be obtained by modifying corresponding data in the file contained in REIDAC. Functional graphical user interfaces (GUIs) provided by REIDAC facilitates calculations of the

dosimetric quantities, as shown in Figure 5. In addition, we proposed a provisional biokinetic model used for wound contamination and incorporated the model into REIDAC. The provisional calculations are shown in Figure 6.

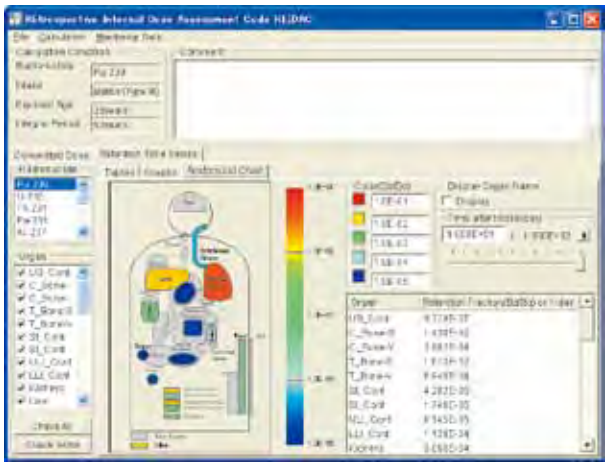


Figure 5 Screen of resulting display of REIDAC.

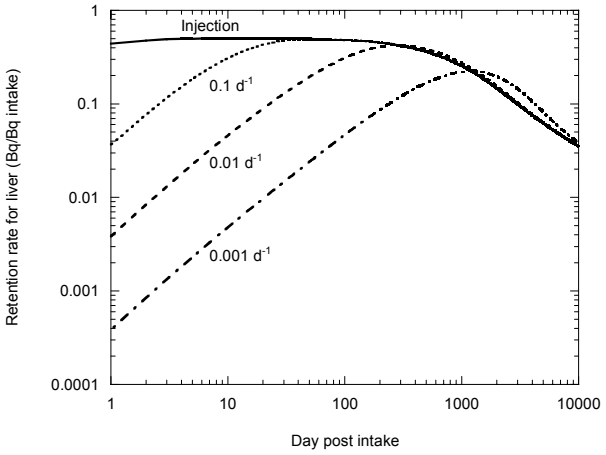


Figure 6 Retention rate of the liver in the case of ^{241}Am wound contamination with different transfer rates

Another system called RAPUTA was also developed at our laboratories [18]. A unique feature of RAPUTA is to perform the general biokinetic model calculations (Th, Np, Pu, Am, Cm) with time-dependant transfer rates between compartments. A step-wise function of the desired transfer rate can be designated in the system. This feature is intended to be used for evaluating the dose reduction by chelation therapy for cases where plutonium compounds are taken in. Examples of provisional calculations are shown in Figure 7. We evaluated the effectiveness of the therapy for different routes of intake or different absorption types, assuming that chelating agents completely accompany plutonium in the body. These results served well as an explanation of the experimentally proven effectiveness of the chelating agents.

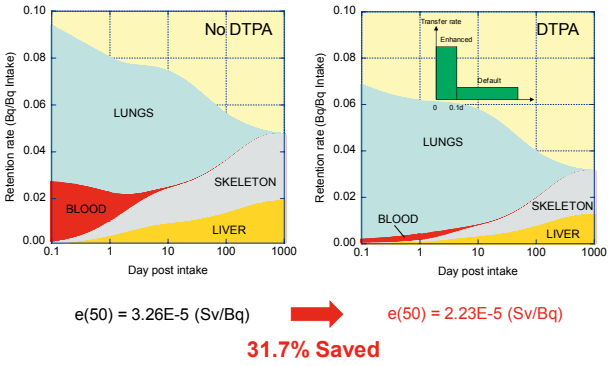


Figure 7 Provisional calculations of biokinetic behaviors of plutonium without/with the chelating agent in case of inhalation of Type M compounds and the corresponding averted dose.

Summary

We introduced various techniques of measurement and treatment for contamination by alpha emitters which were performed in JAEA Nuclear Fuel Cycle Engineering Laboratories. Our developed techniques have been transferred to the commercial reprocessing plant in Rokkasyo, Aomori prefecture through the corporation agreement between JAEA and JNFL.

We hope that the method introduced here would be useful for the people concerned with radiation emergency medicine.

Recently, many ISO/IEC standards relating to radio bioassay and contamination measurements are under development and some of us contributed to these activities. We should consider harmonization these standards and our methodologies in the future.

