Radiotoxicology
51. Effects of D-penicillamine and Ca-DTPA on Removal of Radiocobalt in Rats

Satoshi Fukuda, Haruozo Iida, Yumi Abe* and Hiroki Yoshida*
(*Tokyo Nuclear Service Co. Ltd.)

**Keywords:** D-penicillamine, Ca-DTPA, removal, radiocobalt, daily recommended human dose

Based on a medical treatment schedule for humans, the effects of D-penicillamine and Ca-DTPA on the removal of radiocobalt were examined in rats. Rats were pre-injected with radiocobalt and then treated with D-penicillamine alone via an oral route, Ca-DTPA alone via an intraperitoneal injection, or both compounds at the same time at doses equivalent to the daily recommended human dose. The compounds were administered for 3 days, beginning with or 1 h after radiocobalt injection on the first day. The radioactivity levels of the whole body of rat, urine and feces were measured at intervals of 24 h. On day 4, the rats were sacrificed in order to obtain blood and organs. When D-penicillamine was administered with and 1 h after injection of radiocobalt, the whole body activity was reduced to 9.6 and 79.0% of that of the control, respectively, in the Ca-DTPA-alone groups and to 54.8% in the group in which both compounds were administered 1 h after radiocobalt. In the D-penicillamine-alone groups, the activity levels were reduced to 33.6 and 56.6% with and 1 h after radiocobalt injection, respectively (Fig. 24). In conclusion, the results of this study indicate that D-penicillamine is useful in treating a person contaminated with radiocobalt in an accident.

![Fig. 24. Whole-body retention of radio-cobalt after administration of Ca-DTPA and D-Penicillamine. Values are mean and bars are standard deviation.](image)

52. Effects of CBMIDA on Removal of Uranium in Rats

Satoshi Fukuda, Haruozo Iida, Xueming Yan* and Yuyuan Xie*
(*Shanghai Institute of Materia Medica, China)

**Keywords:** CBMIDA, uranium, rat

The effects of a chelating agent, CBMIDA, on the removal of uranium in rats were determined. Thirty rats were divided into six groups. Group 1 (G1): rats injected intraperitoneally with uranium in a solution of pH 3.2 and injected with sodium bicarbonate 30 min later; Group 2 (G2): uranium compounded with CBMIDA before injection; Group 3 (G3): uranium in a pH 3.2 solution; Group 4 (G4): uranium in a pH 3.2 solution and injection of 1200 μmol/kg CBMIDA 30 min later, Group 5: uranium in a pH 6.8 solution; and Group 6: uranium in a pH 6.8 and injection of 1200 μmol/kg CBMIDA. The uranium activities in the excreta and organs in rats 24 hrs after injection were measured.

The rates of uranium excreted were 1.05 % of the injected dose in group G1, 1.00% in G3, and 0.24% in G5, whereas they were 11.26% in group G2, 9.49% in G4, and 10.13% in G6, respectively (Table 1). The amount of activity in excreta (urine and feces) was highest in group G2. A significant difference of activity in the excreta between groups G 5 and G6 was found. The retention rates in the liver, femur, and spleen in groups G2, G4, and G6, except for the value of spleen in G4, decreased more than those of the corresponding G1, G3 and G5 groups, respectively. However, the retention

Table 1. The rates of uranium activity excreted in urine and feces 24 hr after injection of uranium; the values are presented as percentages of injected doses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Excreted rate in urine (%)</th>
<th>Excreted rate in feces (%)</th>
<th>Total rate in excreta (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: U pH 3.2 and NaHCO₃</td>
<td>1.05±0.20</td>
<td>2.06±1.27</td>
<td>3.11</td>
</tr>
<tr>
<td>G2: compound CBMIDA and U</td>
<td>11.26±3.29</td>
<td>12.65±3.00</td>
<td>23.92</td>
</tr>
<tr>
<td>G3: U pH 3.2</td>
<td>1.00±0.16</td>
<td>15.69±4.02</td>
<td>16.69</td>
</tr>
<tr>
<td>G4: U pH 3.2 and CBMIDA</td>
<td>9.49±3.21</td>
<td>6.13±2.34</td>
<td>15.62</td>
</tr>
<tr>
<td>G5: U pH 6.8</td>
<td>0.24±0.11</td>
<td>0.13±0.03</td>
<td>0.37</td>
</tr>
<tr>
<td>G6: U pH6.8 and CBMIDA</td>
<td>10.13±2.70</td>
<td>1.69±0.22</td>
<td>11.72</td>
</tr>
</tbody>
</table>
rate in the kidney in groups G2 and G4 was higher than that in groups G1 and G3 respectively, but the rate in the G6 group was lower than that of G5.

