6. Research Center for Radiation Emergency Medicine

Outline of Research Career:
Dr. Akashi started his medical career at Jichi Medical School (Tochigi Prefecture) as a junior resident of internal medicine in 1981. He next worked as a senior resident in the Division of Hematology of Jichi Medical School and in 1987 moved 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 has been a staff member of NIRS since 1990. His major studies 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 Research Center had the unique experience of receiving three victims heavily exposed 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 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 a radiation emergency from the point of medicine.

Our required aims to satisfy the plan are as follows:
• to receive victims exposed to radiation who require specialized diagnosis and treatment;
• to dispatch a radiation emergency medical team to local emergency medical headquarters;
• 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;
• to maintain and reinforce an efficient radiation emergency medicine system under usual conditions;
• to promote technical development and research on radiation emergency medicine; and
• to develop skilled manpower for a radiation emergency.

Other objectives of the Center are related to research on radiation emergency medicine. Details are given elsewhere; only subjects are presented here.

1. Research for diagnosis and treatment of exposure to high-dose radiation
   1-1 Studying mechanisms of radiation injuries leading to developing new agents for treatment with focus on the skin and gastrointestinal tract
   1-2 Studying indicators of radiation exposure dose from biological specimens

2. Research on dose assessment for victims in radiation accidents

Overview
After the nuclear accident at Three Mile Island in 1979, the Central Disaster Prevention Council (CDPC) in the Prime Minister’s office reinforced emergency preparedness for dealing with a nuclear power station emergency and issued a report "Urgent Disaster Countermeasures to be taken for Nuclear Facilities by Governmental Agencies" in July, 1979. 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 radionuclides due to nuclear or radiological accidents. From January 2004 the Research Center has served as a liaison institution of WHO/REMPAN (Radiation 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 the tertiary radiation emergency hospital.

1) Network System
   The primary goal is strengthening its institutional system to prepare for radiation emergencies by establishing three nation-wide network councils, for medicine, chromosome analysis as bio-dosimetry, and physical dosimetry.
   a) NIRS Radiation Emergency Medicine Network Council
      This is a group of experts and medical organizations from which NIRS asks for help to treat
the victims at the time of a nuclear disaster or a radiological accident. The cooperation involves sending an expert in the specific field in an emergency, arrangement of acceptance of patients at medical facilities affiliated with the expert's organization, and provision of advice. Such collaboration is expected to reinforce the functions of NIRS. NIRS will call the Radiation Emergency Medicine Network Council to solicit cooperation when it is requested by authorities (or when NIRS thinks the necessity arises) to respond to radiation emergencies. This council worked effectively at the time of the JCO criticality accident in 1999.

b) Chromosome Network Council
This council forms a network among a limited number of experts having dose evaluation capability based on chromosome analysis. Through this network, NIRS can strengthen the capability of the dose estimation by chromosome analysis, and also establish the technical standards of dose estimation method by chromosomes.

c) Physical Dosimetry Network Council
This council is a network of experts in physical dose evaluation. The network is expected to respond to emergencies through collaboration among experts for prompt and precise dose estimation. It is also responsible for accumulating dose evaluation technology and for fostering followers.

d) Local Medicine Network Council
In Japan, medical systems for radiation emergencies are currently being constructed in accordance with disaster prevention plans of local governments that have nuclear facilities in their territories. Within the framework of each local nuclear disaster prevention plan, establishment of a specific collaboration system with NIRS is mandatory and the system must specify the steps to be performed in the smooth transfer of patients from a site to a hospital, including radiation protection management at the hospital.

2) Training
The primary goal for training is the development of radiation emergency medicine skills for medical professionals and disaster responding personnel; these include doctors and nurses involved in nuclear disaster medical care, emergency crews, and nuclear establishment employees. For that purpose the following training courses are regularly held 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.

a) Radiation emergency medicine course (hospital course)
This 3-day course is designed for physicians, nurses, radiologic technologists who may receive victims exposed to radiation and/or contaminated with radionuclides. The course is held three times a year with 20 participants in each course. More than 320 participants have been trained so far. Many of them are working actively in primary or secondary levels of radiation emergency hospitals and playing an important role in local radiation emergency exercises.

b) Emergency rescue training course (pre-hospital course)
This 3-day course is primarily designed for first responders such as fire or police personnel, paramedics, and emergency planners at nuclear facilities. The course is held four times a year with 30 participants in each course.

c) Training course for the WBC
This 3-day course is intended for personnel working in health physics, medical physics, radiation safety and others who have radiation dose assessment responsibilities. The course presents an advanced level of information on radiological/nuclear event reconstruction and dose assessments/estimations, focusing on internal contamination. Topics related specifically to radiation emergency medicine include internal and external contamination. Other topics covered include internal and external dosimetry and bioassay techniques. This course is held once a year with 18 participants.

3) Emergency Exercises
National and local governments annually hold drills for nuclear emergency. NIRS sends staff members to these drills to give advice from the medical or radiation protection point. On 24 October 2007, the Japanese government conducted a nuclear drill at Japan Nuclear Fuel Ltd.’s reprocessing plant for spent nuclear fuel (Rokkasho-mura, Aomori Prefecture) to enforce readiness for a criticality accident. About 1,800 people from some 70 organizations participated in the drill, including medical doctors and experts on radiation protection from NIRS. The daylong drill was conducted assuming nuclear chain reactions were triggered from mishandling fuel and equipment that caused radioactive leaks. A mock victim was transferred from the plant to NIRS using a plane of the Japan Self Defense Force and a helicopter of Fire Department, the Chiba City government. Following the drill, NIRS conducted an additional exercise to simulate emergency handling, especially dose assessment.

4) Follow-up Studies
The Research Center for Radiation Emergency
Medicine conducts research work in a wide range of areas: medical care, radiation measurement and investigation, health physics, cytogenetics, and psychology. In addition, we study dose evaluation which facilitates decision-making in treatment for high-dose exposure or reduction of high-dose exposure effects, identification of radionuclides, and rapid evaluation of population exposure.

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

a) Follow-up examination of the victims of the Bikini nuclear test

On March 1, 1954, the 23 crew members (18 to 39 years old at the time) of the Japanese fishing vessel Daigo Fukuryu Maru, or "Lucky Dragon", out of Yaizu City, Shizuoka Prefecture watched a bright light in the South Pacific as the sun began rising. 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 dropped on Hiroshima. All 23 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. personnel involved in the tests, were also injured from the nuclear fallout.

This medical follow-up examines the health states of these patients over a long period of time in order to study late radiation effects. 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 6.0 Gy. Among the Lucky Dragon victims, 12 have now died. Causes of death are as follows: liver cancer, 6; liver cirrhosis, 2; liver fibrosis, 1; colon cancer, 1; heart failure, 1; and traffic accident, 1.

This year, physical check-ups of survivors were conducted at NIRS and Yaizu City Hospital. Six persons were checked at these facilities. Malignancies were suspected in two of these people. Many of them have evidence of infections by hepatitis viruses since all victims received transfusions in 1954.

NIRS held a symposium in Chiba on "The Fifth Lucky Dragon at the Bikini Atoll" in collaboration with the Japanese Radiation Research Society and discussed aspects of the accident.

b) Follow-up examination of patients with thorotrastosis

Thorotrast is an alpha particle-emitting thorium dioxide colloid, which was used clinically in the 1930s and 1940s as a radiographic contrast medium. It was injected intravascularly for the visualisation of vascular structures. Long-term retention of thorotrast in the reticuloendothelial system, in the liver, spleen and bone marrow produces lifetime alpha particle irradiation of these organs. Considerable epidemiological follow-up has been performed on patients given the contrast, mainly German, Danish and Japanese patients and it has been found that the incidence of leukaemia among them has increased.

In Japan, the product was used from 1932 to 1945 for 10,000 to 20,000 patients, the majority of whom were injured in World War II. This follow-up examination program 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, the medical check-up was carried out for only one patient.

5) Database

Since radiation accidents requiring medical care are extremely rare, as much medical information as possible must be collected from each accident and accumulated to help medical professionals to making 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 related countries. Under the supervision of the World Health Organization (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. NIRS registered the Daigo Fukuryu maru accident in the SEARCH database. In addition, our center is constructing a database by collecting medical data on the victims of radiation accidents and exchanging information with countries that have developed radiation accident medicines. In FY2007, medical data on treatment of internal contamination
with radionuclides were collected from the United Kingdom, Italy, Belgium, and Germany.

6) Special topics
   a) Establishment of 24 hour emergency call system and telephone consultation for radiation effects system

   For more than 10 years, NIRS has provided medical assistance to hospitals, radiation facilities, companies, and others. However, phone calls could not be answered at night, on weekends, or on national holidays, since a 24-hour call emergency system had not been established. In FY2007, NIRS established the 24-hour on-call emergency system for hospitals and first responders including fire departments. This the 24-hour on-call emergency system is for direct or consultative assistance regarding medical and health physics problems associated with radiation or nuclear accidents. After normal business hours, phone calls are automatically transferred to 3 or 4 staff members of the Research Center for Radiation Emergency Medicine at NIRS (who include a medical doctor and a health physicist).