References

[1] DOE-STD-1136-2004, Guide of Good Practices for Occupational Radiological Protection in Uranium Facilities (2004)

[2] DOE-STD-1128-2008 Guide of Good Practices for Occupational Radiological Protection in Plutonium Facilities (2008)

[3] IAEA SAFETY STANDARDS SERIES No. RS-G-1.1, Occupational Radiation Protection (1999)

[4] IAEA SAFETY STANDARDS SERIES No. RS-G-1.2, Assessment of Occupational Exposure to Intakes of Radionuclides (1999)

[5] IAEA SAFETY REPORTS SERIES No. 9, Safe Handling and Storage of Plutonium (1999)

[6] Uezu Y, Hashimoto T. New aspects of time interval analysis method for the determination of artificial alpha-nuclides, Journal of Radioanalytical and Nuclear Chemistry, Vol. 255, No. 1 (2003) 87–89

[7] Hashimoto, T., Sanada, Y. and Uezu, Y., Simultaneous determination of radionuclides separable into natural decay series by use of time-interval analysis, Anal. Bioanal. Chem.,379, 227-233 (2004)

[8] Sanada Y.,Kobayashi H., Furuta S., Nemoto K.,Kawai K., and Hashimoto T.,Selective Detection Using Pulse Tome Interval Analysis for Correlated Events in Rn-progeny with a Microsecond Lifetime, RADIOISOTOPES, 55, 727-734 (2006)

[9] Koarashi J., Saito F., Akiyama K., N. M. Rahman, Iida T., A new digital autoradiographical method for identification of Pu particles using an imaging plate, Applied Radiation and Isotopes 65 (2007) 413–418

[10] Takasaki K., Sagawa N., Kurosawa S., Shioya S, Suzuki K., Horikoshi Y. and Mizuniwa H, Development of Image-Analysis Method of Plutonium Samples by Imaging Plate - Measurement of Plutonium Samples in Mixed Oxide Fuel Fabrication Facility, JAEA-Technology 2008-028(2008)

[11] Takasaki K, Miyabe K, Izumi U, Yasunaka H. A Study of the Decontamination Effect of Commercial Detergents on the Skin, Hoken Buturi,38(2), 140-147(2003)

[12] Kurihara O., Momose T.,Tasaki T., Hayashi N., and Shinohara K., Early faecal excretion of inhaled plutonium, Radiation Protection Dosimetry, 102, 2, p.137 (2002)

[13] IAEA-TECDOC-1334, Intercalibration of in vivo counting systems using an Asian phantom, Results of a co-ordinated research project 1996 - 1998 (2003)

[14]Hirota M, Kurihara O, Takada C, Takasaki K, Momose T, Deji S, Ito S, Saze T, Nishizawa K. Development of ^{241}Am Lung Monitoring System using an Imaging Plate. *Health Physics* Vol. 93, Number 1:28-35 (2007).

[15] Tobita K.,Watanabe H., Shimizu T., Sumiya S., Morisawa M., Morita S., Yoshida M., Nakano M., Manual of Standard Procedures for Environmental Sampling and Analysis, PNC TN8520 94-009(1990)

[16] Uezu Y. Watanabe H.,Maruo Y. and Shinohara K., Analytical Methods for Bioassay Samples in Order to Evaluate Internal Exposure by Reprocessed Uranium, Hoken Buturi,36(3),207-212(2001) (in Japanese)

[17] Kurihara O., Hato S., Kanai K.,Takada C., Takasaki K., Ito K., Ikeda H., Oeda M., Kurosawa N.,Fukutu K., Yamada Y., Akashi M. and Momose T., REIDAC - A Software Package for Retrospective Dose Assessment in Internal Contamination with Radionuclides, Journal of Nuclear Science and Technology, Vol.44, No.10,pp.1337-1346(2007)

[18]Proceedings of Second JAERI-JNC Joint Conference on Nuclear Conference on Nuclear Safety Research –February 6, 2004, Tokyo-, JNC TN1200 2004-002 (2004) (in Japanese)

Preparedness for Emergency Medical Response at Rokkasho Nuclear Fuel Cycle Facilities

Toshiharu Miyakawa[1*] and Yutaka Jin[1]

[1] *Japan Nuclear Fuel Limited, Rokkasho-mura, Japan*

Abstract. Japan Nuclear Fuel Limited (JNFL) is currently focusing on a Nuclear Spent Fuel Reprocessing Plant (RPP) operation during the final commissioning test, called active test, in Rokkasho-mura, Kamikita-gun, Aomori prefecture. JNFL's facilities employed a total of approximately 7000 workers in 2008. JNFL has prepared a program for emergency medical response that is to be implemented in the event of any incidents of radiation. JNLF has concluded agreements for emergency medical response with three hospitals in order to ensure special medical aid that we are unable to provide at our clinics. JNFL has kept good relations with these organizations to enable prompt emergency response to incidents by training their staff and inviting them to visit our facilities every year periodically.

Key Words: emergency medical response, Rokkasho nuclear fuel cycle facilities,

1. Introduction

Japan Nuclear Fuel Limited (JNFL) currently operates three types of nuclear fuel cycle facilities at its location in Rokkasho-mura, Kamikita-gun, Aomori prefecture. These include a Uranium Enrichment Plant, a Vitrified Waste Storage Center, and a Low Level Radioactive Waste Disposal Center. JNFL is also focusing on a Nuclear Spent Fuel Reprocessing Plant (RPP) operation during active test and is planning construction of a Mixed Oxide (MOX) Nuclear Fuel Fabrication Plant as well. These facilities employed a total of approximately 7000 workers in 2008.