In conclusion, the results indicate that CBMIDA can remove uranium at pH 6.8 from the body, while its has no effect for uranium at pH 3.2 solution and shows undesirable characteristic of accumulating uranium in the kidney. Further study of the analogues of CBMIDA will be necessary in order to enhance its removal effects and reduce the associated accumulation of radioactive materials from the kidney.

Publication:

53. DTPA Treatment for Removal of Inhaled Plutonium Nitrate in Rats.

Satoshi Fukuda and Haruo Iida

**Keywords**: plutonium, inhalation, Zn-DTPA, uranium-DTPA compound

This study was performed in order to determine the maximum removal rate of plutonium by DTPA treatment. Aerosols of a complex of plutonium-239 and DTPA (Pu-DTPA) and plutonium nitrate were inhaled by 40 female 3-month-old Wistar rats. Ten rats from the inhaled Pu-DTPA and Pu nitrate group were sacrificed in groups of five at 3 and 7 days. Subsequently, in the period from 7 to 28 days after inhalation, five rats in each group were orally administered Zn-DTPA via their drinking water. The activity of plutonium which was retained in the urine and feces collected at 24 hr-intervals and in all organs was measured. The body retention rates of plutonium in the plutonium-nitrate and plutonium-nitrate + Zn-DTPA groups observed for 28 days were 30.4%, and 23.5%, respectively, of total intake, while those in the Pu-DTPA and Pu-DTPA + Zn-DTPA groups were reduced to 2.3%, and 1.5%, respectively. The administration of Zn-DTPA in the plutonium-nitrate group enhanced the excretion of plutonium in the excreta (Fig. 25), whereas such increases in the Pu-DTPA group were not observed. The greater part of the plutonium retained in the bodies of rats in the plutonium-nitrate group was deposited in the lung. The results indicate that DTPA essentially has the effect of reducing the amount of plutonium in the body, up to about 2% of intake. The problem might be that the effects of DTPA treatment might actually be low if DTPA were administered to persons contaminated with plutonium in an accident. In conclusion, attempts to enhance the effects of DTPA and the development of new chelating agents will be necessary to adequately reduce the risk of plutonium inhalation in the future.

Publication:

54. Immunohistochemical Study on Cellular Origins of Rat Lung Tumors Induced by Inhalation Exposure to Plutonium Dioxide and by X-ray Irradiation

Yoichi Oghiso and Yutaka Yamada

**Keywords**: immunohistochemistry, rat, lung tumors, plutonium-exposure, X-ray irradiation

Immunohistochemical examinations were done on rat lung tumors induced by inhalation exposures to plutonium or by X-ray irradiation to identify and compare cellular origins or, in turn, target cells at risk for radiation carcinogenesis. Female Wistar (W/M) strain rats were either exposed to submicron-sized $^{239}$PuO$_2$ aerosols, or irradiated locally or systemically by X-rays at 100 to 120 days after birth. Primary lung tumor specimens were selected for the present examinations from 135 plutonium-exposed and 41 X-ray irradiated rats. All of those lung tumors appeared to occur from the lower respiratory tract epithelium through bronchioles into alveoli and were histopathologically diagnosed as adenoma, adenocarcinoma, adenosquamous carcinoma, and squamous cell carcinoma. Immunohistochemical staining of neoplastic lesions using rabbit
polyclonal antibodies to rat surfactant apoprotein A specific for alveolar type II pneumocytes and Clara cell antigen specific for nonciliated bronchiolar Clara cells, showed that most adenomatous and adenocarcinomatous lesions from plutonium-exposed or X-ray irradiated rats were positive for either or both antigens, while in contrast, adenomasquamous and squamous lesions were mostly negative for both antigens. Even though there were some differences in the proportions and distributions of immunoreactive cells between plutonium- and X-ray-induced tumors and among neoplastic lesions, the results indicated that radiation-induced pulmonary adenomas and adenocarcinomas mostly originate from either alveolar type II pneumocytes or bronchiolar Clara cells, while adenomasquamous and squamous carcinomas may be derived from other epithelial cell components, or might have lost specific antigenicity during their transforming differentiation.

Publication:

55. Induction of Micronuclei in Rat Alveolar Epithelial Cell Line by Alpha Particle Irradiation

Yutaka Yamada, Yoichi Oghiso, Hiroko Enomoto and Nobuhito Ishigure

*Keywords*: micronucleus, alpha particle, rat, alveolar type II cell line, biodosimetry

A life-span study has been conducted on rats that inhaled plutonium dioxide aerosols and it was observed that plutonium deposited in lung induces lung tumors in a dose-dependent manner. The absorbed dose in lung was calculated from radioactivity in thorax by whole body counting procedure, but the absorbed dose in target cells' level remains unclear. The lung model of ICRP publication 66 describes cells at risk of respiratory tract tissues as secretory and basal cells in bronchial airways, and Clara and Type II cells in the alveolar interstitial region. Therefore, dose estimations in the tissue layers containing the target cells are required in the model. Recently, a biodosimetric approach has been developed and applied to estimate absorbed dose in respiratory cells. In the biodosimetry, responses of isolated cells that have been previously exposed to alpha emitters *in vivo* are compared to the responses obtained from similar populations of cells that were irradiated *in vitro* using alpha sources. As a preliminary experiment for biodosimetry in respiratory epithelial cells of rat, the dose-response relationships of radiation-induced micronucleus formation were investigated utilizing an immortalized rat alveolar Type II cell line (SV40T2).

The SV40T2 cells were exposed to either alpha particles ($^{241}$Am: 3.2 MeV, 128 keV/$\mu$m, 0.093 Gy/min at cell-Mylar interface) or X-rays (200 kVp, 1 Gy/min). The frequency of micronucleus formation was 1.6 - 3.0% for unirradiated subjects but increased for irradiated ones in a dose-dependent manner. A linear relationship between the dose and the micronucleus formation was observed until 1 Gy for alpha particles and 4 Gy for X-rays. The linear slope for alpha particles was 28.5 % per Gy and the relative biological effectiveness (RBE) was 4.3, as calculated from the slopes. The slope for alpha particles was dependent on the energy of the alpha particles. These results indicate that the micronucleus assay is available for biodosimetry of alpha particle irradiation in the rat alveolar epithelial cells.

56. Involvement of p53-Dependent Apoptosis in Radiation Teratogenesis and in the Radioadaptive Response in the Late Organogenesis of Mice

Bing Wang, Harumi Ohyama, Masako Nose, Kaoru Tanaka, Tetsuo Nakajima, Osami Yukawa, Takeshi Yamada, Shirou Aizawa and Isamu Hayata

*Keywords*: apoptosis, p53, teratogenesis, adaptive response, organogenesis

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 malformation, namely, radiation-induced apoptosis in the predigital regions of embryonic limb buds is responsible for digital defects in mice. An examination of embryonic C57BL/6J mice with different p53 (trp53) status enabled us to further find that susceptibility to radiation-induced apoptosis in the predigital regions and digital defects depend on both the p53 status and the radiation dose; p53 wild-type mice appeared to be the most sensitive, while p53 knockout mice were the most resistant. These results indicate that p53-dependent apoptosis mediates radiation-induced digital defects in the later organogenesis period. The existence of a radioadaptive response in embryonic mice, which has not been reported so far, was found by irradiating embryos with either 5 cGy or 30 cGy on
embryonic day 11 prior to a challenging irradiation at 3 Gy on embryonic day 12. p53-heterozygous embryos did not show the radioadaptive response, indicating the involvement of p53 in the radioadaptive response in embryogenesis.

Publications: