6.1 The Study for Medical Treatment for High Dose Exposure

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Outline of Research Career

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

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Objectives

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

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

Progress of Research

1) Effect of FGFC on intestinal injuries due to high doses of radiation

Fibroblast growth factors (FGFs) play important roles in numerous biological events such as angiogenesis, wound repair and so on, suggesting their ability to protect the intestines against radiation injuries. FGF receptor 2 IIb (KGFR) is expressed only in epithelial cells and serves as a high-affinity receptor for FGF1, FGF7 and FGF10, while FGF1 binds to all subtypes of FGFs. However, the structural instability of wild-type FGF1 and its dependence on exogenous heparin for optimal activity diminishes its potential for practical use. We have created an FGF1:FGF2 chimera (FGFC) that is able to stimulate heparan-bearing cells in the absence of exogenous free heparin. This study aimed at evaluating the protective activity of FGFC against radiation-induced intestinal damage. Using BaF3 transfectants overexpressing each FGFR subtype, we showed that FGFC was able to activate all of the FGFR subtypes similar to FGF1. When FGF1, FGF7, or FGF10 was administered with heparin intraperitoneally to BALB/c mice at 24 h before total body irradiation (TBI) at a dose ranging from 8 to 12 Gy, FGF1 most effectively increased crypt survival at 3.5 days after TBI. In the same setting FGFC was equally as effective as FGF1, whereas it was even superior to FGF1 when administered without heparin. Finally, the effectiveness of FGFC was also observed without heparin when it was administered 24 h after irradiation. These findings suggest that FGFC is useful in clinical applications for both prevention and post-treatment of radiation injuries.

2) Cell-permeable PIDD (773-917)-TAT protein inhibits ionizing radiation-induced activation of pro-death caspase-2

PIDD (p53-induced protein with a death domain) plays a critical role in the activation of caspase-2 to trigger DNA damage and to induce apoptosis through the formation of a PIDDosome, which contains the adaptor protein RAIDD and caspase-2. We found that transcription of PIDD was induced by exposure of ionizing radiation in rat small intestinal epithelial cell line (IEC6). Yeast two-hybrid analysis indicated that the death domain of PIDD interacts with RAIDD. Overexpression of rat C-terminal PIDD fragment (residues 773-917) containing the death domain dominantly and negatively inhibited the PIDD-mediated activation of caspase-2 after ionizing irradiation. In order to use the PIDD (773-917) fragment as an anti-apoptotic drug, we purified a recombinant PIDD (773-917) fragment fused with a basic 11-amino acid peptide derived from HIV-TAT which facilitates the uptake of the protein into mammalian cells with high efficiency. When PIDD (773-917)-TAT was added to the IEC6 cells, PIDD (773-917)-TAT was delivered into the cells within 1 hour. Furthermore, we observed the inhibition of caspase-2 activation when PIDD (773-917)-TAT was added to the IEC6 cells 1 hour after irradiation. These results suggest possibility of PIDD (773-917)-TAT for protection from ionizing radiation-induced gastrointestinal cell death.

3) The roles of endogenous TNFα in leukemia cells and mice exposed to radiation

Tumor necrosis factor alpha (TNFα) is a unique pro-inflammatory cytokine whose signaling pathways are linked to both pro- and anti-apoptotic responses in many types of cells and tissues, and it is produced upon radiation exposure. Previously we have shown that radiation induces apoptosis through the caspase pathway requiring TNFα production in human Jurkat T leukemia cells lacking functional p53. TNFα expression is regulated by a transcription factor, early growth response-1 (Egr-1) in cell lines lacking p53. To better understand the mechanism of TNFα expression after high dose radiation, we used inhibitors of the MEK (PD98059), p38 MAPK (SB203580), PI3K (LY294002) and JNK (SP600125) pathways and examined Egr-1 and TNFα expression in these cells. Pretreatment of these cells with an inhibitor of MEK, p38 MAPK or JNK blocked the expression of Egr-1 and TNFα mRNAs by 10 Gy radiation. In contrast, inhibition of PI3K blocked the TNFα but not Egr-1 mRNA expression induced by radiation. Furthermore,
cAMP response element-binding protein (CREB) linking to the transcription of Egr-1 was phosphorylated by radiation. Radiation-induced phosphorylation of CREB was blocked by pretreatment of each inhibitor. Our results suggest that the radiation-induced TNFα expression is mediated through the MEK, p38 MAPK, PI3K or JNK pathway via Egr-1 induction requiring activation of CREB in Jurkat cells. Further studies on mechanisms are in progress.

We also compared the wild-type of TNFα (WT) and its knockout (K/O) bablc mice and found that the survival durations in WT were significantly longer than those in K/O mice and administration of TNFα increased the survival rate in K/O mice. Since autopsies failed to find difference in causes of death between both groups, we compared injuries of bone marrow and small intestine. Numbers of red blood cells were significantly reduced in K/O mice 15 days after exposure with concomitant higher levels of serum iron and lower unsaturated iron binding capacity as compared to those in WT mice. Administration of TNFα significantly improved those in irradiated K/O mice. Assays for crypt microcolony and apoptosis in small intestine showed no difference between both irradiated groups. Interestingly, administration of either TNFα or lipopolysaccharide (LPS) significantly inhibited the apoptosis in WT but not in K/O mice. The serum levels of TNFα were increased following the TNFα challenge in both groups, but that in WT was higher than that in K/O mice. LPS increased the levels of TNFα in WT but not in K/O mice. We also studied the expression of Bax and Bcl2 proteins in intestinal crypt cells. Radiation increased the Bax/Bcl2 ratio in both mice. However, administration of TNFα before radiation reduced the ratio in irradiated WT but not in K/O mice. Our results suggest that endogenously-produced TNFα plays an important role in radiation injury.

4) Lithium chloride protects and rescues the small intestinal epithelial cells from radiation-induced apoptosis through PI3K/Akt and MEK/ERK pathways

High dose radiation induces apoptosis of intestinal epithelial cells and subsequent depletion of the cells, resulting in lethal intestinal injury. However, effective treatment of this injury has not been established yet. Lithium chloride (LiCl) is well known as an inhibitor of glycogen synthase kinase 3 (GSK3), which has been shown to be associated with apoptosis. We studied the effect of LiCl on intestinal radiation injury. Rat small intestinal epithelial cell line, IEC-6 cells and intestinal epithelial cells in primary culture obtained from fetal rat duodenum were treated with LiCl for 1 h and then exposed to γ- radiation of 20 Gy; 24 h after irradiation, the apoptosis was evaluated by Hoechst staining.

Pretreatment with 10 mM of LiCl markedly inhibited radiation-induced apoptosis in both cells. Furthermore, addition of LiCl after irradiation blocked the apoptosis. Inhibition of either phosphoinositide 3-kinase (PI3K)/Akt or mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK)/ERK pathway abrogated the anti-apoptotic effect of LiCl. We administered LiCl to bablc mice 1 h before total-body irradiation (TBI) with 8 Gy. Administration of LiCl blocked the radiation-induced apoptosis in intestinal crypts. Moreover, either pre- or post-administrations of LiCl increased the number of surviving crypts in mice 3.5-day after TBI. In the present study, we found that LiCl protects and rescues intestinal epithelial cells from radiation-induced apoptosis through activation of pathways involving PI3K/Akt and MEK/ERK. Our results also showed that LiCl prevents radiation-induced intestinal injury in vivo.

5) Suppressive effect on ionizing radiation-induced intestinal epithelial cell apoptosis by a cell penetrating peptide bound Survivin

Survivin is a member of the inhibitors of apoptosis (IAP) family and contains signature motifs termed baculovirus IAP repeat (BIR). Survivin is aberrantly expressed in cancer but undetectable in normal differentiated adult tissues. Although the precise mechanism remains to be elucidated, it has a role in cell division and apoptosis (caspase-dependent and caspase-independent apoptosis). In response to inducers of cell death, mitochondrial survivin is rapidly released into the cytosol, where it prevents caspase activation and inhibits apoptosis. These findings suggest that survivin might be effective for protection from ionizing radiation-induced gastrointestinal cell death. We used rat small intestinal epithelial cell line (IEC-6) to investigate the effect of survivin. When survivin was over-expressed, it localized in mitochondria and it functioned as an inhibitor of caspase-9 activation, indicating that it might function as an inhibitor of caspase-3 and caspase-2 activation after ionizing radiation. We synthesized survivin bound to a cell penetrating peptide (TAT-survivin), which is known to facilitate the uptake of the protein into mammalian cells with high efficacy. An investigation of the effect of the protein on the intestinal injury induced by high dose radiation is now in progress.

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

The aim of the study is to obtain basic results to select favorable pharmaceutical agents against intestinal damage caused by exposure to high-dose radiation in accidents. We examined drugs which contribute to recovery from lethal intestinal damage
following radiation exposure using an experimental animal model.