JNFL has prepared a program for emergency medical response under a plan developed by Aomori prefecture and government regulatory agencies that is to be implemented in the event of any incidents of radiation. In addition, JNFL has strived to maintain a working environment where workers can feel safe by obtaining cooperation from neighboring hospitals and fire stations. The outline of the program is as follows.

2. Rokkasho nuclear fuel cycle facilities and number of workers

The Uranium Enrichment Plant and the Low Level Radioactive Waste Disposal Center commenced operations in 1992, the Vitrified Waste Storage Center started operations in 1995, and the Spent Fuel Storage Facility which is part of RPP has been operating since 2000. Furthermore, the RPP has now been under active test since 2005.

The number of workers in our facilities has increased over the past ten years as shown in Figure 2. The total number was more than 7,000 workers in 2008.

[*] Corresponding to: Toshiharu Miyakawa, Director, Japan Nuclear Fuel Limited, 4-108, Okitsuke, Obuchi, Rokkasho-mura, Aomori 039-3212, Japan. E-mail: toshiharu.miyakawa@jnfl.co.jp

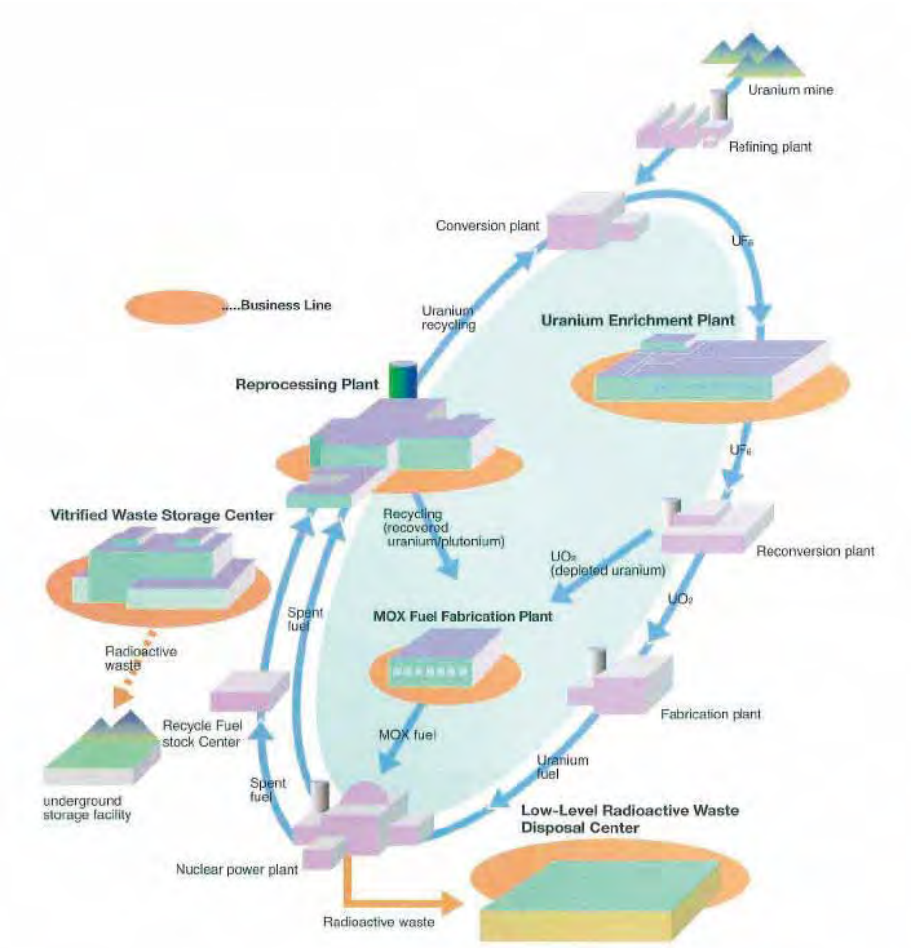


Figure 1. Nuclear Fuel Cycle in Japan

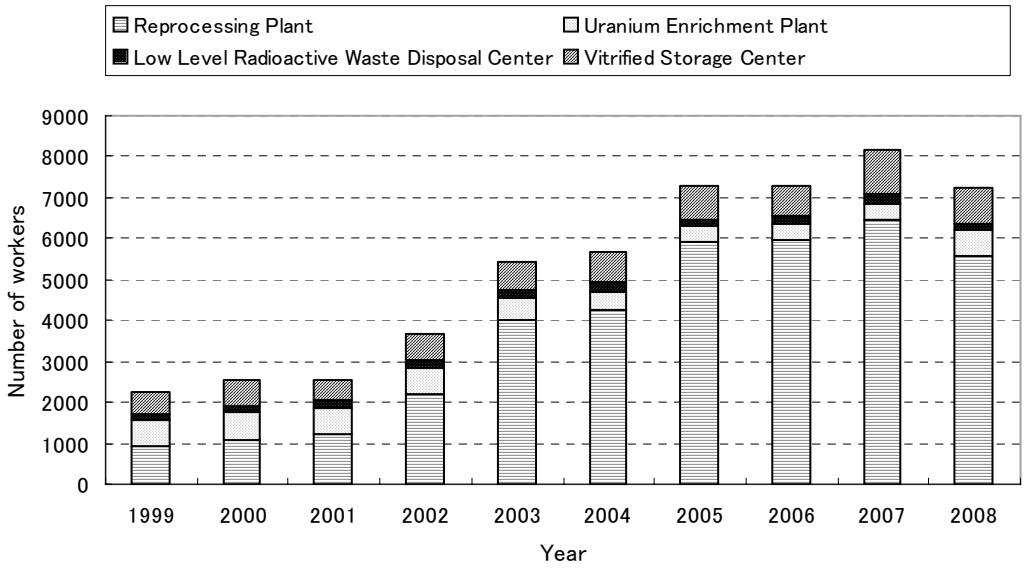


Figure 2. Number of workers at Rokkasho nuclear fuel cycle facilities, 1999-2008

3. For better health care

We have two clinics where one doctor and five nurses are working. One clinic is located in the RRP site and the other is located in the Obuchi area near the site in Rokkasho-mura.

We always staff a nurse at the clinic located on the site during daytime hours on weekdays and holidays. Should an incident occur at night, we will contact a nurse who is on stand-by.

Figure 3. Facade of Gennen Clinic

Figure 4. Operating room in Iyasakatai Clinic

4. Cooperative relationship with regional hospitals

We have concluded agreements for emergency medical response with three hospitals (Primary Hospital: Aomori Rosai Hospital, Secondary Hospital: Hachinohe City Hospital, Tertiary Hospital: Hirosaki University School of Medicine & Hospital) in order to ensure special medical aid that we are unable to provide at our clinics. We have also made an agreement for fire-fighting, including transport by ambulance, with the local fire station.

We have kept good relations with these organizations to enable prompt emergency response to incidents by training their staff and inviting them to visit our facilities every year periodically under the catch phrase “face to face relationship.”

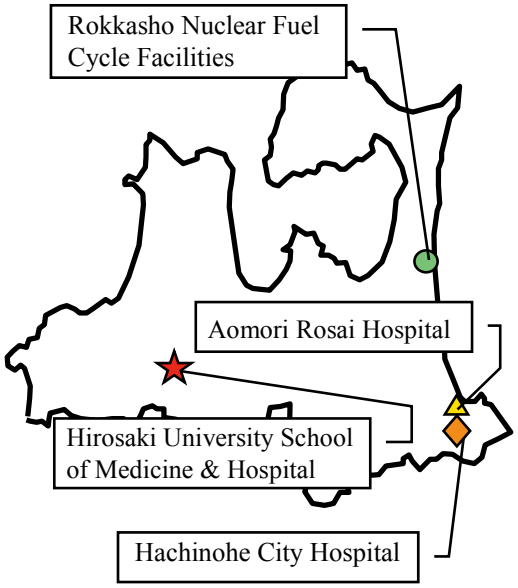


Figure 5. Hospitals for emergency medical response

	2003	2004	2005	2006	2007	2008
Aomori Rosai Hospital	△▲	△	△	△	△	△
Hachinohe City Hospital			△▲	△	△	△
Hirosaki University School of Medicine & Hospital				△	▲△	△
Construction Schedule for RRP			Active Test (Final Commissioning Test)			
			Uranium Test			

