

# **BIO-MEDICAL SCIENCES**

**Radiotoxicology**

## 50. Spin Trapping Reagents as Radioprotectors against Whole Body X-Ray Irradiation of Mice

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*Keywords: spin trapping, radioprotector, X-ray irradiation, mouse*

Spin trapping reagents are used for ESR measurements of short-lived free radicals by extending the lifetime of the radicals via formation of spin adducts. Since the spin trapping reagents react with free radicals, they can be regarded as scavengers of free radicals from another viewpoint. They may function as antioxidants to protect injuries related to reactive oxygen species. In the present study, therefore, we examined *in vivo* radioprotection of spin trapping reagents against whole body X-irradiation of mice. The reagents examined are DMPO (5,5-dimethyl-1-pyrroline-N-oxide), DEPMPO (5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide), PBN (N-*t*-butyl- $\beta$ -phenylnitron), and POBN (-(4-pyridyl-1-oxide)-N-*t*-butylnitron) as shown in Fig. 19.

The spin trapping reagents were administered intraperitoneally to mice (C3H, male, 10 weeks old) and the mice were irradiated with X-rays at the total radiation dose of 8.0 Gy with the radiation dose rate of 0.6 Gy/min. PBN and POBN showed significant radioprotection, whereas DMPO and DEPMPO showed only slight radioprotection. The radioprotection by POBN was dose-dependent and the dose of 450 mg/kg, which shows no acute toxicity, was chosen as the standard dose for later experiments. POBN injected at 60-120 min before the X-ray irradiation showed the highest radioprotective activity, whereas injection after the X-ray irradiation showed no effect. Dose reduction factor (DRF) of

POBN administered at 450 mg/kg was measured as 1.3.

Since the radioprotection is observed only when the reagents were administered before the irradiation, the primary action of the spin trapping reagents may be quenching of the free radicals. However, the effect is different among the reagents examined although the spin trapping activity of them is not very different and relatively long period before the irradiation is required for the protective effect. Therefore, some pharmacological action in addition to the radical quenching might be responsible for the radioprotection.

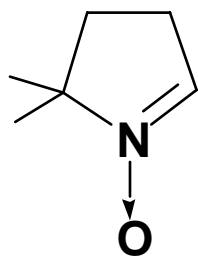
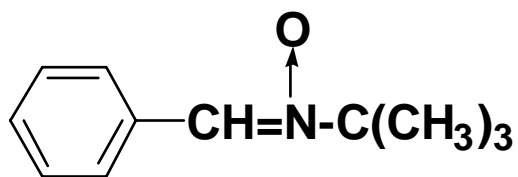
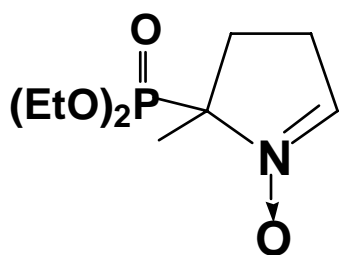
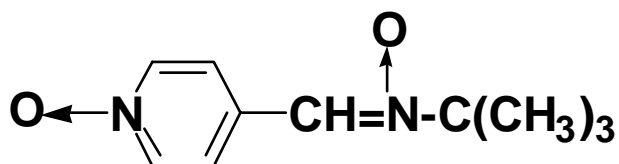
**DMPO****PBN****DEPMPO****POBN**

Fig. 19. Structure of spin trapping reagents used in this study.

**51. Adaptive Response in Embryogenesis: V. Existence of Two Dose-Rate Ranges for the Same Priming Dose to Adapt Fetal Mice**

Bing Wang, Harumi Ohyama, Yi Shang, Masako Nose, Tetsuo Nakajima, Osami Yukawa and Isamu Hayata