   NIRS has another telephone consultation assistance system. The number of phone calls for consultations on radiation effects is increasing. This year we received 76 consultations. Of those, 62 were consultations on radiation exposure. Nineteen cases were about exposure to radiation in medical use and 10 were accidental exposure. None of them needed medical care. However, there were a few cases with persons who believed that they had been exposed to radiation without evidence.

   b) Physical Dosimetry and Chromosome Network Councils

   To smoothly perform dose evaluation of the internal contamination by radionuclides in a radiation emergency, the in-vivo and in-vitro measurement system including internal dose evaluation code of each member organization was reviewed in the physical dosimetry network. While the measurement method and the equipment composition were various in each organization, it was confirmed that each organization can respond to radiation emergencies. As for the computer codes used, MONDAL which was developed by NIRS was highly evaluated. On the other hand, the "Livelink" solution which has been adopted by IAEA, was found to provide accurate information that was key in a radiation emergency. The validation methodology for feasibility study is being considered now. Agreement concerning cooperation in radiation emergencies will be obtained regarding internal exposure dose evaluation based on these results. This year, the investigation of WBCs was carried out at 6 radiation emergency hospitals of second level using standard BOMAB phantoms. There was a big difference in results of measurements depending on the kind of the phantom used when the manufacturer tested it. Among them, the best one was ±3% error from the standard but the worst one was -80%.

   The chromosome analysis network was established after the JCO critically accident at Tokai-mura in 1999. The first version of a standard curve for dose estimation by chromosomes was established by the common criteria for dicentric analysis. This year, an exercise for the dose estimation by chromosomes was performed. NIRS sent two samples of radiated blood to collaborating laboratories. No information on the dose was provided. However, the estimated dose by each collaborating laboratory in the network was almost identical. The first consultation meeting for the establishment of international cytogenetic dosimetry network was held in Geneva Switzerland, December 17-18, 2007 by WHO. Two members from the biodosimetry section attended this meeting where the general scope and concept-of-operations framework were discussed for the establishment of a global biodosimetry laboratory network for radiation emergencies.

7) International Cooperation
   a) Training courses for foreign medical staff organized by NIRS

   Two training courses were held for medical professionals of Asia. From December 4 to 6, 2007, a training course for Taiwanese medical professionals on radiation emergency medical preparedness was held upon request and 26 persons attended from Taiwan. The Korean Institute of Radiological and Medical Sciences (KIRAMS) asked NIRS to provide a training course for Korean medical staff. From December 11 to 13, 2007, a training course for Korean medical professionals was held and 23 persons attended from Korea.

   b) Organization of meetings

   - 'NIRS/NSC Workshop on Medical Response to Nuclear Accidents in Asia' in collaboration with IAEA was organized at NIRS from January 30 to February 1, 2008. Two professionals from IAEA and 19 from China, India, Indonesia, Malaysia, Sri Lanka, South Korea, Pakistan, Philippines, Thailand, and Vietnam attended the workshop and discussed the network for radiation emergency medical preparedness in Asia.
   - Special lecture by Dr. Bill McBride (from US) about research for treatment of high-dose exposure on 9 January 2008.
   - Special lecture of Dr. Volker List (from Germany) about internal contamination with Pu on 9 January
c) Invited lectures
Our staff was invited to give lectures in the following meetings and training courses.

- "The 6th Asia Pacific Burns Congress" held in Seoul, Korea from June 3 to 5, 2007.
- "KIRAMS regional exercise at KINS" held in Daejeon, Korea October 23, 2007.
- "BATAN-JAERI Joint Training Course on Radiological Emergency Preparedness and Response" held in Jakarta, Indonesia from October 28 to November 3, 2007 sponsored by the JAEA.
- IAEA/RCA Regional Training held in Daejeon, Korea from November 12 to 17, 2007

d) International meetings / Conferences
NIRS staff attended the following meetings.

- IAEA/RCA meeting on "Sustainability of Regional Radiation Protection Infrastructure" held in Colombo, Sri Lanka from May 7 to 11, 2007.
- Consultants Meeting on Overview of Radiotherapy Infrastructure Worldwide (DIRAC) held in Vienna, Austria from June 12 to 15, 2007.
- IAEA/RCA Regulation Meeting held in Vienna, Austria from October 15-19, 2007.
- India-Japan specialists meeting held in Mumbai, India from November 13 to 15, 2007.
- IAEA/RCA (RAS/9/042) Asian ARALA Network Meeting held in Daejon, Korea from December 2 to 5, 2007
- WHO Information Gathering Concerning Treatment Method of Radiation Skin Trouble held in Geneva, Swiss from December 17 to 18, 2007.

e) Members of international committees
NIRS staff participated in the following committees.

- Annual Meeting of International Commission on Radiation Units and Measurements (ICRU) held in Florence, Italy from October 10 to 14, 2007.

f) Other Visitors
- Upon a request from a hospital in Singapore, we accepted one medical staff member from September 23 to October 3, 2007 to see a domestic pre-hospital training course on emergency response for first responders held at NIRS. The participant learned about the system supporting nuclear disaster prevention drills in Japan.
- Three professionals from the National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention (Chinese CDC) visited our facility to see a domestic pre-hospital training course on emergency response for first responders from November 26 to 28, 2007. In addition, we discussed a future cooperative project for radiation emergency medicine between NIRS and Chinese CDC, and we exchanged a memo of understanding.
- Four professionals from the Beijing Institute of Radiation Medicine visited our facility and discussed a future cooperative project for radiation emergency medicine with NIRS staff on December 17, 2007.
6.1 The Study for Medical Treatment for High Dose Exposure

Outline of Research Career

Dr. Akashi started his medical career at 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 and moved 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 works are: 1) Establishment of radiation emergency medical preparedness, 2) Research on radiation injuries, including molecular and cellular mechanisms, 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 group 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 group tries to clarify the mechanism of injuries in cells and tissues exposed to high doses of radiation and their effects on survival, repair, and maintenance of function. In this study, we evaluated candidate substances for therapeutic drugs particularly for gastrointestinal and skin injuries. For gastrointestinal injuries due to radiation, the group uses experimental animals, primary cultured cells, and tissues to develop quantitative evaluation systems. In addition, the groups studied medical treatment with cytokines, natural products, and synthetic compounds that decrease the severity of injury.

To develop accurate diagnostic dose assessment for high-dose exposure to radiation, this group also tries to find markers for radiation exposure from biomolecules contained in samples which can be collected less invasively, such as blood. The group tries to determine genes, proteins, and other constituents of the living body that can provide a guide to treatment for radiation exposure.

Progress of Research

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

Ionizing radiation at high doses causes skin damages and hair loss, and apoptosis of hair follicles is one of the major prognostic factors. However, it is difficult to examine the skin and hair loss in small animals such as mice, because total body irradiation (TBI) causes their death with a very high dose. Therefore, we aimed to establish the in vivo assay system of radiation-induced apoptosis in plucking-induced anagen hair follicles and we used this assay to evaluate preventive effects of FGF1. A portion of the dorsal skin of seven-week-old male BALB/c mice harboring uniform telogen phase hair follicles was depilated for induction of anagen. BrdU incorporation and PCNA staining confirmed that the follicle keratinocytes were markedly proliferating at the following anagen phase. The mice received TBI with gamma-rays at doses from 8-16 Gy at anagen V 6 days after depilation. TUNEL assay was performed on paraffin-embedded sections of the dorsal skin to evaluate apoptosis over time after irradiation. The results in this study were as follows: (1) Radiation induced few instances of apoptosis in the non-depilated skin. (2) Radiation drastically increased apoptotic cells in the hair follicles of the depilated skin 8 to 24 h after irradiation. (3) It induced an active form of caspase 3-positive cells in the hair bulbs 8 h after irradiation. (4) It induced hair follicle dystrophy and regression from anagen to catagen in the follicles of the depilated skin in a radiation dose-dependent manner. (5) FGF1 significantly decreased the proportion of apoptotic cells in the hair follicles after irradiation.

Our findings revealed the induction of anagen by plucking the hairs was useful for evaluating the radiation-induced apoptosis, and it enables us to analyze a new substance for effects on radiation skin damage and hair loss in the mice irradiated at a lethal dose.

2) Inhibition of the ionizing radiation-induced activation of pro-death caspase-2 by a C-terminal PIDD (773-917) fragment

PIDD (p53-induced protein with a death domain) plays a critical role in the activation of caspase-2 to trigger DNA damage-induced apoptosis through the formation of a PIDDosome, which contains the adaptor protein RAIDD and caspase-2. PIDD also plays an essential role in DNA damage-induced activation of the antiapoptotic transcription factor NF-kB through the
formation of an alternative PIDDosome, consisting of PIDD, RIP1 and NEMO. Thus, PIDD acts as a molecular switch to turn life and death pathways on and off after DNA damage. We found that transcription of PIDD was induced after exposure of ionizing radiation in rat epithelial cell line (IEC6) cells, suggesting that PIDD might be a drug target for protection from ionizing radiation-induced gastrointestinal cell death.

Yeast two-hybrid analysis indicated that the death domain of PIDD interacts with RAIDD. When a rat C-terminal PIDD fragment (residues 773-917) containing the death domain was overexpressed, it dominantly-negatively inhibited the PIDD-mediated activation of caspase-2 after ionizing irradiation. In order to use the PIDD (773-917) fragment as an antiapoptotic drug, we purified a recombinant PIDD (773-917) fragment fused with 11-arginine which facilitates the uptake of the protein into mammalian cells with high efficiency.

3) The roles of TNFα in cells or mice exposed to radiation

Exposure to high dose radiation results in radiation injury that is a serious problem in accidental exposure and also in radiation therapy. Radiation activates the production of tumor necrosis factor α (TNFα) in various cells. However, the role of TNFα has not been fully understood in radiation exposure.

