6. Research Center for Radiation Emergency Medicine

Outline of Research Career:
Dr. Fujimoto received a Bachelor of Science degree from Kyoto University and obtained a Doctoral Degree in Engineering at the University of Tokyo. He has spent most of his career in studies on natural environmental radiation, especially terrestrial gamma radiation and indoor radon. After the criticality accident at JCO in Tokai his major involvement shifted to dose estimation for radiation emergencies. He was at the Harvard School of Public Health as a visiting scientist from 1981 to 1982 and in the International Atomic Energy Agency as an environment protection specialist from 1990 to 1994. He also served as a lecturer at the University of Tokyo from 1989 to 1996. He is now Director of the Research Center for Radiation Emergency Medicine (since 2003), an International Editorial Adviser of the Journal of Radiological Protection and an Advisory Editorial Board Member of Nuclear Technology & Radiation Protection.

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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 a medical viewpoint. Our required aims are as follows:
To accept 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 dealing with a radiation emergency.

Other objectives are to carry out research on radiation emergency medicine. Details are given elsewhere; only subjects are presented here.
1. Research for diagnosis and treatment of high-dose exposure to radiation,
   1-1 Studying mechanisms of radiation injuries leading to developing new agents for treatment, with the focus on skin and gastrointestinal tract,
   1-2 Studying indicators for dose of radiation exposure from biological specimens, and

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.

1.1. 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 or a radiological accident. The cooperation involves dispatch of 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.

1-2. Chromosome Network Council

This council forms a network among a limited number of specialists having dose evaluation capability based on chromosome analysis. Through this network, NIRS can strengthen the capability of the dose estimation by chromosome aberration, and also enhance the technical standards of chromosome aberration dose estimation method.

1-3. Physical Dosimetry Network Council

This council is a network of experts in physical dose evaluation techniques. 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.

Topic: Investigation on WBC in the secondary levels of hospitals for radiation emergency

The whole body counter (WBC) is used for measurement of internal contamination. However, it cannot provide meaningful measurements without calibration. The secondary levels of hospitals for radiation emergency have WBCs and victims internally contaminated with radionulides are transported to them. The committed effective dose is important for deciding whether these victims should be transferred to NIRS. We checked WBCs in four hospitals by using BOMAB phantom containing the standard radioactivity source of either $^{137}$Cs, $^{60}$Co, $^{133}$Ba or $^{40}$K which NIRS had developed based on the ANSI standard. Using the phantom with $^{137}$Cs, we found that the deviation from the standard ranged from -87.5% to + 35%. One of the main reasons for this variation is that the methods of calibration were different among makers of WBCs; phantoms used by these makers were too small for calibration of these WBCs. Thus, a committee has been constructed for establishment of a standard calibration method in Japan.

1-4. Local Medicine Network Council

In Japan, medical systems 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, set up of a specific collaboration system with NIRS is mandatory and it must specify the steps to be performed in the prompt transfer of patients from a site to a hospital, including radiation protection management at the hospital.

2) Training

The primary goal is the development of radiation emergency medicine skills for medical professionals and disaster prevention 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 disseminate the relevant information and skills to deal with a radiation emergency.

(A) Radiation emergency medicine course (hospital course)

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)

The course is held four times a year with 30 participants in each course. The duration of the course is 3 days.

(C) Training course for the "whole body counter" measurement

The persons who are responsible for estimation of internal exposure dose in the case of a radiation emergency are trained to be able to measure and estimate internal dose by themselves.

3) Emergency Exercises

National and local governments in Japan regularly organize emergency exercises to which we send our staff to take roles in emergency medicine and radiation protection. On 25-26 October 2006 the Japanese government conducted a nuclear disaster prevention exercise in Ehime prefecture. Our staff participated in this. Moreover we conducted an additional exercise to simulate emergency handling, especially dose assessment.

4) Follow-up Studies

The center also carries on follow-up clinics for the victims of the thermonuclear weapons test on the Bikini Atoll, patients with thorotrastosis and the surviving JCO accident victim.