**Keywords:** embryogenesis, radiation, adaptive response, dose-rate range, mouse

Radioadaptive response (AR) manifests an important phenomenon in radiobiology. Research on the essential conditions for RA induction is of critical significance, and has with an impact on understanding novel biodefense mechanisms against hazardous effects from irradiation. This paper reports the first time evidence on the existence of two dose-rate ranges for the same priming dose of irradiation to successfully induce the AR *in utero* in fetal mice. A dose of 0.30 Gy given to the fetuses on embryonic day 11 (E11) was adopted as the priming dose according to our previous study. A dose of 3.50 Gy administered to the fetuses on E12, which alone could lead to the death of all neonates within the first postnatal week, was chosen as the challenging dose. Induction of apoptosis in the predigital regions of fetal limb buds, incidences of fetal limb malformations and prenatal fetal death, and postnatal survival of neonates were employed as the biological endpoints. The dose-rate effects in a range from 0.06 Gy/min to 5.00 Gy/min of the priming irradiation on RA induction were investigated. The effectiveness of AR induction was correlated to the dose rate in neither a normal nor a reverse dose-rate effect manner, namely, priming irradiation at a dose-rate range either from 0.18 to 0.98 Gy/min or from 3.50 to 4.20 Gy/min could successfully adapt the fetuses, while giving another dose rate always lead to failed AR induction. Results indicated that dose rate of priming irradiation played a crucial role in AR induction. These findings provided specific information on dose rate to complement the current

understanding of AR induction and they suggested that influence of dose rate should be taken into consideration regarding making radiotherapeutic protocols as well as setting standards of radiation protection.

**52. Radiation-Induced Teratogenesis in the Late Period of Organogenesis in Mice: Dose Rate Effects**

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**Keywords:** *dose-rate effect, apoptosis, teratogenesis, organogenesis, mouse*

The irradiation of fetuses at the late period of organogenesis has been known to induce a dramatic increase in malformations. The mechanisms involved, however, have remained unclear. Using the mouse limb bud system, we first found that radiation-induced apoptosis is involved in the induction of limb malformation, namely, radiation-induced apoptosis in the predigital regions of embryonic limb buds is responsible for digital defects in mice. To investigate the possible dose-rate effects on these radiation-induced phenomena, 3.5 Gy of X-rays at dose rates ranging from 0.06 to 5.00 Gy/min was given to ICR mice on gestation day 12. The dose rate of radiation dramatically affected the consequences of the experiment. Percentages of alive fetuses, malformed fetuses among the alive fetuses, and postnatal survival were significantly higher in the ICR mice irradiated with 3.5 Gy at the dose rates from 2.82 to 3.50 Gy/min when compared to those that received the same dose but at other dose rates. The biological effect as a function of dose rate appeared like a U shape curve. This phenomenon could not be described or evaluated by calculating the dose-rate effectiveness factor. Contrary to both normal and inverse dose-rate effects, which have a regular order of tendency, the dose-rate effects observed in the present study seemed to have an irregular order. As the duration of irradiation at different dose rates in the present study was within 1 h, the

results could hardly be simply attributed to the change of cell kinetics. Further studies need to be done on the mechanisms underlying the phenomenon.

### 53. Comparative Study on *Tp53* Mutations in Rat Lung Tumors Induced by Inhalation Exposure to Alpha Emitters and X-ray Irradiation

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among the rat lung tumors induced by different irradiation, and G:C to A:T transition is a common mutation in *Tp53* of the tumors. The fact that low frequencies of *Tp53* mutation were observed in the radiation-induced lung tumors indicates that the abnormalities of the *Tp53* might not be so critical for the pulmonary carcinogenesis, although other carcinogenic process for genetic and/or epigenetic changes might also be related to the rat lung tumors induced by alpha-emitters and X-rays.

**Keywords:** *Tp53*, mutation, plutonium, neptunium, radon, X-ray irradiation, rat, lung tumor

The purpose of this study is to compare the frequency and type of *Tp53* mutations in rat lung tumors after inhalation exposures to three different alpha-emitters, <sup>239</sup>PuO<sub>2</sub> (Pu), <sup>237</sup>NpO<sub>2</sub> (Np) and <sup>222</sup>Rn (Rn). In addition, *Tp53* mutations of X-ray-induced lung tumors are also compared with the high LET radiation-induced tumors. Genomic DNA was extracted from tumor sites of paraffin-embedded sections of the tumors. Exon 5 to 8 of *Tp53* were amplified individually from the extracted DNA by PCR method. The exons were amplified in 16 cases of Pu-, 22 cases of Np-, 15 cases of Rn- and 33 cases of X-ray-induced tumors, respectively. PCR products were analyzed for mutations utilizing SSCP and direct sequencing method. Point mutations within exon 6 at codon 219 (G to A transition) and exon 8 at codon 266 (C to T transition) were detected from Pu-induced tumors. Only one point mutation was found in Np-induced tumors within exon 5 at codon 175 (C to T transition) and in X-ray-induced tumors within exon 6 at codon 224 (C to T transition). No mutations, however, were found in Rn-induced tumors (See Table 2). These results indicate that the mutation frequencies of the *Tp53* are inconsistent

Table 2. *Tp53* mutation in rat lung tumors after inhalation of alpha emitters and X-ray irradiation

Radiation source	No. examined by PCR-SSCP	No. of mutations in direct sequences (%)	Mutation types
$^{239}\text{PuO}_2$	<b>16</b>	<b>2 (12.5)</b>	Exon 6: codon 219, G A G -A A G Exon 8: codon 266, G A C -G A T
$^{237}\text{NpO}_2$	<b>22</b>	<b>1 (4.5)</b>	Exon 5: codon 175, C C C -T C C
$^{222}\text{Rn}$ (+BNF)	<b>15</b>	<b>0 (0)</b>	
X-ray	<b>33</b>	<b>1 (3.0)</b>	Exon 6: codon 224, G G C -G G T

(BNF: beta-naphthoflavone)

#### **54. Pulmonary Carcinogenesis in the Rat Following Inhalation Exposure to Plutonium Dioxide in Comparison to X-ray Irradiation**

Yoichi Oghiso and Yutaka Yamada

**Keywords:** lung tumor, rat,  $^{239}\text{Pu}$ , X-ray, relative effectiveness

Radiation-induced pulmonary carcinogenesis was compared in a total of 1,200 female Wistar rats following either inhalation exposure to alpha-emitting  $^{239}\text{PuO}_2$  aerosols, or whole-body and thoracic X-ray irradiation. No significant differences from the unexposed control rats in survival periods and lung carcinoma induction were observed at the lowest dose of 0.16 Gy in  $^{239}\text{Pu}$ -exposed rats, but dose-dependent survival reduction was correlated with increased malignant lung carcinomas at the doses over 0.45 Gy, reaching the maximum incidence of 90 % at 6.6 Gy. While differential dose responses for each histopathological type of tumors were noted, almost 70-80 % were carcinomas among all the primary tumors from  $^{239}\text{Pu}$ -exposed rats. As the dose response curves for lung carcinomas were compared, the slope of the fit linear equation and the calculated relative effectiveness for 50 % incidence of lung carcinomas were approximately 11 times as high in  $^{239}\text{Pu}$ -exposure as those of thoracic X-irradiation. The numbers of tumor lesions distributed in the lung per tumor-bearing animal were about 2-fold more in  $^{239}\text{Pu}$ -exposed rats, while the proportions of their histopathological types were almost similar between  $^{239}\text{Pu}$ -exposure and X-irradiation. These results indicate that magnitudes of relative effectiveness or risk for pulmonary carcinogenesis are greater in  $^{239}\text{Pu}$ -exposure than X-irradiation, and that radiation-induced lung tumor types appear to originate mostly from the same target epithelial cells.

Publications:

- 1) Oghiso, Y. and Yamada, Y.: J. Radiat. Res. **43**, 301-311, 2002.
- 2) Oghiso, Y. and Yamada, Y.: J. Radiat. Res. **44**, 271-280, 2003.

## 55. Specific Induction of Osteosarcomas in Different Mouse Strains Following Injection of Plutonium Citrate

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**Keywords:** *bone tumor, soluble  $^{239}\text{Pu}$ , strain difference, mouse*

Lifetime bone tumor induction by injection of a bone-seeking alpha-emitter,  $^{239}\text{Pu}$  citrate, was compared among a total of 630 female mice from three strains (C3H/He, C57BL/6 and B6C3F1) showing different genetic background for spontaneous and radiation carcinogenesis. Bone tumors, mostly osteosarcomas, appeared relatively early during the periods from 200 to 600 days after the injection of  $^{239}\text{Pu}$ , showing an almost similar dose responsiveness with a peak incidence of 50 - 63 % at the skeletal doses of 2-3 Gy, in all the strains of mice. The primary sites of bone tumors from these strains of mice were also predominantly distributed in 80 - 90 % of the skeletal bones including vertebra, femur and tibia which had well-developed trabecular bone surfaces and large vascular sinusoids. Histological appearances of osteosarcomas from three strains of mice were commonly characterized by an irregular growth of osteoblasts along or inside endosteal bone surfaces accompanied by trabecular bone formation. The frequency of lymphoid neoplasms was significantly lower than the control levels, while some appeared earlier at the higher injected doses than those of the controls. Fewer or no myeloid leukemias were found in all the control and  $^{239}\text{Pu}$ -injected animals, and the incidences of other solid tumors rather decreased, reaching zero at doses where the maximum incidences of bone tumors were noted. These findings indicate that osteosarcoma is the only specific tumor commonly observed among different mouse strains following the injection of soluble plutonium compounds.

Publication:

Oghiso, Y. and Yamada, Y.: *J. Radiat. Res.* **44**, 125-132, 2003.

## 56. Induction of DNA Double Strand Breaks in Scid Cells by Carbon Ions

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and Yutaka Okumura\*  
(\*Kumamot Univ.)

**Keywords:** DNA double strand break, scid cell, DNA-PK, repair, high-LET radiation

The DNA double strand breaks (DSBs) induced by X-ray and carbon ion beam irradiation in scid cells were analysed using pulsed-field gel electrophoresis. Scid cells and hybrid cells were ideal to study the DNA DSB repair mechanisms, because their genetic backgrounds were identical except DNA-PK activity. Induction of DNA DSBs was determined after exposure to X-rays and carbon beams. DNA DSB repair was by biphasic kinetics with a fast and a slow component. For scid cells only a slow component was observed, whereas the kinetics of DSBs repair was biphasic with a fast and a slow component. It was concluded from the experimental data that the induced DSB rejoining in scid cells was due to the lack of DNA-PK activity.

### **Publication:**

Shimasaki, T., Ihara, M., Furusawa, Y., and Okumura, Y.:  
*Radiat. Protec. Dosim.* **99**, 155-157, 2002.

**57. Effects of a Human Dose of Ca-DTPA on  
Removal of Different Amounts of Plutonium  
from the Rat**

Fukuda, S., Yan, X. and Iida, H.: *Jpn. J. Health  
Phys.*, **37**, 158-161, 2002.

Satoshi Fukuda, Xuemig Yan and Haruzo Iida

*Keywords: daily recommended human dose of  
Ca-DTPA, plutonium removal, chelation therapy,  
rat, urinary plutonium excretion*

The effects of the daily recommended human dose (1 g per 70 kg body weight=30 mol/kg) of calcium diethylenetriaminepentaacetic acid (Ca-DTPA) on removal of plutonium from rats injected with different amounts of plutonium were examined. Sixty female wistar rats were preinjected with doses of 0.185, 0.37 and 3.7 x10<sup>5</sup> Bq/kg of plutonium as a nitrate, and half of the rats of each dose group were injected with 30 mol/kg of Ca-DTPA for 3 days, beginning 1 h after plutonium injection on the first day. The 24-h urine and feces of rats were collected. The amount of urinary plutonium excretion in the Ca-DTPA groups was found to be significantly greater than that in the respective corresponding control groups on days 1-3. The amount of urinary plutonium excretion in the Ca-DTPA group on the 1st day was increased in response to the plutonium injected dose (correlation coefficient: r=0.628, vs. r=0.573 in the control group, that accumulated in the Ca-DTPA group for 3 days was r=0.800 vs. r=0.669 in the control group), while their rates of plutonium injected dose were decreased. Such findings were not obtained in the feces measurements. In conclusion, the recommended human dose of Ca-DTPA as chelation therapy enhances plutonium excretion for increasing plutonium intake, however the excreted rate of plutonium intake is decreased.

Publication: