6. Research Center for Radiation Emergency Medicine

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Outline of Research Career

Dr. Akashi started his medical career at the Jichi Medical School (Tochigi Prefecture) as a junior resident of internal medicine in 1981. He worked as a senior resident at the Division of Hematology of Jichi Medical School before moving to the Division of Hematology/Oncology at UCLA School of Medicine in 1987. He received a Ph. D. from Jichi Medical School in 1988. He became a staff member of NIRS in 1990. His major interests are: 1) establishment of radiation emergency medical preparedness; 2) research on radiation injuries, including molecular and cellular mechanisms; and 3) development of methods for mitigation of radiation injuries. He has treated patients of the Tokai-ura criticality accident.

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Objectives
This Research Center had the unique experience of receiving three victims heavily exposed to radiation at the JCO criticality accident of Tokai-mura in September 1999, because the Center has been assigned as the National Center for Radiation Emergency Medical Preparedness and Response by the Nuclear Disaster Prevention Plan of the Japanese government since 1980. The Center is responsible for, and has established a solid system for dealing with radiation emergency from a medical viewpoint. Our required missions are as follows:
1) To receive victims exposed to radiation and/or contaminated with radioactive materials who require specialized diagnosis and treatment.
2) To dispatch a radiation emergency medical team to local emergency medical headquarters.
3) To facilitate exchange of information, research activities, and human resources, by constructing networks in cooperation with other organizations who could deal with a radiation emergency.
4) To maintain and reinforce an efficient radiation emergency medicine system under usual conditions.
5) To promote technical development and research on radiation emergency medicine.
6) To build skilled manpower for a radiation emergency.

As an additional objective, we are carrying out fundamental research on radiation emergency medicine. Details are given elsewhere; only the subjects are presented here.
1. Research for diagnosis and treatment of exposure to high-dose radiation and/or contamination with radioactive materials.
1-1) Studying mechanisms of radiation injuries leading to development of new agents for treatment, with focus on the skin and gastrointestinal tract.
1-2) Studying indicators of radiation exposure dose from biological specimens.

Overview
In 1997, the Central Disaster Prevention Council (CDPC) in the Prime Minister's office added a section on emergency preparedness for dealing with a nuclear power station emergency to the Basic Plan for Disaster Prevention. This plan was reinforced in 2000 following the criticality accident at Tokai-mura in the previous year. The plan was also revised in 2008 after the Niigata-Chuetsu-Oki Earthquake caused damage to a nuclear power plant in 2007.

In June 1980, the Nuclear Safety Commission (NSC) came up with a guideline entitled "Off-site Emergency Planning and Preparedness for Nuclear Power Plants." This guideline nominated NIRS as a tertiary radiation emergency hospital that serves as the final stage hospital for receiving victims heavily exposed to radiation and/or contaminated with radioactive materials due to nuclear or radiological accidents. In 2000, NSC published the guidelines for radiation emergency medical preparedness and revised it in 2008 to clarify the role of hospitals for radiation emergencies.

From January 2004 the Research Center has served as a liaison institution of WHO/REMPAN (Radiotherapy Emergency Medical Preparedness and Assistance Network). The Research Center carries out the following activities to maintain and enhance or strengthen the emergency preparedness system required to fulfill its role as a tertiary radiation emergency hospital.

1) Network System
The primary goal is to strengthen the institutional system to prepare for radiation emergencies by establishing three nation-wide network councils, for medicine, chromosome analysis as bio-dosimetry, and physical dosimetry.

1-1) NIRS Radiation Emergency Medicine Network Council
This is a group of experts in radiation emergency medicine or health physics for treatment of patients in cooperation with NIRS at the time of a nuclear disaster or a radiation accident. In an emergency, the cooperation involves sending an expert to NIRS, arrangement of acceptance of patients at medical facilities affiliated with the expert's organization, and providing advice. Such collaboration is expected to reinforce the functions of NIRS. This is called the Radiation Emergency Medicine Network Council to solicit cooperation when it is requested by authorities (or when NIRS considers the necessity arises) to respond to radiation emergencies. This council worked effectively at the time of the JCO criticality accident in 1999. In FY 2009, a communication exercise was performed for members of the council as a general drill for radiation emergencies on 22 December and the council annual meeting was held on 15 January 2010.

1-2) Chromosome Network Council
The Chromosome Network Council forms a network among nearly 10 experts on cytogenetic radiological dosimetry to strengthen its capability and establish technical standards of dose estimation methods using chromosomes. The members are from six areas of Japan and will cooperate with NIRS to do cytogenetic dosimetry when a number of people are involved in a radiation accident. An inter-
comparison study on the dose estimation by chromosome analysis is performed among the council members when the national drill for radiation emergencies is held every year.

In FY2009, prematurely condensed chromosome (PCC) preparations from two blood samples experimentally exposed to six different doses (0-30 Gy) were analyzed by member institutions. Each member scored PCC-ring chromosomes of the samples without knowing of the true doses and estimated the doses based on a common calibration curve. By comparing the results, it was found that there was still a problem in sample preparation for the PCC-ring biodosimetry. This year, NIRS held a "Symposium on Radiation Emergency Medicine: Biodosimetry Based on Chromosomal Aberrations" on 22 January 2010 (Tokyo, in Japanese) and 66 researchers participated. In this symposium, recent progress in radiation cytogenetics and biodosimetry was presented by five researchers and actively discussed.

1-3) Physical Dosimetry Network Council

This council is a network of experts for radiation detection and/or physical dose assessment. The network assists and provides advice to NIRS for physical dose evaluation at radiation/nuclear accidents. In FY 2009, a real-time communication system with high security was developed. Using transmission function of measurement data such as gamma energy spectrum of Whole Body Counter (WBC), it enables us to perform prompt and precise dose evaluation through real-time discussion among members in a remote place. Since the main server installed at NIRS has huge storage, members of other Network Councils can share information. Analysis of dicentric chromosome aberration is possible on the website. In the annual meeting, various levels for decision making in triage were discussed.

1-4) Local organizational system for radiation emergency medicine

In Japan, the medical system for radiation emergencies is currently being constructed in accordance with disaster prevention plans of local governments where nuclear facilities have been established. Within the framework of each local nuclear disaster prevention plan, establishment of each-collaboration system with NIRS is mandatory and it must specify the steps to be performed in the smooth transfer of patients from an accident site to the hospital whose staff are well trained.

On 13 February 2009, the Ministry of Internal Affairs and Communications (MIC) pointed out to the Ministry of Education, Culture, Sports, Science and Technology (MEXT) that the appropriate system for transportation of contaminated victims from on site to NIRS has not been established in some local governments, and recommended that such a system has to be built soon and that support from the Ministry of Defense (MOD) should be included in the system. Based on the recommendation, NIRS discussed with local governments on transportation of patients and clarified a role of the MOD in transportation of patients in a radiation emergency. To discuss the issues, meetings were held in Hokkaido, Aomori, Miyagi, Niigata, Ibaraki, Fukushima, Kanagawa, Ishikawa, Fuku, Kyoto, Osaka, Okayama, Ehime, Shimane, Saga, Nagasaki and Kagoshima Prefectures and the information regarding treatment of internally-contaminated victims was also provided there. Moreover, a desktop drill using a several scenarios including combined injury was introduced in each meeting. In the annual meeting held in October in Tokyo with 19 local governments with nuclear facilities, discussion focused on transport of contaminated victims to NIRS in cooperation with the MOD. Relevant ministries and agencies such as MEXT, and the Fire and Disaster Management Agency (FDMA) also attended this meeting.

2) Training

The primary goal for training is the development of radiation emergency medicine skills for medical professionals and disaster response personnel; these include doctors and nurses involved in nuclear disaster, medical first responder crews, and nuclear establishment employees. For that purpose, NIRS holds the following courses regularly in addition to our participation in nuclear disaster prevention training, seminars on medical response and other activities conducted by local governments to provide the relevant information and skills to deal with a radiation emergency. From FY 2009, response to malicious events and transport accidents of radioisotope were newly added to the following course's curriculums.

2-1) NIRS Course "Radiation emergency medicine (hospital course)"

In FY 2008, this 3-day course was designed for physicians, nurses, and radiological technologists who may receive victims exposed to radiation and/or contaminated with radionuclides. The course was held from 18-20 November with 25 participants. Some of them are working actively in primary or secondary levels of radiation emergency hospitals and playing an important role in local radiation emergency exercises.
In addition to this course, upon a request from Hiroski University School of Health Sciences, another hospital course was organized for medical professionals with 20 participants from 31 August to 2 September 2009. Aomori Prefecture has a reprocessing factory for nuclear fuel in addition to nuclear power plants. The Hiroski University Hospital is one of the main hospitals and is responsible for radiation emergency medicine in Aomori Prefecture. Therefore, NIRS and the Hiroski University have exchanged a memorandum of understanding (MOU) for radiation emergency medicine. Based on the MOU, this course was held.

2-2) NIRS Course "Radiation Emergency for first responders (pre-hospital course)"

This 3-day course was primarily designed for first responders such as fire or police department personnel, paramedics, and emergency planners at nuclear facilities. The course was held from 8-10 February 2010 with 24 participants including personnel from the Japan Coast Guard and the Japan Ground Self-Defense Force.

3) Exercises for Radiation Emergency

National and local governments annually hold drills for nuclear emergencies. NIRS sent staff members to these drills to give advice from the viewpoints of medical care and radiation protection. On 21-22 December 2008, the Japanese government conducted a nuclear drill at the Tokai No. 2 Power Station of the Japan Atomic Power Company (Ibaraki Prefecture) to enforce readiness for an accident; 3,020 people from 113 organizations participated and some experts from foreign countries including France and Korea observed the drill. The 2-day long drill assumed that a trouble occurred in the cooling system, which caused radioactive leaks. From NIRS, medical doctors and experts on radiation protection participated. In this drill, a mock victim was transferred from the plant to NIRS by a helicopter of the Chiba City Fire Department. Following the drill, NIRS conducted an additional exercise to simulate emergency handling of a patient, especially decontamination and dose assessment. The drill activities at NIRS were open to media representatives.

4) Follow-up Studies

The center carries out a medical follow-up for victims who were exposed to radiation in the thermonuclear weapon tests on Bikini Atoll, patients with thorotrastosis, and the surviving JCO accident victim.

4-1) Follow-up examination of the victims of the Bikini nuclear test

On 1 March 1954, the 23 crew members (18 to 39 years old at the time) of the Japanese fishing vessel Daigo Fukuryu Maru (which means "Lucky Dragon") from Yaizu City, Shizuoka Prefecture saw bright light in the South Pacific resembling a sun rise. Seven or eight minutes later there was a terrific sound. They did not know what it was at the time. The blast, equivalent to about 12 million tons of TNT, was 750 to 1,000 times more powerful than the atomic bomb exploded over Hiroshima. All 23 people were hospitalized after returning to Japan. One of them died of liver failure seven months later. Several hundred inhabitants of the Marshall Islands in the Pacific, as well as nearly 30 U.S. Army personnel involved in the tests, were also injured from the nuclear fallout. Their medical follow-up aims to study late radiation effects by examining the health states of these victims over a long period of time. The follow-up examinations that have been conducted for 50 years provide important information. The type of exposure was external and also internal, although internal doses were thought to be relatively small. The estimated whole body doses were 1.7 to 64.9 Gy. Among 23 victims, 14 have already died. In FY 2009, a medical check-up of survivors was conducted for 6 victims at Yaizu City Hospital. Details on the cause of death are as follows: 6 died of liver cancer, 2 of liver cirrhosis, 1 of liver fibrosis, 2 of colon cancer, 1 of heart failure, 1 in a traffic accident, and 1 of an aortic aneurysm rupture. Malignancies were suspected in two of these people. Many of them have evidence of infection with hepatitis viruses. Since all 23 victims received transfusions in 1954, transfusion might be the most important factor for infection by hepatitis viruses, although transfusion was one of the best treatments for bone marrow suppression at that time.

4-2) Follow-up examination of patients with thorotrastosis

Thorotrast is an alpha emitting thorium dioxide colloïd, which was used clinically in the 1930s and 1940s as a radiographic contrast medium. It was injected intra-vascullly for the visualization of vascular structures. Long-term retention of thorotrast in the reticulo-endothelial system, in the liver, spleen and bone marrow produces lifetime alpha particle irradiation of these organs and considerable epidemiological follow-up work has been performed. The major cohorts that can be used for risk evaluation are German, Danish and Japanese patients subjected to thorotrast. The incidence of leukemia has increased among these persons. In
Japan, the product was used from 1932 to 1945 for 10,000 to 20,000 patients, the majority of whom were killed in World War II. This follow-up examination estimates the amount of thorium deposited in surviving patients, investigates their clinical symptoms, analyzes the relationship between the deposited amount and carcinogenesis, and elucidates the effects of long-term internal radiation exposure on human bodies. This year, a medical check-up was carried out for only one patient.

5) Database
Since radiation accidents requiring medical care are extremely rare, the medical information must be collected from each accident and accumulated to help medical professionals to make decisions for strategies to treat victims, and establish and improve therapeutic methods. A medical database including the cases of radiation exposure at Bikini Atoll in the South Pacific and cases of thorotrastosis is being constructed. Today, there are many database systems on radiation accidents and their victims, but most are only accessible from the related countries. Under the supervision of the WHO, an international program called REMPAN exchanges information on radiation accidents, including those in the database owned by the US REAC/TS (Radiation Emergency Assistance Center/Training Site). REMPAN has a collaborating center at Ulm University in Germany and manages a SEARCH database of patient information. It aims to construct an international database by registering cases that are attributable to the Chernobyl accident and other radiation accidents. The NIRS registered the Daigo Fukuryu Maru accident in the SEARCH database. In addition, the center is constructing a database by collecting medical data of the victims of radiation accidents and exchanging information with countries that have developed radiation accident medicine.

6) Operation of 7 days/24 hours Radiation Emergency Call System
Since FY2008, the NIRS has been operating 7 days/24 hours on call radiation emergency system for hospitals and first responders, including fire department personnel. This system is for direct or consultative assistance regarding medical and health physics problems associated with radiation or nuclear accidents. This consultation assistance on a 24-hour basis can be reached by phone. After business hours, the phone call is automatically transferred to a staff member of the Research Center for Radiation Emergency Medicine (which include a medical doctor and a health physicist).

7) Other consultation for health effects of radiation
The NIRS receives consultations on health effect of radiation. The number of phone calls for consultation of radiation effects is increasing. This year we received 27 consultations. Of those, 11 were consultations on radiation exposure (10 cases were about exposure to radiation in medical use and 1 was accidental exposure). Nine were questions about radiation or the radiation emergency medicine system. 7 cases were from persons who believed that they had been exposed to radiation without reasonable evidence. Since some events occurred in Japan last year which were about uncontrolled or stolen radioactive sources, some consultations or questions we received were associated with these cases. To deal with these situations, the NIRS also released important information about each event to the public on it's homepage.

8) International Cooperation
8-1) Training courses for foreign medical professionals organized by NIRS
Upon a request from the Korea Institute of Radiological & Medical Sciences (KIRAMS), NIRS held a NIRS Training Course for Korean Medical Professionals on Radiation Emergency Medical Preparedness from 9-11 December 2009 and 19 Korean medical professionals attended the courses.

8-2) International workshop
The NSC/NIRS workshop on medical response to nuclear accidents in Asia was held from 19-21 January 2010 organized by the Nuclear Safety Commission (NSC) and NIRS in cooperation with the IAEA and the WHO. As part of this workshop, information on internal contamination and other topics was exchanged; total of 22 people (10 from 8 Asian countries, 8 from other area countries, and 4 from IAEA and WHO) were invited. In this workshop, one person from Australia and two from IAEA participated in the workshop via a TV system.

8-3) Invited lectures
Our staff were invited to give lectures in the following meetings and training courses.
- IAEA Workshop on Infrastructures Needed for Off-site and On-site Emergency Preparedness and Response Activity, and on Medical Treatment held in Kuala Lumpur, Malaysia, 11-16 November 2009.
- 1st International Seminar on Biodosimetry held


f) IAEA Regional Training Course on Medical Response to Radiation Emergencies held in Doha, Qatar, 12-17 December 2009.

g) International Workshop on Acute and Protracted Radiation Biodose Studies and International Networking System in Taipei, Taiwan, 4-5 February 2010.

8-4) International meetings/conferences

NIRS staff members attended the following meetings and exercises.
a) American Association for Cancer Research 100th Annual Meeting held in Denver, CO, USA, 18-22 April 2009.

8-5) Members of international committees

NIRS staff members participated in the following committees.

b) IAEA Consultancy Meeting to Finalize the Training Material on Medical Response to Radiation Emergencies held in Vienna, Austria, 25-29 May 2009.

c) IAEA Consultancy Meeting to Completing Development Materials for Medical Response to Malicious Events with Involvement of Radioactive Materials held in Vienna, Austria, 7-9 October 2009.


e) Global Health Security Initiative: GHSI Meeting held in Washington, D.C., USA, 3-6 November 2009.

f) The International Commission on Radiation Units and Measurements (ICRU) Annual Meeting held in Dresden, Germany, 11-16 September 2009.

g) ICRU low dose report committee held in Bethesda, MD, USA, 9-12 January 2009.

h) RANET 2nd Technical Meeting on Guidelines for National Assistance Capabilities held in Vienna, Austria, 15-19 February 2010.

i) IAEA Reviewing the draft manual on Biodosimetry application in Radiation Emergency held in Vienna, Austria, 1-8 November 2009.

8-6) Other Visitors

a) A registered nurse from Chulalongkorn University Hospital visited our facility to get a lecture on 16 April 2009.

b) A chemist from Université Paris 13 was invited to NIRS from 22-24 July 2009 to discuss and give a lecture regarding rational design and syntheses of powerful Uranyl ligands.

c) Two medical professionals from the Hôpital d'Instruction des Armées Percy in France were invited to NIRS on 30 July 2009 to give lectures regarding mesenchymal stem cell transplantation and an accident report in Chile, 2005.

d) A scientist from the Mahidol University in Thailand visited NIRS to study radioprotective agents against gastrointestinal injury, from 1-26 March 2010.

8-7) Establishment of REMAT (Radiation Emergency Medical Assistance Team)

Today, radiation is widely used in our lives. Potential sources of radiation 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. However, once an accident involving radiation occurs, much anxiety and fear arise in society, based on the fact that such accidents, fortunately, are not common, but then, paradoxically, and there are also few chances to become knowledgeable about radiation. Radiation cannot be seen by the human eye, smelled, heard, or otherwise detected by our normal senses, nor do symptoms/signs appear soon after exposure. Therefore, dose assessment is essential for taking care of patients involved in radiation accidents, providing appropriate treatment including administration of decontamination agents. Since the practice of medicine is based on science as well as past experience, the knowledge of triage, assessment, initial diagnostic methods and general treatment protocols has to be shared among medical professionals throughout the world.

In January of 2010, the NIRS has established a medical assistance team, called the Radiation Emergency Medical Assistant Team (REMAT), which consists of physicians, nurses, radiation protection experts, and health physcists ready to respond to radiation emergencies. Upon request by foreign governments of countries affected or international organizations such as the International Atomic Energy Agency (IAEA) or the World Health Organization (WHO), NIRS activates REMAT. REMAT provides rapid dose assessment, radiological and medical triage, diagnosis and management in radiation incidents. REMAT is
equipped with radiological and medical equipment and devices that are transportable to affected sites.

8-8) Exchange of human resources and information
a) A medical doctor from NIRS is working as a consultant at the IAEA Incident and Emergency Centre (IEC) to exchange information about radiation/nuclear accidents.

b) Based on the MOU between NIRS and the Institut de Radioprotection et de Surete Nucleaire (IRSN), one health physicist from NIRS is studying bioassay for radiation emergency at IRSN.

c) Staff members visited the Shanghai Institute of Materia Medica, Chinese Academy of Sciences to exchange information concerning medicines for internal contamination with radionuclides on 4 March 2010.

8-9) Memorandum of Understanding
a) NIRS and KIRAMS agreed to extend the MOU signed in 2004 for another 5 years. Eight Korean delegation visited NIRS to attend a signing ceremony on 4 November 2009.

b) The NIRS exchanged a MOU on radiation emergency medicine with King Abdulaziz City for Science and Technology (KACST) on 1 March 2010.