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

During the nuclear test on Bikini Atoll on 1 March, 1954, 23 crew members (18 to 39 years old at the time)
of the Lucky Dragon (Dai-go Fukuryu-maru) out of Yaizu City, Shizuoka Prefecture, were exposed to radiation. This follow-up survey aims to examine the physical states of these patients over a long period of time to study late radiation injuries. The follow-up examinations that have been conducted for 50 years provide precious data. The type of exposure was external and also internal, and the estimated dose was 1.7 to 6.0 Gy. A physical checkup of still living survivors was conducted at NIRS and Yaizu City General Hospital this year.

Follow-up examination of patients with thorotrastosis

Thorotrast is a radioactive contrast medium for angiography. The main constituent is thorium dioxide. A German company started sales of this medium in 1930. 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. Thorotrast is deposited in the liver and spleen and causes internal radiation exposure over a long period of time. 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.

Database

A database including the cases of radiation exposure on Bikini Atoll and cases of thorotrastosis is being constructed. Since radiation accidents are rare, the maximum amount of information must be collected from each accident and accumulated to help medical professionals decide strategies to treat patients, and establish and improve therapeutic methods. Today, there are various databases on radiation accidents and their victims, but most are not accessible from other 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. The NIRS registered the Dai-go Fukuryu-maru accident in the SEARCH database. In addition, our center is constructing a database by collecting the medical data of the victims of radiation accidents and exchanging information with countries that have developed radiation accident medicine.

International Cooperation

1. Six professionals from Beijing Institute of Radiation Medicine visited our facility and discussed a future cooperative project for radiation emergency medicine with NIRS staff on 29 June 2006.

2. Invited lectures

Staff members were invited to give lectures in the following meetings and training courses.

(1) A training course held in Jakarta, Indonesia from 6 to 11 November 2006 sponsored by the JAEA.


(3) International Symposium on Emergency Medical Response for Radiation Accidents held in Ulsan, Korea from 18 to 20 April 2006.

(4) A lecture regarding Internal Dosimetry in Inhalation Intakes held at Fudan University in
3. Organization of meetings


(2) Meeting on Radiation Emergency Medical Preparedness and Response in Asian countries was organized from 30 January to 1 February and from 27 February to 1 March 2007. The former was held with 4 participants from South Korea, and the latter was held with 16 participants from Indonesia, South Korea, Mongolia, Philippines, Thailand, and Vietnam.


5. Members of international committees

(1) IAEA Assistance Work Group (AWG) and Expert Groups (EG) meetings held in Milan, Italy on 6-8 June and in Vienna, Austria on 6-8 June 2006.

(2) The 11th Coordination and Planning Meeting of the WHO REMPAN Collaborating Centers and Liaison Institutions held in Kiev, Ukraine on 25-28 April 2006.

(3) Meeting for medical database of radiation accidents held Beijing and Xiamen, China from 29 November to 1 December 2006.
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 activities 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:
Study on injuries and therapeutic measures in high-dose radiation exposure
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.

1) Research on mitigation of radiation injuries caused by high-dose exposure
The group tries to clarify the mechanism of injuries in cells and tissues exposed to high doses of radiation and dose effects on survival, repair, and maintenance of function. It evaluates candidate substances for therapeutic drugs particularly for gastrointestinal and skin injuries. Regarding gastrointestinal injuries due to radiation, the group uses experimental animals, primary cultured cells, and tissues to develop quantitative evaluation systems. For the study of skin injuries, cultured skin cells and the skin model system using feeder and epithelial cells are used to explore basic mechanisms of injury and fibrosis due to high-dose radiation exposure.

2) Research on diagnostic measurements for high-dose exposure
To develop diagnostic measurements in high-dose exposure to radiation, this group tries to find markers for radiation exposure from bio-molecules contained in samples which can be collected less invasively, such as cells and 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) Research for mitigation of radiation injuries caused by high-dose exposure
1-1. Study on treatment of skin in high-dose radiation exposure

Recently, it has been found that fibroblast growth factors (FGFs) protect and enhance repairing radiation-induced tissue damage. They need the binding of heparan sulfate proteoglycan (HSPG) to activate their target receptors. We investigated the effect of ionizing radiation on the biosynthesis of HSPG in human HaCaT keratinocytes. Levels of two sulfotransferases, HS2ST1 and HS6ST1, were significantly increased in the HaCaT cells 24 hr after X-ray irradiation. Addition of hydrogen peroxide decreased the levels of these enzymes in HaCaT cells. Transfection of the catalase gene also induced an increase of HS2ST1 and HS6ST1 expression in the cells. In addition, Western blot analysis showed that the smeared bands of HSPG were clearly shifted to higher-molecule-weight in the transfectants owing to glycosylation. These enzymes are known to play a critical role in the formation of binding complexes of HSPG with FGFs and their receptors. Indeed, siRNA-mediated repression of catalase or HS2ST1 significantly inhibited FGF7-induced proliferation of HaCaT cells. These findings suggest that hydrogen peroxide inhibits sulfation of specific sites in HSPG in irradiated cells. Thus, hydrogen sulfate may play a role as a negatively signaling molecule to promote the regeneration of irradiated skin.

1-2. Study on mitigation of gastrointestinal tract (GIT) injuries by high-dose radiation exposure
Exposure to high dose radiation causes lethal GIT injuries. This is largely due to apoptosis of intestinal epithelial cells and subsequent depletion of the stem cells. We found that lysophosphatidic acid (LPA) and ginsenosides Rd and Rg1 prevent and rescue radiation-induced apoptosis in vitro and in vivo.

LPA, a biogenic phospholipid, is linked to a variety of cellular responses including differentiation, proliferation, migration, or inhibition of apoptosis. Pretreatment
of the rat small intestinal epithelial IEC-6 cells with LPA significantly inhibited apoptosis by radiation. Addition of LPA to these cells after radiation also blocked the apoptosis. Inhibition of either PI3K/Akt or MEK pathway significantly attenuated anti-apoptotic effect of LPA. In contrast, treatment with an inhibitor of p38 MAPK pathway did not affect the apoptosis. LPA activated phosphorylation of Akt and ERK1/2 but not p38 MAPK in irradiated cells. Moreover, LPA inhibited activation of caspase-3 and -9 in irradiated cells. LPA increased levels of Bcl-2 and Bcl-xL without affecting Bax levels. Thus, these results suggest that LPA protects and rescues IEC-6 cells from radiation-induced apoptosis through activation of a pathway involving PI3K/Akt and ERK. We also show that the mitochondrial pathway is important for anti-apoptotic effects of LPA.

Ginsenosides are the main pharmacoactive molecules of ginseng, the root of Panax ginseng C. A. Meyer. Ginsenosides Rg1 and Rg1 have been identified as two of the most effective compounds responsible for pharmaceutical actions of ginseng. Rg1 blocked radiation-induced apoptosis of these cells in a dose-dependent manner. Inhibitor of either MAP kinase kinase (PD98059) or p38 mitogen-activated protein kinase (SB230580) significantly decreased the number of apoptotic cells, whereas an inhibitor of the phosphoinositide-3 kinase (LY294002) pathway, attenuated the anti-apoptotic effects of Rg1. Radiation activated phosphorylation of the extracellular signal-regulated protein kinase (ERK), p38, and Akt proteins. Treatment with Rg1 either before or after irradiation inhibited phosphorylation of ERK and p38 MAPK, but enhanced phosphorylation of Akt. Rg1 also decreased levels of Bax and caspase 3, and increased Bcl-2 expression. Studies of immuno-histochemistry found that Rg1 significantly improved recovery of crypt architecture and also reduced the number of apoptotic bodies in the crypt of irradiated mice. In conclusion, Rg1 protects and rescues intestinal injuries in vitro and in vivo through activation of the PI3K/Akt pathway and, via inhibition of the MEK/p38 MAPK pathway.

1-3. Study on the mechanism for radiation-induced GIT injuries

The mechanisms of GIT injuries by radiation was studied using mice. Mouse intestinal epithelial cells were obtained subsequently along the crypt to villus axis by the modified method of Weiser. Western blot analysis showed that proteins of the proliferating cell nuclear antigen (PCNA), Bax and Bcl2 were constitutively expressed in the crypt and their levels were reduced toward the villus axis. On the other hand, active form of caspase 3, and cytochrome c were accumulated in the villus tip. Radiation increased levels of Bax, active form of caspase 3, and cytochrome c with concomitant reduced levels of PCNA and Bcl2 in the crypt. In contrast, radiation did not affect the caspase 3 and cytochrome c in the villus tip. The p53 and p21 proteins were not detected in intestinal epithelial cells without radiation. However, radiation increased the levels of these proteins toward the crypt but not in the villus. On the other hand, the p27 protein was constitutively expressed in the crypt, and radiation decreased its level in the crypt. Our results suggest that the cell proliferation and response to radiation is differentially regulated in the crypt from the villus; p53 may play an important role in the crypt.

Tumor necrosis factorα (TNFα) has both apoptotic and anti-apoptotic properties depending on the activation of signaling pathways. The human T cell line Jurkat cells were transfected with TNFα short interfering (si) RNA (siRNA). Studies of competitive PCR showed that an increased level of TNFα mRNA was observed immediately after irradiation in control cells. However, the knock-down of TNFα using siRNA significantly abolished the irradiation-induced accumulation of TNFα mRNA. We also investigated the mRNA levels of Bax, Bcl-2, and Bcl-XL. Levels of Bcl-2 and Bcl-XL mRNA were similar in control cells and siTNFα cells. However, the level of Bax mRNA was apparently higher in siTNFα than control cells but radiation failed to increase the level in both cell lines. On the other hand, staining with Annexin-V and detection of DNA fragmentation showed that irradiation at a dose of 10 Gy induced apoptosis more frequently in the control cells than TNFα siRNA cells. These results indicate that irradiation induces apoptosis through a pathway requiring TNFα production. Our results also suggest that apoptosis by irradiation occurs independent of the Bax expression.

1-4. Study on endothelial cells exposed to high-dose radiation

The balance between survival and cell death of vascular endothelial cells is regulated by a homeostatic mechanism. This homeostatic mechanism is deregulated by exposure to radiation. It is known that vascular endothelium is a principal target for radiation injury to the GIT, lung, brain, and skin. Thus, vascular damage is a key mechanism in radiation injuries, and death and proliferation of the vascular endothelial cells are the primary lesion leading to stem cell dysfunction.

We examined the effects of vascular endothelial growth factor (VEGF) and copper/zinc superoxide dismutase (Cu/ZnSOD) on cell growth in radiation using human primary umbilical vein endothelial cells (HUVEC). Studies of cell growth and proliferation showed that radiation suppressed the cell growth in a dose-dependent manner. Moreover, assays of WST1 and colony formation showed that treatment of these
cells with VEGF or Cu/ZnSOD before or after radiation, rescued the cell growth suppression and both factors activated the phosphorylation of ERK1/2 in irradiated cells. We also found that VEGF or Cu/ZnSOD restored endothelial damage caused by radiation. Thus, our results indicate that VEGF and Cu/ZnSOD may be candidates of therapeutic agents for radiation-induced injury.

VEGF or fibroblast growth factor-2 (FGF-2) significantly increased viability, up-regulated telomerase activity, and inhibited apoptosis in irradiated HUVECs. VEGF synergistically suppressed apoptosis with FGF-2 in irradiated cells. VEGF was more effective than FGF-2 in inhibition of radiation-induced apoptosis. This anti-apoptotic effect of VEGF occurred through up-regulation of telomerase activity and subsequent activation of PI3K/Akt pathway.

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 so-called PIDDosome, which contains the adaptor protein RAIDD and caspase 2. PIDD also plays an essential role in DNA damage-induced activation of the anti-apoptotic transcription factor NF-kB through the formation of an alternative PIDDosome, consisting of PIDD, RIP1 and NEMO. We found that transcription of PIDD was induced after exposure to ionizing radiation in rat epithelial cell line (IEC6) cells, suggesting that PIDD might be a drug target for protection from radiation-induced gastrointestinal cell death. When IEC6 cells were exposed to radiation, ubiquitinated and SUMO-modified NEMO was observed, which is required for the activation of NF-kB. The modification of NEMO was also observed even in the unirradiated cells when full-length human PIDD cDNA was overexpressed in IEC6 cells. Yeast two-hybrid analysis indicated that the death domain of PIDD interacts with RAIDD. In order to inhibit the PIDD-mediated apoptosis with designed synthetic peptides, we are currently pursuing fine mapping of regions involved in the interaction of PIDD with RAIDD using yeast two-hybrid analysis.

The aim of this project is to establish the methods for evaluating the effective dose and other radiation effects using biological samples based on the molecular and biochemical technology. It is already known that the parasites such as endogenous viruses in the microorganisms are activated by lethal damage of the host microorganisms. Since mammalian genome possesses thousands of copies of DNA elements that encodes endogenous retrovirus-like transposon, we focused on retrotransposon, intracisternal A-particle (IAP) DNA element in mouse. To distinguish from the endogenous IAP, synthetic IAP DNAs that possess nucleotide sequence markers, were constructed and stably introduced into the RAW264.7 mouse cells. Based on the real-time PCR method to quantitate very low levels (approx. 0.001 copy/ haploid) of reverse-transcripts that are generated by the activation of IAP, a method was established. When the cells were irradiated at 3 to 5 Gy of X-ray, the levels of these reverse transcripts were significantly increased. This suggests that the effective dose of radiation at the cellular level is measurable by specific quantification of a trace amount of nucleic acid.

Major Publications:
3) K Suzuki, I Tanaka, I Nakashii, A Kurematsu, H Yakumaru, N Ikota, H Ishihara: Drastic effect of several caffeic acid derivatives on the induction of
heme oxygenase-1 expression revealed by quantitative real-time RT-PCR., BioFactors, 28, 151-158, 2006


5) K Yoshida, Y Hirabayashi, F Watanabe, T Sado, T Inoue: Caloric restriction prevents radiation-induced myeloid leukemia in C3H/HeMs mice and inversely increases incidence of tumor-free death: implications in changes in number of hemopoietic progenitor cells, Experimental Hematology, 34, 274-283, 2006


7) M Chikamori, J Fujimoto, N Nishizumi, T Yamamoto: Identification of multiple SNT-binding sites on NPM-ALK oncoprotein and their involvement in cell transformation, Oncogene, in press.
6.2. Research on Radiation Dose Assessment for Radiation Emergency Medicine

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 28 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 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.

Also 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, have been made for radiation emergency medicine.

Progress of Research:
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 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 persons in all cases. It is necessary to find other human tissues or substances around exposed persons that can be used to estimate personal exposures. Nail samples are more easily obtained from exposed persons than teeth enamel samples. Therefore, nail samples were applied to ESR dosimetry in the case of γ-irradiation. Stability of free radicals in nail clippings was found to differ among individuals. Decreasing of radicals (fading) produced in the nail sample after irradiations of 3.75, 7.5, 11.3, and 15 Gy was measured by ESR for one week. A linear relationship between ESR sensitivity and absorbed dose (Gy) was obtained to be linear by curve-fitting of the fading conditions. Unknown dose of γ-exposed nail clipping was estimated within 30% errors using the obtained calibration curve. However, analysis time was too long for radiation emergency purpose. A more rapid method must be developed for practical use.

Chromosome aberration analysis
For cytogenetic dosimetry in radiological emergencies, it is important to keep the quality level of the chromosome analysis in the biological dosimetry laboratory. Therefore, we analyzed the chromosome abnormality of human lymphocytes irradiated at the doses of 0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 Gy. The frequencies of dicentric chromosomes at each dose points in these analyses were almost identical with those previously obtained by other investigators.

The Metaphase Finder, one part of the chromosome analysis system, has been developed. It consists of a motorized microscope, camera and computer. The finder scans the slide glass and finds metaphase cells in which chromosomes can be seen, records their positions and relocates them to be seen by the
cytogenetists or machine analyzer. New features of the software for the Metaphase Finder were added this year as follows:

a) The metaphase position converter was added to adjust inter-microscope difference.
b) Digital cameras were found to be usable for image processing.
c) A new algorithm for binarizing the cells was developed.
d) A new metaphase finding program was tested for fluorescent images.

In the protocol for the chromosome analysis, it is important to distinguish clearly the centromere. From this viewpoint, we have also analyzed the relationship between the degree of chromosome condensation, the treatment period and the concentration of Colcemid. There were no differences in the chromosome condensation at the concentration of 0.02 - 0.1 \( \mu g/ml \) for 48 hrs treatment. In the present project, we also analyzed the chromosome abnormality in the embryonic fibroblasts, because of the preparedness of the biological dosimetry in persons who had been irradiated partially. Unfortunately, the metaphases suitable for the analysis were not obtained in the experiment, because there are some problems in the timing of slide preparation after irradiation of cells.

Nose swab for alpha emitters

Alpha emitters are the most dangerous materials for internal exposure. A nose swab method is a useful way to detect lung contamination just after accidental inhalation of alpha emitters. It is quite difficult to determine the quality of an inhaled radioactive volume yet. Filter paper or cotton swab is used for the nose swab method. For determine the quality of nose swab samples, detection efficiency for alpha radiation was tested on several filter papers. Plutonium oxide suspension or plutonium nitrate solution was dropped onto the filter paper as simulated nose swab. Detection efficiency was calculated from the alpha activity measured by an alpha scintillation counter. The detection efficiencies for some filter papers were compared with that of stainless steel disk. All detection efficiency for filter paper showed lower than that for stainless steel disk. This result indicates that the activity of nose swab is underestimated when counting efficiency is determined by the standard calibration method using a stainless steel disk source. The detection efficiency for plutonium oxide was higher than that for plutonium nitrate. This indicated that plutonium oxide stayed on the surface of the filter paper as aerosol particle. This result suggested that physical and chemical forms for plutonium affected determination of the quantity needed for nose swab.

Development of integrated WBC and basic study on phantoms for calibration

Instead of the conventional whole body counter (WBC) using the 8''\( \times \) 4'' NaI (Tl) scintillator which has a scanning bed geometry, an integrated WBC was developed. This integrated WBC is intended for use in identifying nuclides in multiple contamination cases. This counter has ten detectors which are located just above the target organ in order to improve the identification of nuclides. Two sets of p-type high efficiency coaxial Ge detectors are used for intestine. Similarly, the n-type coaxial detector is used for thyroid and other p-types are used for skull, liver and gastrointestinal tract, respectively. An electric cooling system based on adiabatic expansion was adopted as the cooling apparatus for all detectors with the objectives of preventing asphyxia and operating continuously.

In the case of lung measurement, the chair is set at a loco position to prevent deformation of lungs. All other measurements are performed at a decubitus position to reduce a burden of a patient.

As for the efficiency calibration, a JAERI phantom with newly-designed tissue equivalent lungs which contain \( ^{241}\text{Am} \) homogeneously was applied to lung detector and a BOMAB phantom regulated by ANSI was also applied to detectors for trunk. This system shows 2.55 times improvement of MDA compared to that of previous NaI (Tl) system for \( ^{137}\text{Cs} \). It demonstrates the new system is a considerable improvement in radiation emergency preparedness.