▲ : Conclusion of Agreement △ : Training

Figure 6. Result of training demonstration at regional hospitals

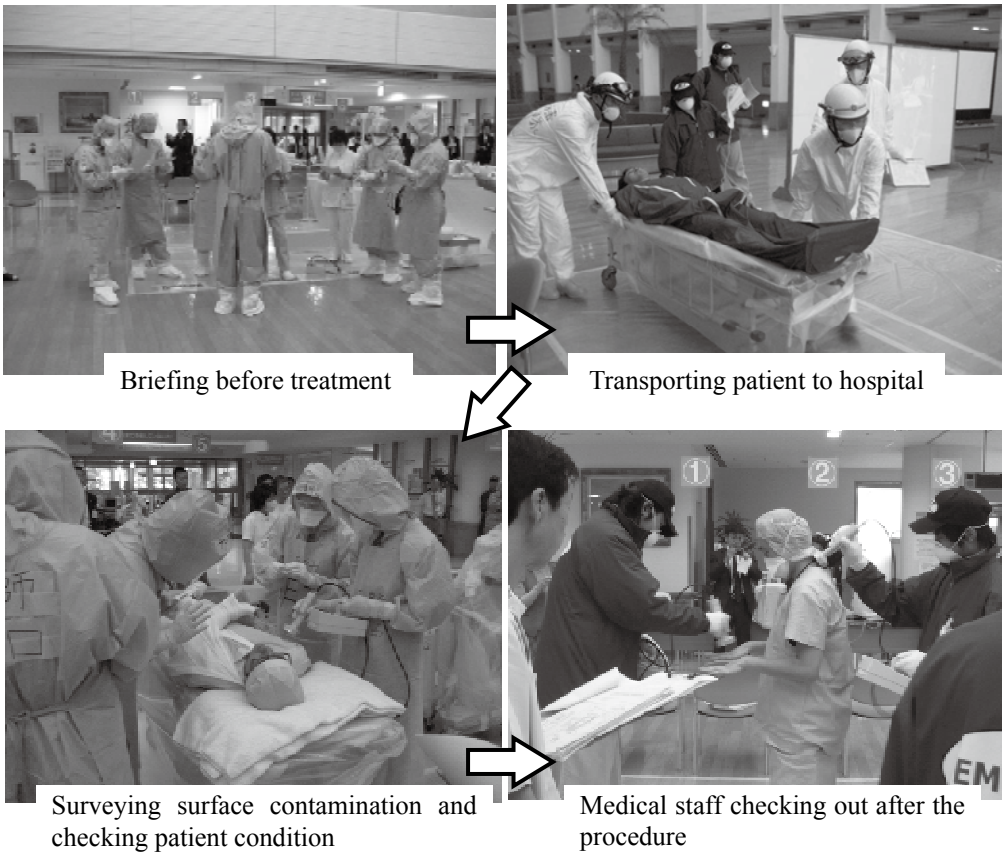


Figure 7. Training at a regional hospital

5. Future view

It is an important issue to be able to transport patients quickly in order to ensure the provision of quality treatment. Recently, Aomori Prefecture began operation of a medical helicopter service that operates with a medical doctor on board. We plan to request the related organization that operates the medical helicopter service to make it applicable for emergency medical response.

We should maintain a relationship of emergency medical response where anybody will be able to get the best treatment anytime and anywhere, even if an incident occurs at our facilities.

We also desire to receive support and advice from Hirosaki University staff and all relevant parties.

DTPA ADMINISTRATION METHODS FOR ACCIDENTS OF α PARTICLE CONTAMINATION

Yutaka Jin
Emergency Medicine Team, Japan Nuclear Fuel Ltd

SUMMARY

Diethylene triamine pentaacetate (DTPA) is a calcium or zinc salt that is specifically used for treating people who have been internally contaminated with plutonium, americium and curium. The DTPA administration protocol using newly designed electric mesh nebulizer for contaminated workers was discussed.

Keywords

diethylene-triamine-pentaacetate (DTPA), plutonium, ultrasonic mesh nebulizer

DTPA ADMINISTRATION PROTOCOL

DTPA administration methods include inhalation, intravenous administration and application to wound. As inhalation is the easiest among these administration methods, it enables administration at an early stage in the radiation controlled area.

In the event of plutonium inhalation, a nose smear test of radionuclides is performed. This method measures the amount of radioactive material adhering to a filter paper with which the nostril is swabbed. Although this method is easy to perform, the calculation of intake is inaccurate. Therefore, in the event that nose smear test shows any significant count, DTPA inhalation is conducted immediately. 50mg of DTPA is administered by inhaling it for 1 minute. DTPA solution is sticky, but electric mesh nebulizer can produce sufficient mist without dilution. As it produces mist without leaking even if turned upside down, it is usable in confused circumstances(1,2,3) .

Following the nose smear test, lung monitor and bioassay testing are performed. A lung monitor is a device that detects gamma rays and X rays by germanium semiconductor. Bioassay is a method to analyze urine and stool. Additional administration of DTPA by injection is based on these data of lung monitor and bioassy.

Treatment with DTPA for contaminated workers should be made as early as possible, because the effectiveness of treatment can drop dramatically within a few hours. A promptly administered DTPA aerosol would have the advantage that plutonium would be chelated in the respiratory tract, thus minimising subsequent deposition in systemic tissues. Moreover, combined treatment involving early inhalation of DTPA followed by repeated intravenous injections is likely to be the most effective treatment for workers who have accidentally inhaled plutonium and americium .

DISCUSSION

Treatment with DTPA for contaminated workers should be made as early as possible, because the effectiveness of treatment can drop dramatically within a few

hours. A promptly administered DTPA aerosol would have the advantage that plutonium would be chelated in the respiratory tract, thus minimising subsequent deposition in systemic tissues (5). Moreover, combined treatment involving early inhalation of DTPA followed by repeated intravenous injections is likely to be the most effective treatment for workers who have accidentally inhaled plutonium and americium (4).