8-10) Other topics
a) Staff members attended the IAEA general meeting and introduced the center's activities by poster presentation.

b) The NIRS has the Radiotoxicology Research Building, which is the only biological research facility for accidents involving actinoids in Japan. The NIRS has started to re-organize the facility for research on radiation emergency medicine. The NIRS has developed research for biological effects of actinides for over than 20 years. In order to enhance the research project and to facilitate cooperation with other institutions, the NIRS established a management office in October.
6.1. The Study for Medical Treatment for High Dose Exposure

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Outline of Research Career
Dr. Akashi started his medical career at the Jichi Medical School (Tochigi Prefecture) as a junior resident of internal medicine in 1981. He worked as a senior resident at the Division of Hematology of Jichi Medical School before moving to the Division of Hematology/Oncology at UCLA School of Medicine in 1987. He received a Ph.D. from Jichi Medical School in 1988. He became a staff member of NIRS in 1990. His major interests are: 1) establishment of radiation emergency medical preparedness; 2) research on radiation injuries, including molecular and cellular mechanisms; and 3) development of methods for mitigation of radiation injuries. He has treated patients of the criticality accident in Tokai-mura.

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**Objectives**

This department conducts studies that are usually not performed by other research institutions, emphasizing the diagnosis and treatment of radiation injuries due to high-dose exposure. The members try to clarify the mechanism of injuries in cells and tissues exposed to high doses of radiation and its effects on survival, repair, and maintenance of function. In these studies, we are evaluating candidate substances for therapeutic drugs particularly for gastrointestinal and skin injuries. For gastrointestinal injuries due to radiation, we use experimental animals, primary cultured cells, and tissues to develop quantitative evaluation systems. In addition, we studied medical treatments with cytokines, natural products, and synthetic compounds that decrease the severity of injury.

To develop accurate diagnostic dose assessments for high-dose exposure to radiation, we also try to find markers for radiation exposure from bio-molecules contained in samples which can be collected less invasively, such as blood. We are attempting to determine genes, proteins, and other constituents in a living body that can provide a guide for treatment to radiation exposure.

**Progress of Research:**

1) Study on treatment for intestinal injuries due to high doses of radiation

Several Fibroblast growth factors (FGFs) are able to protect against radiation-induced intestinal damage. An FGFI:FGF2 chimera (FGFC) showed greater structural stability than FGFI. FGFC was capable of stimulating epithelial cell proliferation much more strongly than FGFI or FGF2 even without heparin. In this study we evaluated and compared the protective activity of FGFC and FGFI against radiation-induced injuries. FGFC and FGFI were administered intraperitoneally to BALB/c mice 24 h before or after total body irradiation (TBI). The numbers of surviving crypts were determined 3.5 days after TBI with γ-rays at doses ranging from 8 to 12 Gy. As a result, the effect of FGFC was equal to or slightly superior to FGFI with heparin. However, FGFC was significantly more effective in promoting crypt survival than FGFI (P < 0.01) when 10 μg of each FGF was administered without heparin before irradiation. In addition, FGFC was significantly more effective at promoting crypt survival (P < 0.05) than FGFI even when administered without heparin at 24 h after TBI at 10, 11, or 12 Gy. FGFC post-treatment significantly promoted BrdU incorporation into crypts and increased crypt depth, resulting in more epithelial differentiation. However, the number of apoptotic cells in FGFC-treated mice decreased to almost the same level as that in FGFI-treated mice. These findings suggest that FGFC strongly enhanced radioprotection with the induction of epithelial proliferation without exogenous heparin after irradiation and is useful in clinical applications for both the prevention and post-treatment of radiation injuries.

2) A cell-permeable C-terminal fragment of PIDD inhibits ionizing radiation-induced activation of pro-death caspase-2

PIDD (p53-induced protein with a death domain) plays a critical role in the activation of caspase-2 to trigger apoptosis induced by DNA damage through the formation of a so-called PIDDosome, which contains the adaptor protein RAIDD and caspase-2. We found that transcription of PIDD was induced after exposure to ionizing radiation in rat small intestinal epithelial cell line (IEC6). Yeast two-hybrid analysis indicated that the death domain of rat PIDD interacts with RAIDD. Interestingly, a rat C-terminal PIDD fragment (residues 773-917) containing the death domain interacts with RAIDD much more tightly than the longer PIDD fragment (residues 610-917). When the PIDD (773-917) fragment was overexpressed in these cells, the PIDD-mediated activation of caspase-2 was dominant-negatively inhibited. In order to use the PIDD (773-917) fragment as an anti-apoptotic drug, we purified a recombinant PIDD (773-917) fragment fused with a basic 11-amino acid peptide derived from the HIV-Tat protein which facilitates uptake of the protein into mammalian cells with high efficiency. When PIDD (773-917)-TAT was added to the IEC6 cells, the protein was efficiently delivered into the cells within an hour. Furthermore, we observed that ionizing radiation-induced activation of caspase-2 and caspase-9 was inhibited when PIDD (773-917)-TAT was added to the IEC6 cells. These results suggest that PIDD (773-917)-TAT could protect gastrointestinal cells from ionizing radiation-induced cell death.