We also investigated LLNL phantom for the lung monitor. When a transuranic element such as \( ^{239}\text{Pu} \) is inhaled, we must measure the low energy LX-rays which are difficult to measure accurately with the lung monitor. However, LX-rays are influenced strongly by properties of the medium which they passed through. The LLNL phantom which is referenced to Westerner was used to calibrate the lung monitor. That phantom was very different from Japanese. So, we developed the phantom for calibrating the lung monitor which was fitted to the Japanese physical size. Now, we confirm the phantom was agreed with the formation which was designed. The formation of the lung models in the phantom agreed with MRI data for individual lung as well, and the characteristics for radiation penetration also had good agreement. One of the lung models was sliced, and the uniformity of the internal structure was actually confirmed, and a chemical composition analysis was done. Additionally, the uniformity of the distribution of \( ^{241}\text{Am} \) (LX-rays and \( \gamma \) ray sources) was confirmed by imaging plate analysis as well as the structure of polyurethane.
A rapid analysis technique of uranium in urine samples

Internal dose evaluation is more complicated than external dose evaluation. Especially internal dose estimation due to α- and β-emitters is more difficult compared with that of γ-emitters. For this purpose, chemical analyses of urine and feces (bioassay) are conducted to estimate the input and accumulation volumes of radioactive nuclides of human bodies. However, the chemical analyses are usually complicate and time consuming. In a radiation emergency, the earliest possible analytical results are requested for medical treatment of exposed persons.

In this study, the strontium specific resin column, microwave digestion (MW) technique and inductively coupled plasma mass spectrometry (ICP-MS) were combined to develop a rapid urine analysis of $^{238}$U in human urine samples. An aliquot of $^{238}$U standard solution was added to get final concentrations of 1.2–10 μg per fresh urine samples of 20ml (63–500ng/ml-urine). The spiked urine samples were digested by the MW technique. A good recovery (above 86–99%) was obtained. The total analysis time was ca. 8 hr. This would be an effective bioassay method in a radiation emergency and also a good way to prevent a detector contamination of ICP-MS instrument by the high matrix urine samples.

Computer code for internal dose evaluation

Computer code MONDAL3 has been released for all users including non-specialists to estimate committed effective dose based on measurement results of individual monitoring. Also the preparation for database update was performed on the basis of a new human alimentary tract model and revision of the ICRP recommendations for radiological protection. On the assumption that the characteristics of the intake pathway, chemical form or AMAD (Activity Median Aerodynamic Diameter) of inhaled aerosols would be unidentified at an early stage in radiation emergency medicine, the committed effective doses were calculated for various conditions under a certain monitoring measurement quantity. The doses for radionuclides were calculated using imaging plate technology : A case study of leaves affected by the Chernobyl nuclear power plant and JCO criticality accidents, Nuclear Technology & Radiation Protection, 21, 41-47, 2006

Clinical diagnostic indicators for uranium toxicity, and tests of chelating agents on removal of uranium, comparison of acute toxicities of plutonium and uranium for radiation emergency medicine

The first work was carried out to clarify the usefulness of clinical diagnostic indicators of renal and bone damages in rats intravenously injected with 0.2–2.0mg/kg depleted uranium. The serum and urinary biochemical markers of renal function, such as N-acetyl-b-D-glucosanidase, blood urea nitrogen and creatinine increased, but might not distinguish the chemical safety limit of 3mg/g of kidney weight from that of control group. Also, the bone marker, osteocalcin responded sensitively to the lowest dose, compared with TRAP, pyridinoline, and PTH. The second work was to examine the effects of deferiprone, an iron chelator, on removal of uranium. The result indicated that the deferiprone was effective in increasing initial excretion of uranium in urine, suggesting that this drug may possibly to excrete uranium little by little in a long-term treatment. The third work was to examine the effects of chelating agents, CBMIDA (catechol-3,6-bis (methyleneiminodiacetic acid) and EHB (ethan-1-hydroxy-1,1-bisphosphonate) on removal of uranium in rats when the chemical forms of uranium were varied by pH. The results indicated that CBMIDA was more effective in excreting uranium than EHB, particularly preventing damages. The fourth work was carried out to compare the acute toxicity of plutonium with that of depleted uranium in mice, to prepare the assessment of mixed plutonium and uranium induced-chemical toxicities and the method of treatment. The results indicated that, in the ranges of 2-32mg/kg, plutonium was more toxic than uranium.

Major Publications:
5) S. Fukuda, M. Ikeda, M. Nakamura, X. Yan, Y. Xie : Effects of pH on DU intake and DU removal by CBMIDA and EHB, Health Physics, 92, 10-14, 2007