Administration method of DTPA aerosol by inhalation using ultrasonic nebulizer is useful for an emergency treatment (6). Electric mesh nebulizer named Microair is based on a principle of passing liquid through a vibrating mesh of micronsized holes. Vibrations of an ultrasonic horn are used to force liquid through the mesh, which vibrates in sympathy with the horn (1,3). MMAD of mist is 5 μm and 80% of the aerosol is within 1 - 8 μm. As this device is very small and it produces mist without leaking even if turned upside down, it is usable in confused circumstances of radiological emergency.

REFERENCES

1. Newman S. The Omron Microair vibrating mesh technology nebuliser, a 21st century approach to inhalation therapy. Journal of Applied Therapeutic Research 2005; 5(4): 29-33

2. Yoshiyama Y. The nebulization of Budesonide suspension by a newly designed mesh nebulizer. Respiratory Drug Delivery 2002: 487-489.

3. Newman SP, Pitcairn GR, Pickford, Gee-Turner A, Asai K. The MicroAir electronic-mesh nebuliser deposits aerosol in the lungs more efficiently than a conventional jet nebuliser. Drug Delivery to the Lungs XV, The Aerosol Society, London, 2004:228–231

4. Stather JW, Stradling GN, Gray SA, Moody J, Hodgson A. Use of DTPA for increasing the rate of elimination of plutonium-238 and americium-241 from rodents after their inhalation as the nitrates. Hum Toxicol. 1985; 4(6): 573-582

5. Menetrier F, Grappin L, Raynaud P, Courtay C, Wood R, Joussineau S, List V, Stradling GN, Taylor D, Berard Ph, Morcillo MA, Rencova J. Treatment of accidental intakes of plutonium and americium: Guidance notes. Radiation and Isotopes 2005; 62(6): 829-846

6. Koizumi A, Fukuda S, Yamada Y, Iida H, Shimo M. Administration Method of Ca-DTPA by Inhalation as an Emergency Medical Treatment. Japanese Journal of Health Physics 2001; 36(1): 45-50

Shandong Radiation Exposure October 21, 2004

Yutaka JIN[1], Xiaohua CHEN[2]

[1] Emergency Medicine Team, Japan Nuclear Fuel Ltd

[2] Beijin Institute of Radiation Medicine

Accident Summary:

It was at 5:30 in the evening on October 21, 2004 when the radiation source control was short-circuited that caused aberrant conditions while two workers from a food irradiation plant in Shandong engaged in vegetable freshness-preserving process using radiation source Co60 (0.3 million curies of radiation). The radiation source control failed to lower to a safety position. Patients A and B transported vegetables in the field while the radiation source was in action (1.5 meters above the ground) and worked for a few minutes 0.8 to 2.0 meters away from the radiation source before walking away from the field.

With Patient A, the first symptoms appeared 3 minutes after getting out of the field. The following symptoms had developed: nausea attended with continuous abdominal pains, vomiting, headaches, languor, and bleariness. Vomiting 7 times in a row and no diarrhea was observed.

With Patient B, the first symptoms appeared 10 minutes after getting out of the field. The following symptoms had developed: nausea attended with continuous abdominal pains, vomiting, headaches, languor, and bleariness. Vomiting 5 times in a row inducing gastric emptying and diarrhea twice was observed.

The said two patients were admitted to the hospital at 7 p.m. on October 21. Findings on admission with the patients included drowsiness, mental instability, body temperature of 38.1 to 39.2ºC, heart rate of 98 beats a minute, blushing face and hands, and parotid and abdominal tenderness. The symptoms slightly subsided in response to appropriate medical treatment, but abdominal pains and mental instability persisted. The patients were transferred to the Beijing Institute of Radiation Medicine Hospital at midnight on October 24, 2004.

Reference Dose:

Patient A

Physical dose: 20 to 25Gy; Biological dose: 12 to 25Gy; Exposure dose integrated evaluation: 16 to 25Gy

Patient B

Physical dose: 9 to 15Gy; Biological dose: 8 to 12Gy; Exposure dose integrated evaluation: 8.5 to 13.5Gy

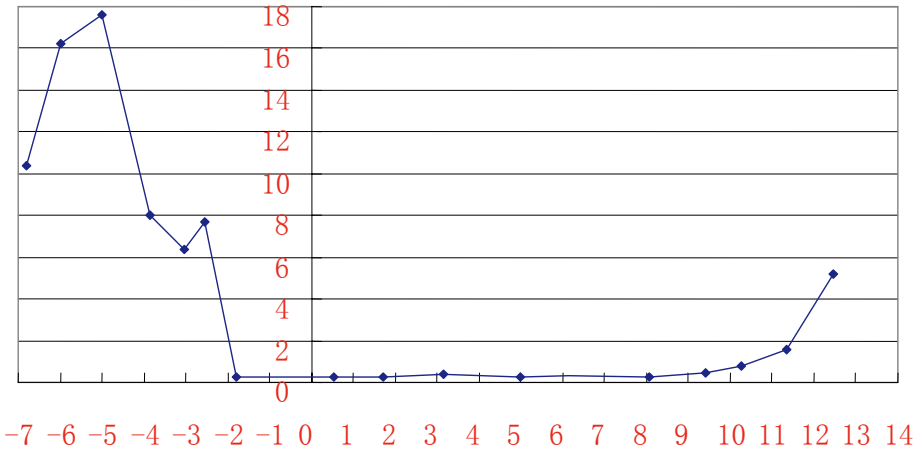
Clinical Therapy:

A hematopoietic stem cell transplant was practiced due to a relatively large quantity of exposure and a slim chance of hemopoiesis self-recovery. The first day at the hospital, (+4d) HLA matching was carried out to identify the degree of matching between the patients and their respective family members (donors). As to Patient A, his 51-year-old sister: DR/DQ matched and A/B loci half-matched; as to Patient B, his 52-year-old brother: HLA fully matched. The following table shows the pre-transplant processing plan.

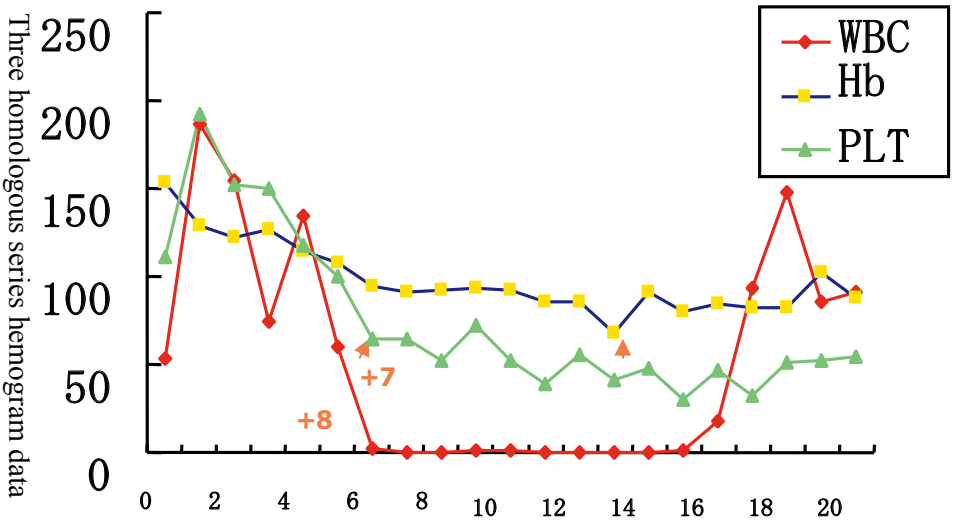
Transplant Date	-3 10.25	-2、 10.26	-1 10.27	0 10.28	+1 10.29	+2 10.30
Flu	100mg	50mg				
CTX		3.4g				
ATG		100mg		100mg		
CSA	200mg	200mg	200mg	200mg	200mg	200mg
MMF	2.0	2.0	2.0	2.0	2.0	2.0
SCT				**Infusion**	**Infusion**	

Variations in the white blood cell count before and after hematopoietic stem cell transplant are presented in graphical form below.

Variation diagram of WBC count before/after transplant



Variations in three homologous series hemogram in sample blood



Elapsed time in days after exposure

Patient A was diagnosed as having severe lung infections with chest x-rays and CT scans that he underwent several times eight days after the transplant. It was clinically-convincing fungal infections and microbism of the lungs, which led to the use of amphotericin B and itraconazole as an antifungal agent and of Tienam and Vancocin as an antibacterial agent in treatment. These drugs, however, produced little effect, which allowed the patient to develop complications of hypoactivity in lungs, heart, kidney, and liver. Although the patient was attached to a respirator by cutting

open his trachea 30 days after the exposure, he died of a multiple organ failure on the 33rd day despite a life-saving measure.

Patient B had been suffering from severe lung fungal infections since the 17th day after the exposure and later was diagnosed as Trichosporon asahii and Aspergillus terreus in sputum culture examinations conducted several times. In combination with Caspofungin, Amphotericin B, itraconazole, and fluconazole, antifungal treatment improved fungal infection noticeably. Treatment using antibacterial agents including Tienam, Vancocin, and piperacillin and antiviral agents including ganciclovir enabled control of microbism to some extent despite septic symptom (Gram-positive and -negative sepsis), and cytomegalovirus test turned out negative.

However, the patient showed signs of his lung infections, total-body radiation injury, and organ functions worsening, which heavily limited his respiratory function and developed respiratory distress syndrome. He was attached to a respirator by cutting open his trachea 45 days after the exposure. Consecutive occurrences of complications, such as arrhythmia, cardiac failure, hepatic failure, kidney failure, gastrointestinal paralysis, and intestinal obstruction, caused unstable vital signs and brought him to a low oxygenation level and an exaggerated hypotensive state. On the 73rd day, the patient went into cardiac arrest and developed third-degree AV block. AV block failed to be treated with cardiac pacing, and he died of a multiple organ failure at 9:10 p.m. on January 4, 2005 (75th day) despite a life-saving measure.

A New Therapeutic Approach for Radiation Burns combining Surgery and Mesenchymal Stem Cell Administrations: About four cases

***Eric Bey*[1] *and Jean-Jacques Lataillade*[2]**

[1] Hôpital d'Instruction des Armées Percy, Service de Chirurgie Plastique, Avenue Henri Barbusse, 92141 Clamart, France. Phone : 33 1 41 46 62 05 ; email : bey.eric@yahoo.fr
[2] Hôpital d'Instruction des Armées Percy, Centre de Transfusion Sanguine des Armées Jean Julliard, Département Recherches et Thérapies Cellulaires, BP 410, 92141 Clamart, France. Phone : 33 1 41 46 72 60 ; email : jjlataillade@ctsa-armees.fr

The physiopathological mechanisms of severe radiation burns are well described and therapeutic process is well codified but very difficult with an important functional and vital risk. We present four patients with local radiation burn and propose a new therapeutic approach combining surgery and local stem cell therapy. It's a preliminary report but the results are very promising.

The first patient had local radiation burns of left fingers and left buttock.
We performed early excision of the irradiated part of the buttock after dosimetric reconstruction. We covered the buttock and the fingers with full thickness skin graft and autologous Mesenchymal Stem Cells were locally administrated in the lesion at the same time.
The second patient had a very important radiation-induced skin necrosis located to the left arm from the shoulder to the elbow. The surgical procedure used a pedicle latissimus dorsi muscle flap and a proximal forearm ante bracchial flap after a very large excision of skin and triceps muscle. Several Stem Cell administrations were combined to the surgery after many bone marrow collections.
The third patient had a local radiation burn of the hands.
Full thickness skin grafts were combined with local stem cells administrations.
The fourth current case presents a local radiation-induced burn of the limb and is hospitalized and treated in our Hospital following the same procedure.
All these cases are radiation accidents occurred in the world: Chilli, Senegal, Tunisia and Equator.
We obtained a complete and stable healing in the three first cases. Stem cell therapy using autologous expanded MSC has to be considered as an adjuvant treatment of the surgery corresponding to excision of necrosis tissues and flap reconstructions.

Our results demonstrate that this new therapeutic procedure of local radiation burns using surgery and local stem cell therapy is very promising. We believe that this innovative approach could improve the treatment of local radiation burns in term of functional and vital results.

Mesenchymal Stem Cells as Drug Cells for Radiation Burn Treatment

Eric Bey[1] and Jean-Jacques Lataillade[2]

[1] Hôpital d'Instruction des Armées Percy, Service de Chirurgie Plastique, Avenue Henri Barbusse, 92141 Clamart, France. Phone : 33 1 41 46 62 05 ; email : bey.eric@yahoo.fr
[2] Hôpital d'Instruction des Armées Percy, Centre de Transfusion Sanguine des Armées Jean Julliard, Département Recherches et Thérapies Cellulaires, BP 410, 92141 Clamart, France. Phone: 33 1 41 46 72 60 ; email : jjlataillade@ctsa-armees.fr

Local radiation syndrome is marked by necrosis that may extend to the deep subcutaneous structures. Today, treatment is surgery, excision, graft and flap with sometimes bad results. It has been suggested that Mesenchymal Stem Cells (MSC) therapy could be used in order to treat numerous tissue lesions. We have performed a novel therapeutic approach of local radiation syndrome by using local autologous MSC therapy combined to surgery. For this purpose, autologous bone marrow cells were collected from the unexposed iliac crest. For GMP production, MSC were expanded in a closed system (MacoPharma partnership) containing an innovative serum free medium supplemented with human platelet lysate as previously described (Doucet et al., *J. Cell Physiol.*, 2005). Quality control assays evidenced that expanded cell population retained typical MSC characteristics and did not exhibit chromosomal abnormalities. As previously demonstrated, MSC produced many cytokines and growth factors which could have a critical role in improving the healing process by counteracting the local inflammatory waves and by promoting the autologous skin engraftment.

We believe that MSC act as drug cells delivering *in situ* in the lesion growth factors which contribute to the healing of the lesion. We have also demonstrated that after *in vitro* cell activation, the conditioned medium of MSC exhibited a similar effect on wound healing than that obtained with freshly expanded MSC. In case of caryotypic abnormalities occurring after *in vitro* MSC expansion, the use of MSC conditioned medium could be considered as a relevant alternative of MSC therapy. Other sources of MSC such as adipose tissue, gingival mucosa are also taken in consideration in view of setting up an allogeneic stem cell bank.

The 2009
Hirosaki University International Symposium
The 1st International Symposium on
Radiation Emergency Medicine at Hirosaki University

平成22年７月12日　印刷
平成22年７月16日　発行（非売品）

編　集　　弘前大学大学院保健学研究科
〒036-8564　弘前市本町66番地１

発行所　　弘前大学出版会
〒036-8560　弘前市文京町１
電話 0172（39）3168　Fax 0172（39）3171

印刷所　　やまと印刷株式会社