Since it was difficult to find the regions of lethal damage in the large intestine, survival rate was used as an indicator. Damage to the whole intestine was induced by abdominal exposure of anesthetized C3H/He mice to 15.7 or 17.6 Gy of x-rays. Parenteral nutrition and drugs were concomitantly injected to the mice from day-1 to 10 after the irradiation. Even though the nutrition was administered to the mice, the body-weight decreased until day-7. Although mice that showed increasing weight on day-8 survived at least until day-28, others died within 10 days. Using at least 3 different lines of mice, the effects of the drugs on the survival rate were determined. Various drugs including alpha-adrenergic receptor stimulators (salbutamol and phenylephrline), parasympatholytic agents (scopolamine butylbromide and atropine) and antispasmodic (papaverine) showed an effect on the survival rate. In contrast, their antagonists such as sympatholytic drugs (reserpine and propranolol), parasympathomimetic agents (pilocarpine and neoestigmine) and benzodiazepine derivatives (such as antianxiety agents diazepam and tofisopam) decreased the survival rate. These results showed that relaxation of smooth muscle during the recovery of damaged mucosal tissue of intestine may inhibit recovery of radiation-induced damages.

Major publications


3) T. Tamura, X. Cui, N. Sakaguchi, M. Akashi: Ginsenosides Rd Prevents and Rescues Rat Intestinal Epithelial Cells From Irradiation-Induced Apoptosis, *Food and Chemical Toxicology*, 46[9], 3080-3089, 2008


5) T. Yamamoto, N. Sakaguchi, M. Hachiya, F. Nakayama, M. Yamakawa, M. Akashi: Role of catalase in monocytic differentiation of U937 cells
6.2. Research on Radiation Dose Assessment for Radiation Emergency Medicine

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[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. At NIRS, he has had over 30 years of experience in research on radioactive aerosols and their internal exposure. 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.

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 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 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 more easily obtained from exposed persons than tooth enamel samples. Therefore, nail samples were applied to ESR dosimetry in the case of γ-irradiation. Relationship of ESR sensitivity and absorbed dose (Gy) in nails was found to be linear. Unknown dose of γ-exposed nail was estimated using the modified calibration curve at room temperature for 1-2 weeks. However, it was found both ambient temperature and humidity have an affect on this calibration curve more than individual sensitivity differences from radiation. Those problems must be solved to establish nail ESR dosimetry.

2) Chromosome aberration analysis

In order to maintain the quality level in chromosome analysis for the dose estimation, the dicentric chromosome was analyzed in the lymphocytes which were irradiated by gamma-rays at the doses of 0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 Gy. The frequencies of the dicentric chromosome at each dose point in these analyses were almost identical with those obtained from the analysis in all previous years. This means that the quality level for the detection of dicentric chromosome was maintained and the standard curve for dose estimation was also not changed.

In the process of dose estimation by dicentric chromosome analysis, the slide preparation seems to be very important to obtain the value of radiation dose more accurately. Therefore, in the present study, we analyzed the effect of the concentration of Colcemid which is the chemical agent to stop the cell cycle at the M-stage on the chromosome condensation. No relationship was observed at concentrations of 0.03, 0.05 and 1.0 μg/ml. However, the chromosomes were significantly elongated at the concentration of 0.01 μg/ml in the case of a 2-hour treatment.

Furthermore, in order to establish an assay system to estimate the radiation dose in the case of partial body exposure, we used the human hair root as the target organ for dose estimation. The comet assay was applied for the detection of DNA damage in the hair root cells after irradiation and we detected a slight relationship between tail length indicating DNA damage and irradiated dose. This suggests the possibility that the comet assay in hair root cells will be useful for dose estimation in partial body exposure.

One of the purposes in our laboratory is to develop and improve automated systems of facilities used for chromosome studies. The metaphase finder is an automated optical microscope system which automatically scans and finds metaphase cells on the slide glass and relocates metaphase cells to the center of the field of view of the microscope to observe chromosomes in high magnification. The software of this metaphase finder uses mathematical morphology filters in most of the image recognition process. In addition, mathematical morphology filters for grey-level images are used in the most recent version. This year, the performance of this metaphase finder was tested and the results were presented at an international meeting. It was proven that the false positive rate was improved to 2%.

3) Development of a semi-tissue equivalent Si semiconductor for local dose estimation

The majority of persons have non-uniform exposure in the case of high dose external exposure. For such a case, it is indispensable to reconstruct the local dose in
the early stage for determining the radiation emergency medicine treatment. One of the optimum methods to estimate the dose is geometrical simulation by Monte Carlo calculation. To construct the basic data, a semi-tissue equivalent Si semiconductor detector to be inserted in a physical phantom for the bench mark test on the Monte Carlo calculation was developed. This detector consists of a Si sensor (1mm square) which is connected to the Si substrate on which a super-thin amplifier circuit is formed directly with the MEMS technique, and a tissue-equivalent medium which is composed of hydrogen (8.2%), carbon (66.2%), nitrogen (2.2%), oxygen (20.7%), chlorine (0.4%), and calcium (2.3%). Using mono-energetic photons from 10keV to 70keV and $^{137}$Cs and $^{60}$Co sources, it was confirmed the Compton spectrum which would be used in an unfolding technique could be measured.

4) Nasal swab for alpha emitters

To improve the first estimation of intake activity, the quality of a nasal swab measurement was experimentally investigated. Alpha spectrometry was used to examine the experimental nasal swab samples which had been added a plutonium solution or particles. It was observed that the alpha energy spectrum had a quite different shape among samples, and it was characterized by the type of contaminant. The detection ratio for samples was almost 30% for the counting efficiency of the detector. To define the reason for the low detection ratio and the different shape of the spectrum for experimental swab samples, an AASI (Advanced Alpha-Spectrometric Simulation) was used. According to the AASI simulation, alpha radiation emitted from more than 80 $\mu$m below the surface hardly penetrated the filter medium. The count for 80 $\mu$m below the surface decreased to about 5% of that for no absorber. This means that the filter is infinitely thick with respect to the alpha particles at 5.15MeV. Since the thickness of the filter paper was 210 $\mu$m, the alpha radiation could be detected up to about 30% in depth. This simulated result supports that the detection ratio for the experimental sample was about 30% for the counting efficiency of the detector. The radioactive contaminant would also soak into the filter medium filled with distilled water. These results suggest that the absorption of alpha radiation should be considered to determine an accurate alpha activity for nasal swab samples. When the activity of particle and solution sample were adjusted, the peak count in the alpha energy spectra showed remarkable difference among them. The peak area count was determined by deducting the count of the solution sample from that of particle sample. The ratio of the peak area count to the total was calculated as 30%. For the simulated spectrum, the ratio of the peak area count to the total was estimated to be 31% when the ratio was determined by deducting 35% as the ratio of the particle from 4% as that of the solution. The 31% for the simulated result was very close to 30% for the experimental result. This means that the difference in the detection ratio reflects the nominal energy without any shielding of the filter fiber. Therefore, the shape of the alpha energy spectrum would give an advantage to distinguish between the particle and solution samples. These results of a simulated analysis indicated that alpha spectrometry would give a sufficient detection ratio result to distinguish between radioactive substances.

5) Development of lung phantom for in-vivo measurements

A thorax model was designed and made for realistic shape of lungs and Japanese body size. This year, we compared the model with Lawrence Livermore National Laboratory phantom (LLNL phantom). First, the distribution of the radioactivity was compared by using the mapping measurement of 59.5keV gamma rays from $^{241}$Am inside a low background room using a one-inch NaI detector that rolled the collimator of the lead 1mm. As a result, LLNL phantom gathers when seeing ahead and the radiation source will have gathered in the narrow area. The difference of the each distribution was caused by the difference of a flat extension of lung models. Moreover, they were measured by the lung monitor and compared by count efficiency. Because the view was expected to be almost the same in both models, a big difference would not be seen in the count efficiency in the lung monitor of NIRS.

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

Internal dose evaluation is more complicated than external dose evaluation. Especially internal dose estimation due to $\alpha$- and $\beta$-emitters is more difficult compared with that of $\gamma$-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 complicated and time consuming. In a radiation emergency, early analytical results are requested for medical treatment of exposed persons.

In this study, three kinds of extraction resin columns and a liquid scintillator or alpha-spectrometer were combined to develop a rapid measurement system for strontium, americium, and uranium in human urine samples. After spiking an aliquot of $^{90}$Sr into the urine sample, the $^{90}$Sr fraction was purified by a Sr-specific resin column and detected by a liquid scintillator. Am and U were separated by UTEVA and TRU resin columns and measured by an alpha-spectrometer. A good recovery (above 80-99 %) was obtained in all cases. The total analysis time for a urine sample was
within a work day (ca. 8 h.) This system would be an effective bioassay method on radiation emergency.

7) Acute toxicity of uranium and the effects of chelating agents in simulated wounds model of rats

The initial behavior and acute toxicity of depleted uranium (DU) via wounds in which uranium was injected into femoral muscles of rats were compared with those by subcutaneous (SC) injection in previous studies. There were differences in the uranium behavior and excretion rates in feces from that of SC injection, probably due to the differences in diffusion speed of uranium from the DU injected site. The CBMIDA by local treatment, in which infused into the DU injected site, particularly for decreasing dysfunction of kidneys, was as effective as that by SC injection. This time, new effects of two lactoferins by oral administration were examined. The lactoferrin with Fe has efficacy for excreting uranium and decreasing uranium accumulation in organs, and the lactoferrin without Fe enhanced the effects of CBMIDA, although either effects were lower than that of CBMIDA. The results indicated that local treatment by CBMIDA has efficacies for decreasing acute toxicity of uranium via wounds, when CBMIDA is infused within 2 h, and lactoferrin might be used in prolonged treatments in radiation emergency medicine. Findings of these studies were published.

Major publications