3) TNFα is required for erythropoiesis in irradiated mice

Tumor necrosis factor α (TNFα) is a pro-inflammatory cytokine that has a wide variety of bioactivities, and over-production of TNFα leads to damages of tissues. To determine the role of TNFα in high-dose radiation exposure, we used wild-type of TNFα (WT) and its knockout (KO) BALB/c mice. The survival duration in KO was shorter than that in WT after irradiation and administration of TNFα to KO before irradiation improved the survival rate, the numbers of red blood cells (RBC), the levels of hemoglobin (Hb), the hematocrit values (Ht) and the unsaturated iron binding capacity (UIBC) that were significantly lower compared with WT. We also showed that the activity of erythroid burst-forming
units (BFU-Es) and erythroid colony-forming units (CFU-Es) was significantly reduced in KO than in WT following irradiation, and that administration of TNFα improved activity in irradiated KO. Furthermore, bone marrow transplantation (BMT) markedly increased the survival rate in both groups of irradiated mice. These results show that irradiation-induced death was mainly caused by myelosuppression. Our results suggest that endogenously-produced TNFα plays important roles in protection and mitigation from radiation injury; an optimal concentration of TNFα effectively enhances the recovery of bone marrow suppression by irradiation, especially erythroid hematopoiesis.

4) Cell-permeable inhibitor of apoptosis (IAP) proteins inhibits radiation-induced cell death

Gastrointestinal syndrome after high-dose radiation exposure is caused by gastrointestinal apoptosis. Inhibitor of apoptosis (IAP) proteins, such as X-linked inhibitor of apoptosis (XIAP) and cellular inhibitor of apoptosis protein 1 and 2 (cIAP1 and 2), are intrinsic cellular inhibitors of apoptosis, inhibit caspase activity directly or indirectly. XIAP is the best-characterized IAP in terms of both its structure and biochemical mechanism. XIAP contains three BIR domains (BIR1, BIR2, and BIR3) and a RING domain. The BIR2 domain of XIAP directly inhibits caspase-3 and caspase-7, whereas the BIR3 domain inhibits caspase-9.

In order to prevent gastrointestinal syndrome, we purified cell-permeable recombinant cIAP2 and XIAP (full-length, BIR2 domain, and BIR3-RING domain with or without mutations of autoubiquitilation sites) proteins fused with 11 amino-acids derived from the HIV-Tat protein and examined the effects of these proteins on radiation-induced cell death in IEC6 cells. When the TAT-conjugated IAP proteins were added to IEC6 cells, these protein were delivered into the cells and inhibited apoptosis after irradiation. Our results suggest that the TAT-conjugated IAP proteins may be useful for protection of gastrointestinal cells from radiation-induced cell death.

5) Diurnal modification of radiation dose-dependent augmentation of mRNA levels for DNA damage-induced genes in mouse hematocytes

Messages for p21 and mdm2 that reflect growth-arrest, and for bax and puma that initiate apoptosis, are expressed in various cells after the exposure of radiation. Although the intracellular levels of the mRNAs seem to reflect the extent of DNA damage, quantitative knowledge is not enough to analyze cellular events particularly in cells from a living body.

For detailed quantification of these mRNAs, we established an accurate real-time RT-PCR method and obtained highly reproducible values among various hematocytes as relative RNA levels of these genes per GAPDH. In x-irradiated murine macrophage RAW264.7 cells, the peak levels of mRNAs of p21, mdm2 and puma strongly correlated to the radiation dose and were consistent with cellular damage. Similarly, the relative RNA levels of p21, mdm2, bax, and puma per GAPDH also increased dose-dependently in peripheral blood and bone marrow cells isolated from whole-body-irradiated C3H/He mice. However, some of this responsiveness in the mRNA levels was strongly affected by circadian rhythm of the irradiated mice. In peripheral blood, induction levels of all messages after nighttime irradiation were reduced by half as compared with daytime irradiation. In normal cells, levels of p21 and mdm2 mRNAs after nighttime irradiation were higher than daytime irradiation. This shows that early-stage cellular responsiveness in DNA damage-induced genes in the isolated cells is modified by the irradiation clock-time of the animals between diurnal and nocturnal irradiation in the cells from living animals.

6) Anabolic steroid stimulates the regeneration of mucosa in small intestine damaged by ionizing radiation

Acute intestinal damage is a serious problem after high-dose radiation. We focused on the regeneration process following irradiation in intestinal mucosa and performed pharmacological studies. To examine the proliferation in IEC-6 cells, hormones clinically used were compared. The most prominent effect on growth was observed by an anabolic steroid, nandrolone (19-nortestosterone). Single injection of 19-nortestosterone ester to C3H/He mice 24 h after abdominal irradiation at a lethal dose of 15.7 Gy of x-ray showed a significant life-saving effect. We also studied the effect of 19-nortestosterone on regeneration of intestinal mucosa in irradiated mice. A microcolony assay in a Brd U-incorporated cell, 19-nortestosterone enhanced regeneration on Day 5 and the expression of c-myc mRNA was stimulated on Day 4 in these mice. The results suggest that this anabolic steroid enhances the regeneration of small intestinal mucosa after radiation exposure.

7) Induction of heme oxygenase-1 by polyphenols from whisky congeners in human endothelial cells

Phenolic compounds are known to induce HO-1 mRNA and protein in various cells. Production of the cytoprotective heme oxygenase-1 (HO-1) protein in endothelial cells would ameliorate vascular injuries.

We investigated the effect of whisky, which contains various phenolic substances in HO-1 expression. A study of quantitative real-time RT-PCR showed the
whisky congeners activated dramatically the transcription of the HO-1 gene in mouse macrophages.

The HO-1 protein was also induced by the whisky congeners in human umbilical vein endothelial cells. The congeners of brandy and beer also induced expression of the HO-1 protein. The congeners of freshly distilled whisky spirit had no activity, while those of whiskies stored from 4 to 30 years in oak barrels induced the HO-1 protein. To determine the compounds with potent HO-1-inducing activity in whisky congeners, several chemicals that had been reported to exist in whisky were screened. We found that coniferaldehyde and syringaldehyde exhibited HO-1-inducing activities. Thus the elements which emerged in whisky during storage in barrels induced the cytoprotective protein, HO-1, in human endothelial cells.

**Major publications**


6.2. Research on Radiation Dose Assessment

Yuji Yamada, Ph.D.
Director, Department of Radiation Dosimetry

Outline of Research Career:
Dr. Yamada received a Ph.D. from Nagoya University in 1989 for his study on collection performance of high efficiency particulate air filters. He has had over 30 years of experience in research on radioactive aerosols and their internal exposure at NIRS. Between 1986 and 1987 he was at the Inhalation Toxicology Research Institute (ITRI) of Lovelace Foundation, USA as a visiting scientist where he studied aerosol deposition within respiratory tracts.

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Objectives:
Radiation accidents can be classified into those resulting from external exposure and those resulting from internal exposure. For heavy exposure, stem transplantation may be considered depending on the external exposure dose received, or administration of medicine may also be considered to inhibit deposition and promote excretion of radioactive materials incorporated into the body. Dose assessment of victims in radiation accidents has to be made within a short time in combination with investigation for the details of the accident to estimate the radiation effects and to initiate appropriate treatment.

Major subjects in radiation dose assessment research are 1) collection and analysis of information on the occurrence of radiation accidents, radiation type, and radioactivity; 2) determination and evaluation of the amount of radioactivity in the body and excreta; and 3) biological evaluation of the effects resulting from exposure to the body. Our aims are to shorten the time needed for analysis and dose determination, and to improve the accuracy of comprehensive assessment, which combines physical and biological dose assessments.

In the area of radiation emergency medicine, we have made basic and applied studies for clinical use of agents in removing radionuclides, especially alpha emitters like plutonium or uranium that are incorporated into the body.

Progress of Research:
1) Development of ESR dosimetry using human nail
Electron spin resonance (ESR) dosimetry is a method to measure radical numbers produced by radiation in substances and to estimate exposure dose. This method is useful for dose estimations when workers are exposed while not wearing personal monitors and when the general public is exposed accidentally. Tooth enamel is typically used for this purpose. However, teeth can not be removed easily from patients in all cases. It is necessary to find out other human tissues or substances around exposed persons for estimating personal exposure. Nail samples are easily obtained from exposed persons compared with tooth enamel samples. Therefore, nail samples were applied to ESR dosimetry in the case of γ irradiation. A protocol was established based on a hypothesis that there was no difference of radical fading rates among personal nails. A fading constant depending on a fading temperature and a background level of sample were used for the dose estimation. Doses (around several gray) were estimated in a 90-180% precision in 2-3 days.

2) Chromosome aberration analysis for dose assessment
Dicentric chromosome analysis (DCA) has been used for biological dosimetry since the mid 1960s. It is now called the gold-standard assay for accurately estimating unknown radiological doses in individuals following radiological or nuclear accidents. However, there is no generally accepted way of deriving its uncertainty. Thus, we are studying differences of calibration curves among individuals to see if there are any permissible limits in a standard curve. By the DCA of four male and three female blood donors aged 20s-40s, no significant differences were detected thus far. Effects of different qualities of radiation and tube voltages on the dose-response curve were also studied. A DCA for one blood sample was performed using 0 to 5.0 Gy-radiation doses with 60Co-γ-rays, 137Cs-γ-rays, and X-rays (120 kV). Although no significant differences were detected among them, more dicentric yields per cell were detected with lower LET at higher doses over 3.0 Gy. By X-ray-irradiation at a constant exposure dose (3.0 Gy) emitted in different tube voltages from 80 to 240 kV for one blood sample, no significant differences of dicentric yields per cell were detected. Now, further analysis is in progress by collecting more blood donors to see individual differences in the dose-response curve.

3) Development of semi-tissue equivalent Si semiconductor for local dose estimation
A system that evaluates dose distribution in homogenous external exposure by a photon spectrum has been constructed. The evaluation of basic performance of the system was completed by using a tissue equivalent semi-conductor detector which measures the Compton spectrum directly. The photon spectrum concerning X-rays, 60Co, 137Cs, or 241Am reconstructed by the unfolding method from the Compton spectrum has already been obtained.

4) Nasal swab for alpha emitters
A nasal swab is good evidence for the possibility of internal contamination just after inhalation accidents. Also it is expected to be a useful method for rapid dose assessment. To improve the preliminary estimation of intake activity by the nasal swab method, sample collection efficiency was experimentally investigated. Focusing on swab materials and aerosol particle sizes, sample collection efficiency was examined for plane disk or sham nasal cavity. Two types of swab materials were used for the experiment: a strip of filter paper or cotton cloth wrapped around the end of a swab stick. Whatman 40 (Whatman International Ltd., England) and Cotton Ciega® (Chiyoda Co., Ltd., Japan) were selected for filter paper and cotton cloth respectively.
Whatman 40 is prepared for nasal swabs in emergency medicine at the NIIRS (National Institute of Radiological Sciences, Japan) considering alpha emitter measurement. Cotton Giceli is no-dust pure cotton used for precision instruments. Fluorescent latex and $^{239}\text{PuO}_2$ particles were prepared for experimental contaminants. The particle number for latex was counted by fluorescence microscopy before and after swabbing, and the alpha activity of $^{239}\text{PuO}_2$ was measured by an alpha spectrometer. The sample collection efficiency was calculated from the ratio of remaining particle number or alpha activity to initial particle number or alpha activity. The sample collection efficiency varied depending on the particle size and swab materials. The collection efficiency of $^{239}\text{PuO}_2$ particles was plotted on an extended curve for fluorescent latex particles. These results suggest that sample collection efficiency depends on particle diameter. Cotton had a higher collection efficiency than filter paper, and the efficiency showed a clear dependence of aerosol particle diameter – either disk or sham nasal cavity. On the other hand, sample collection efficiency for filter paper showed more or less the same tendency for sham nasal cavity, and a lower value (average = 17.7%) than that for disk. Filter paper had advantage: steady efficiency with less dependency of particle size. On the other hand, cotton showed higher efficiency, two-fold or more. Correction of sample collection efficiency would be essential to reduce uncertainties for the nasal swab method. To obtain higher and steady sample collection efficiency, cotton may require a more uniform structure as filter paper.

5) Development of in-vivo measurements

This year, minimum detectable activity (MDA) was obtained by using BOMB phantoms in all whole body counter (WBC). It was confirmed that MDA allows us to use WBC for screening and for detailed measurements of internal contamination. For some kinds of BOMB phantom and point radiosources were used for age dependency and to assess the distribution of radionuclides. Moreover, since surface contamination was assumed, point radiosources were on the BOMB phantom and measured. It is well known that counting efficiency is greatly different depending on energy and ages. After the Ge detector had been set up, volunteers were measured for the first time. Two patients with mesothelioma and five healthy volunteers were measured. We would distinguish relatively easily even radionuclides of natural origin or radionuclides with a small emission ratio.

6) A rapid analysis technique of Sr, Am, and U in urine samples

Dose evaluation for internal contamination is more complicated than that for external dose exposure. Especially, there is difficulty comparing internal dose estimation from α and β-emitters than from γ-emitters. For this purpose, chemical analyses of urine and feces (bioassay) were conducted to estimate the amount of radioactive materials in human bodies. However, chemical analyses are usually complicated and time consuming. In a radiation emergency, analytical results will be required for treatment of exposed persons as soon as possible. In this study, three kinds of extraction resin columns, a liquid scintillator, and an α-spectrometer were combined to develop a rapid measurement system for strontium, americium, and uranium in human urine and feces samples. After spiking an aliquot of $^{90}\text{Sr}$ to the urine sample, the $^{90}\text{Sr}$ fraction was purified by an Sr-specific resin column and detected by a liquid scintillator. Am and U were separated by UTEVA and TRU resin columns and measured by an α-spectrometer and/or inductively coupled plasma mass spectrometry. A good recovery (above 80-95%) was obtained in all cases. The total analysis time per urine sample was within a working day (ca. 8 hrs). It would be an effective bioassay method for radiation emergencies.

7) Effects of chelating agents on removal of uranium in simulated wound model of rats

The effects of a chelating agent, CBMIDA, on removal of uranium via wounds (as the model of uranium contamination with a shallow injury) in which uranium was injected intracutaneously in the rat's back skin and the combination effects with the surgical excision of uranium-injected skin were examined. About 80% of the injected dose of uranium was removed when the skin was excised 30 min after DN injection (uranium nitrate, pH 1), and the uranium in the surrounding skin after the excision was less than 1%. At the same time, the amount of uranium on the surface of the femur decreased. The urinary excretion rate of uranium in the excision + CBMIDA group increased compared to that of the excision alone. The effects of combinations of local (infusion of chelating agents into the uranium-injected site) and systemic administration of chelating agents after the intramuscular injection of uranium in rats were examined. When CBMIDA was administered into the uranium-injected sites and systemically, the amount of uranium in the kidneys, femur, and muscles (uranium-injected site) decreased significantly. We also performed screening tests of newly synthesized agents. The effects of newly synthesized chelating agents, TREN-Bisphosphonate, TREN-methyl-Bisphosphonate, or Hydroxypropyridine-Bisphosphonate on removal of uranium were determined. However, no effects were
observed. One of other nine new agents decreased the concentration of uranium in the liver and femur at the same time.

Major Publications
2. K. Shiraishi, S. Ko, Y. Muramatsu*, P. V. Zamostyan*, N. Y. Tsigankov*: Dietary Iodine and Bromine Intakes in Ukrainian Subject, Health Physics, 96 (1), 5-12, 2009
4. K. Fukutsu, Y. Yamada, M. Akashi: Characterisation of Nasal Swab Samples by Alpha Spectrometry, Radiation Protection Dosimetry, 134(2), 87-93, 2009
5. A. Funukawa, M. Minamihisamatsu, L Hayata: Low-Cost Metaphase Finder System, Health Physics, 98 (2), 269-275, 2010