TNFα is one of the mediators of apoptosis. Previous studies have shown that TNFα expression is regulated by a transcription factor, early growth response-1 (Egr-1), in cell lines lacking p53. To better understand the pathways of TNFα expression after high dose irradiation, we used an inhibitor of MEK (PD98059), p38MAPK (SB203580) or PI3K (LY294002) and examined Egr-1 and TNFα expression in human T cell leukemia cell line Jurkat which lacks functional of p53. Studies of RT-PCR showed that the increased levels of Egr-1 and TNFα mRNA were observed immediately after 10 Gy irradiation; the levels reached a plateau at 30 min. When Jurkat cells were pretreated with an inhibitor of MEK, or p38MAPK, and then irradiated at a dose of 10 Gy, both levels of Egr-1 and TNFα mRNA were reduced. Furthermore, when Jurkat cells were pretreated with an inhibitor of MEK, p38 MAPK or P3K, radiation-induced activation of caspase-8 and caspase-3 was suppressed. In conclusion, our results suggest that ionizing radiation-induced TNFα induction is mediated by MEK, p38 MAPK or P3K via Egr-1 induction in Jurkat cells. Further studies on mechanisms are in progress.

In this study, we also investigated the roles of TNFα in mice exposed to radiation. We compared the wild-type (wt) and the TNFα knock-out (k/o) BALB/c mice. Both groups of mice were subjected to γ-ray radiation. The survival durations in wt mice were significantly longer than those in k/o mice. Furthermore, exogenously added TNFα before radiation increased survival rate. We compared numbers of blood cells, and surviving intestinal crypts, apoptosis in crypt cells and activity of an antioxidant enzyme manganese superoxide dismutase (MnSOD) following radiation. There was no significant difference in numbers of white blood cells after exposure in the two groups. On day 15 after exposure, the numbers of red blood cells in wt mice was higher than those in k/o mice. Moreover, there was also no significant difference in numbers of surviving intestinal crypts after exposure and apoptosis in crypt cells between wt and k/o mice. Activities of MnSOD were lower in liver of k/o mice than that of wt mice. We also studied the expression of apoptosis-related proteins in mouse intestinal epithelial cells along the crypt-villus axis after radiation. The Bcl2 protein was constitutively expressed and its level was reduced by radiation in wt mice. In contrast, Bcl2 was not expressed in k/o mice and radiation did not induce its expression. Our results suggest that endogenously produced TNFα may play important roles in the radiation-induced injuries.

4) Lithium chloride reduces radiation-induced intestinal injury through inhibiting apoptosis in intestinal epithelial cells

High dose radiation induces apoptosis of intestinal epithelial cells and subsequent depletion of the stem cells, resulting in lethal gastro-intestinal injury. However, effective treatment of this injury has not been established yet. Lithium chloride (LiCl) is well known to activate a Wnt signal by inhibiting the activity of glycogen synthase kinase 3 (GSK3). The Wnt signal pathway has been shown to be associated with the maintenance of the stem cells of the intestinal crypt. Moreover, LiCl has been reported to inhibit neuronal apoptosis. The present study was designed to investigate effect of LiCl on intestinal injury induced by high dose radiation. Rat small intestinal epithelial cell line, IEC-6 cells and intestinal epithelial cells in primary culture obtained from 17.5 day fetal rat duodenum were used for in vitro assays. The cells were treated with LiCl 1 h either before or after γ-radiation of 20 Gy, and then cultured at 37°C until 24 h. The apoptosis was evaluated by Hoechst33258 staining of cells. Pretreatment with 10 mM of LiCl markedly inhibited radiation-induced apoptosis in IEC-6 cells. Addition of LiCl to these cells after radiation also blocked the apoptosis. The anti-apoptotic effect of LiCl was also found in intestinal epithelial cells in primary culture. Inhibition of either the phosphoinositide 3-kinase (PI3K) /Akt or mitogen-activated protein kinase (MAPK) /extracellular signal-regulated kinase (ERK) kinase (MEK/ERK) kinase pathway abrogated the anti-
apoptotic effect of LiCl. Western blot analyses showed that LiCl inhibited activation of caspase-3 and an induction of Bax in irradiated cells. Moreover, LiCl increased levels of Bcl-2 and Bcl-xL even in irradiated cells. We also administered LiCl to male Balb/c mice intraperitoneally an hour prior to TBI with 8 Gy. The numbers of surviving crypts were greater in mice treated with 200 mg/kg body weight of LiCl than control mice with PBS. Thus, our results suggest that LiCl protects and rescues intestinal epithelial cells from radiation-induced apoptosis through activation of pathways involving PI3K/Akt and MEK/ERK. We also showed that LiCl reduced radiation-induced intestinal injury in vivo.

5) Study on the mechanisms of radioprotective effect of heat-killed Lactobacillus casei

Molecular mechanisms of the radioprotective effects of the natural substances and medicines in the mice were studied. Heat-killed [Lactobacillus casei] (LBC) showed strong radioprotective effect in C3H/He inbred mouse. Although no mice (n=110) survived 11.25 days in average after whole-body irradiation at supralethal dose (8.0 Gy) of X-rays, 28 day-survival rate reached 0.70 when the mice (n=100) were subcutaneously injected with 30mg/kg of LBC 24 h before irradiation. Simultaneous administration of anti-inflammatory steroid, dexamethasone (Dex) reduced the survival rate to 0.37 (n=30). The reduction in the survival rate was not observed with the use of non-steroid anti-inflammatory drugs (NSAIDs), indometacin (0.80, n=30) nor sulpyrine (0.68, n=40). To reveal the effects of the LBC and Dex on the survival, we measured levels of an inflammatory cytokine, interleukin (IL) -1 beta in the circulation following the injection of LBC. Between 8 and 16 h after LBC-injection, the level of IL-1beta in blood reached maximam at 118 ng/mL (n=30) beyond the baseline level of less than 20 ng/mL. When the Dex was introduced with LBC, the maximal level declined to 40 ng/mL (n=12). The inhibition of the increase in the blood IL-1beta (IL-1β) levels by LBC was not observed when the indometacin or sulpyrine were injected simultaneously. There results suggest that the radioprotective effect of LBC may be mediated by systemic inflammation reflected in the blood IL-1β levels. This also indicates the advantage of simultaneous use of NSAIDs for radioprotection due to their weak systemic effect on the inflammation.

6) Study on the effect of pharmaceutical agents on the recovery of intestine damaged by radiation

The aim of the study was to obtain basic data to choose favorable pharmaceutical agents against intestinal damage caused by accidental or therapeutic radiation. The intestinal damage in C3H/He inbred mouse was generated by single abdominal irradiation of a high dose of X-rays. Three to four days after the irradiation at a dose of more than 19.0 Gy, all the mice were emaciated with hematochezia and had decreased body weight, and finally died within 8 days, showing that intestinal damage contributed to their death. When the X-ray dose of 17.0 Gy was used and concomitant nutrient infusion was subcutaneously injected once per day for 10 days, the body weights recovered to increase on Day 7 to 9 and mean survival rate of 0.60 (n=30) was obtained. Furthermore, we compared the effects of psychotropic agents in mice. Among minor tranquilizers, injection of phenobarbital did not cause significant change of the survival rate (0.53, n=15). In contrast, administration of a benzodiazepine derivative, diazepam drastically reduced the survival rate (0.23, n=20). This suggests several pharmaceutical agents may affect the recovery of intestine damaged due to radiation.

Major publications


6.2 Research on Radiation Dose Assessment for Radiation Emergency Medicine

Outline of Research Curuer
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 30 years of experience at NIRS 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 the Lovelace Foundation (USA) as a visiting scientist where he studied aerosol deposition within respiratory tracts.

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Objectives:
Radiation accidents can be divided into those resulting from external exposure and those resulting from internal exposure. For severe accidents, bone marrow transplantation may be considered depending on the external exposure dose received, or drug administration may also be considered to inhibit deposition and promote excretion of radioactive substances incorporated into the body. Dose assessment of victims in radiation accidents must be made within a short time in combination with the details of the accident to estimate the radiation effects and to initiate appropriate medical 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 on 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 application 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 clippings
Electron spin resonance (ESR) dosimetry is a method to measure radical numbers produced by radiation in substances and to estimate external 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 cannot be extracted easily from the exposed persons in all cases. It is necessary to find other human tissues or substances around exposed persons for estimating personal exposures. Nail clipping 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. The relationship between ESR sensitivity and absorbed dose (Gy) in the nails was found to be linear. An unknown dose of γ-exposed nail was estimated in 3-4 weeks using the modified calibration curve at room temperature. For radiation emergency purposes, the analysis time was succeeded to shorten 3 times by increasing the ambient temperature to 50°C. Several problems must be solved to establish nail ESR dosimetry and we are working on their solutions.

2) Chromosome aberration analysis
In order to confirm the calibration curve for cytogenetic dosimetry, we analyzed the dicentric chromosome in human lymphocytes irradiated at doses of 0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0Gy. The frequencies of dicentric chromosome occurrence at each dose point in these analyses were almost identical with those previously obtained by other investigators. This means that the quality level for the dicentric chromosome assay has not gone down. In the process of dose estimation by dicentric chromosome analysis, preservation of the blood sample may have a profound effect on the frequency of chromosome abnormality. Therefore, in the present study, we have begun analysis of the relationship between the dicentric chromosome frequency and temperature (4, 20 and 30°C) for preservation and also of checking the effect of higher concentration of Colcemid in human
peripheral lymphocytes. We expect results will be obtained in the near future.

Furthermore, in order to establish an assay system to evaluate the dose of partial body exposure, we used the human hair root as the target organ and detected indicators of dose estimation. We have also examined the method of culturing hair root cells for detecting chromosomal aberrations. The epilated hairs were dissected carefully, and single cells were isolated by enzyme treatment. When the hair was irradiated with \( \gamma \)-radiation, DNA damage was detectable in the comet assay, and the cultured hair root cells could be grown in vitro. These results suggest that dose estimation of partial body exposure may be possible using hair root cells.

3) Surface contamination monitor for unknown nuclides

When a patient is accepted at a treatment facility, surface contamination monitoring is carried out with more than one survey meter by reason of detecting multiple kinds of radiation in radiation emergency medicine. When internal contamination is doubtful, rapid deployment becomes possible if unknown nuclides can be identified at this stage. So development of an instrument which can distinguish all kinds of radiation with one detector was targeted to evaluate unknown nuclides promptly in the emergency locale. Different scintillators were adopted for detecting each kind of radiation such as a ZnS (Ag) scintillator for \( \alpha \)-rays, a plastic scintillator for \( \beta \)-rays and a CsI (TI) scintillator for \( \gamma \)-rays. These scintillators are optically coupled on the same axis and the scintillation light is detected by one photomultiplier tube. Here, \( \alpha \) and \( \beta \) detectors are surrounded by a \( \gamma \) detector which also acts as a veto detector to reduce the contribution of environmental \( \gamma \)-rays to the other detectors. The principle of this instrument is the same as that of a Phoswich detector using the decay time difference of each scintillator. Namely, identification of three components from the plastic scintillator (decay time: 1.8ns), ZnS (Ag) (200ns) and CsI (1000ns), can be a trigger signal for each radiation measurement. The detection efficiency is equal to or higher than that of present commercially available survey meters. As a result, when a patient was contaminated by unknown nuclides, we confirmed that separate measurements of \( \alpha \)-rays, \( \beta \)-rays, \( \gamma \)-rays made the identification of the nuclides possible.

4) Nasal swab for \( \alpha \) emitters

It is effective to obtain useful information from the nasal swab sample for prompt internal dose estimation of \( \alpha \)-particle emitting nuclides. Sample activity has generally been measured using gross \( \alpha \) counters such as a ZnS (Ag) scintillation counter because their measurements are sufficient to judge intake of \( \alpha \)-particle emitting nuclide has occurred. In order to obtain more information for emergency medicine, the measuring method using \( \alpha \) spectrometry has been re-examined. Simulated nasal swab samples were prepared. A suspended solution of plutonium oxide (PuO\(_3\) suspension) or a solution of plutonium nitrate (Pu (NO\(_3\)) \(_3\) solution) was dropped onto a filter paper as a simulated nasal swab sample. The PuO\(_3\) suspension and Pu (NO\(_3\)) \(_3\) solution were assumed to simulate dust and mist exposure, respectively. The measured \( \alpha \) spectra had a different shape for the PuO\(_3\) suspension and Pu (NO\(_3\)) \(_3\) solution. The spectrum of PuO\(_3\) suspension had clear energy peaks. The peak energy showed no energy loss to the filter paper fibers. These results suggested that identification of nuclides would be easy using energy peaks even for mixed nuclide exposure. On the other hand, the spectrum of Pu (NO\(_3\)) \(_3\) solution had unclear energy peaks. Almost the same count continued down to the lowest energy and because the solution infiltrated the filter paper fibers, the energy loss by them was large. We thought that this was the reason why the detection efficiency was lowered compared with the simulated dust exposure. These results suggested that \( \alpha \) spectrometry would give useful information to estimate internal dose assessment.

5) Development of lung phantom for in vivo measurements and improvement of thyroid monitor

A thorax model had been designed and made to give a realistic shape of the lungs and Japanese body size. In FY2007, the model’s composition and the distribution of the radioactivity were examined in detail. The material distribution in the model was confirmed by the X-ray tomography and by its actual cutting. Also a non-radioactive lung model, which was made in a similar way to the radioactive lung model, was cut and the composition analysis was done in several places. The result showed that the composition was almost the same, independent of the position. Therefore, the material distribution and the composition were proven to be uniform. Hydrogen, carbon, nitrogen, oxygen, and calcium accounted for 99% of the composition. The lung models were made by uniformly diffusing a radioactive-source in the polyurethane. Because the radioactivity was about 4.3kBq for a whole lung model, the sections of the radioactive lung model were closely set on the imaging plate inside a blackout-curtained area inside a low background room. The 59.5keV \( \gamma \)-rays from \(^{241}\)Am were observed. Moreover, the mapping measurement was done that rolled the collimator of the lead 1mm in one-inch NaI detector. The obtained results confirmed the uniformity of the radiation source as well as the physical structure.
To improve accuracy of the thyroid monitor used at the site of a nuclear accident which is anticipated to release radioactive iodine to the environment, we developed a trial portable thyroid monitor that combined a Compton suppressor with high purity Ge detector. Its basic properties were measured under the optimized conditions at the maximum BG compensation. As a result, we confirmed that the monitor had a performance equal to that of heavy conventional thyroid monitor (detection limit: approx. 13Bq) with a lead shield. Good portability was also obtained.

6) Rapid technique for urine analysis

Bioassays are important method to estimate amounts of radionuclides taken into a human body in the event of a radiation accident that involves internal exposure, especially exposure by $\alpha$- and/or $\beta$-emitters. In the case of internal exposure by soluble radionuclides, urine is used as a sample for bioassays and coprecipitation is mainly used to efficiently collect radionuclides from urine. However, on coprecipitation, it is difficult to estimate exposed dose quickly after a radiation accident happens, since with coprecipitation a lot of sequential chemical procedures must be followed. Then, we have developed a rapid coprecipitation method using a filtering kit which is commercially supplied by Millipore Co. Nitric solution which mainly contains $^{239}$Pu was used as a sample and the radioactivity was measured with a liquid scintillation counter and a high-purity germanium detector (HP Ge). Coprecipitation was completed within 2 h for a 400mL urine sample and soluble radionuclides were collected at the rate of more than 80% from the urine. Detection limits of exposed dose were estimated as 0.065Sv for inhalation and 0.025Sv for ingestion. The time needed for radiation measurements was about 1 h. From these results, it seemed possible to estimate the amounts of soluble radionuclides within 3 h after a urine sample was obtained.

7) Decision of detector efficiency in $\alpha$-spectrometry by ICP-MS

Internal dose estimation due to $\alpha$- and $\beta$-emitters has a difficulty compared with that of $\gamma$-emitters. For this purpose, chemical analyses of urine and feces (bioassays) are conducted to estimate the input and accumulated volumes of radioactive nuclides in a human body. After chemical separation, the final detection of $\alpha$-emitter nuclides is usually by $\alpha$ spectrometer, ZnS-scintillator and ICP-MS. In all case, detection efficiencies of the methods are important to get reliable results. Detection efficiency of $\alpha$ spectrometry usually is determined by standard electro-coated sources. There sometimes is a difficulty to buy the source preparation. In this study, detection efficiency of $\alpha$ spectrometry was decided by using ICP-MS. Uranium-238 concentrations in all steps of electro-deposition (initial sample solution, remaining sample solution after electro-deposition) were determined by ICP-MS. Counts of an electro-coated source was also measured by $\alpha$ spectrometry. The detection efficiency calculated by two measurements (ICP-MS and $\alpha$ spectrometry) was identical to that by a standard source ($^{241}$Am). Detection efficiency could be estimated by ICP-MS, especially in radionuclides having a long-life.

8) Dose calculation from internal exposure

The PC software MONDAL3 has been released for non-specialists users to estimate the committed effective dose from an internal radiation exposure using the results of individual monitoring measurements. Preparations for a database update were made on the basis of a human alimentary tract model for radiological protection published as ICRP Publication 100. Also, the dose calculation for radioactive zinc was carried out for the salivary glands and male reproductive organs. These organs were not included as source organs in the ICRP biokinetic model of Zn, though experiments in rats show relatively high Zn concentrations. The numbers of disintegrations in the testes, the prostate gland, salivary glands and the thymus were calculated from experimental results in rats, and SAFs (specific absorbed fractions) from the MIRD stylized model were determined under some assumptions. The equivalent dose of the testes or the prostate gland may be about ten times higher than the dose of the rest of the body. On the other hand, the dose of the salivary glands or thymus which showed a high density temporarily but rapidly decreased. may compare with that of other organs.

9) Acute toxicity of uranium and the effects of chelating agents in simulated wounds using rat model

The study focused on (1) the examination of the acute toxicity of uranium induced by uranium-contaminated wounds, and (2) the effects of chelating agent CBMIDA in local treatment of these wounds. To clarify the differences in behavior and toxicity at different depth of wounds that depleted uranium (DU) initially entered, different doses and chemical forms of uranium (4 and 16 mg/kg DU in pH=1 or pH=7 solutions) were administered by intracutaneous (IC), subcutaneous (SC), and intramuscular, and intraperitoneal injections to four groups. Uranium (pH=1) injected as IC and SC was retained at a level of about 60-70% in the injected sites for 1-3 h, 76-96% excreted at 24h, and the rest was deposited in liver, kidney and femur; their biochemical markers increased significantly 1 h. Uranium excretion was time-
dependent in urine and feces and digestive duct, and dependence was greater in feces more than in urine.

CBMIDA was infused into the wounds 0, 10, 30, 60, 120 min and 24 h after DU injection. The results indicated that CBMIDA could decrease uranium in the DU injected sites, kidneys, and femur, as well as the serum creatinine and urinary NAG/creatinine, and CBMIDA accelerated excretions of uranium in urine and feces when it was administered 30-120 min after DU injection.