Effects of LIHOPO, DTPP, CAP and DTPA on the Removal of Plutonium in Rats

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Abstract: The effects of DTPP, CAP, LIHOPO and Ca-DTPA in removing plutonium-239 in rats were examined. Twenty-five female Wistar rats, 8 weeks old, were pre-injected with plutonium in the right femoral muscles and divided into five groups; thereafter rats of four groups were injected intraperitoneally with chelating agent CAP, DTPP, LIHOPO or Ca-DTPA at a dose of 30 µmol/kg, once per day for 3 days, beginning 1 hr after plutonium injection on the first day. The fifth group was used as the control. The 24-hr urine and feces were collected. On day 4, rats were sacrificed to obtain organs and serum. In the DTPP group, high urinary excretion on the first day and low concentrations of Pu in the kidney, spleen, femur and muscle of Pu-injected site were observed, but the effect of DTPP was not superior to those of LIHOPO and Ca-DTPA. The results indicated that LIPOHO was the most effective chelating agent for removing plutonium in rats.

1. Introduction

Chelation therapy in emergency medical treatment following a radiation accident is required to reduce the risk of cancer induction and shortening of life span. Diethylene triamine penta acetic acid (DTPA) is prepared as a chelating agent to apply to anyone who becomes contaminated with plutonium while in the hospital for radiation emergency medicine in our institute. However, the effects of DTPA are not always sufficient to remove plutonium quickly from the body. Therefore, a chelating agent that is more effective than DTPA is desired. We have determined that LIHOPO is superior to DTPA for removing plutonium [1]. Two new chelating agents, ethylene diamine tetramethylene phosphonic acid (DTPP) and cyclam amino phosphonates (CAP) were compounded. Therefore, our goal in the present study was to determine the effects of DTPP and CAP, compared with those of LIHOPO and Ca-DTPA, in
removing plutonium in rats.

2. Materials and Methods

2.1 Products

LIHOPO, DTPP and CAP were synthesized by Prof. Burgada, R. and Dr. Bailly, T. Before the administration, these agents were resolved in isotonic saline solution. Ca-DTPA (1g in 4 ml of distilled water) in a vial for human therapy was purchased from Hyle Co. (Germany) and diluted in distilled water. The concentration of each solution was 30 $\mu$mol/kg of body weight, which is equivalent to a daily recommended human dose, at a solution volume of 0.46-0.50 ml. The concentration of each agent was 6 $\mu$mol in 0.4 ml of solution.

2.2 Plutonium

Plutonium-239 in a stock solution (0.27-N nitric acid) at our institute was diluted in 2N-HNO3, 100 mM citrate solution and 1N-NaOH, and adjusted to pH 6.8. The concentration of plutonium for injection was 18,600 Bq/ml.

2.3 Animals and procedures

Twenty-five male Wistar Mishima (WMnrs strain) rats, 2 months old, weighing 142±7g, were pre-injected intramuscularly with plutonium at $3.7 \times 10^4$Bq/kg per rat, and divided into five groups (n=5). The rats of four groups were injected intraperitoneally once per day for 3 days at a dose of 30 $\mu$mol/kg of DTPP, CAP, LIHOPO or Ca-DTPA, beginning 1 hr after plutonium injection on the first day. Rats were kept in individual cages to collect urine and feces separately every 24 hr during this experiment. The feces that had fallen on the net were picked up using a pair of tweezers. The urine on the cage bottoms was gathered with a mixture containing nitric acid, distilled water and hydrofluoric acid (100:150:1). On day 4, the rats were sacrificed to obtain blood, liver, kidney, spleen, femur and muscles of the plutonium-injected site. The samples were incinerated at 700 °C for 24 hr in a crucible. Two milliliters of a solution as described above was added to the crucible. Two milliliters of a solution as described above was added to the crucible. Two milliliters of a solution as described above was added to the crucible. Subsequently, the solution was poured into a counting vial with a scintillator. The alpha activity of plutonium in the vial was measured for 30 min by spectrometry using an alpha liquid scintillation counter (Beckman Instrument, Inc., Model LS5801).

Recovery and counting efficiency were confirmed based on the value measured in the solution containing the plutonium by alpha spectrometry; results were confirmed after a
comparison was made between an electrodeposited sample made of the same plutonium source as that used in this method and a purchased standard source.

3. Results

On the first day, the urinary plutonium excretion of DTPP was highest, followed by Ca-DTPA and LIHOPO, but CAP was not effective (Fig. 1). On the second and third days, Ca-DTPA and LIHOPO were effective. The feces plutonium excretion of LIHOPO and that of Ca-DTPA were effective for 3 days (Fig. 2).

Total amounts of excreted plutonium in urine and feces of LIHOPO-administered rats were the highest, followed by Ca-DTPA and DTPP, while CAP had no effects (Table 1).

![Fig. 1 Urinary-excreted plutonium](image1)

![Fig. 2 Feces-excreted plutonium](image2)

Table 1 Amount of excreted Pu in urine and feces for 3 days (percent of Pu injected dose)

<table>
<thead>
<tr>
<th>Group</th>
<th>Urine</th>
<th>Feces</th>
<th>Urine and Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.206±0.139</td>
<td>0.147±0.027</td>
<td>0.353±0.125</td>
</tr>
<tr>
<td>DTPP</td>
<td>0.743±0.198*</td>
<td>0.439±0.443</td>
<td>1.181±0.584</td>
</tr>
<tr>
<td>CAP</td>
<td>0.122±0.032</td>
<td>0.053±0.011*</td>
<td>0.307±0.088</td>
</tr>
<tr>
<td>LIHOPO</td>
<td>0.435±0.103</td>
<td>2.021±0.280*</td>
<td>2.379±0.427*</td>
</tr>
<tr>
<td>Ca-DTPA</td>
<td>0.997±0.014*</td>
<td>0.550±0.097*</td>
<td>1.547±0.108*</td>
</tr>
</tbody>
</table>

*Significant difference from the control value (p<0.05)

LIHOPO: significant difference from all of the other agents (p<0.05)
The concentrations of plutonium in the liver, femur and serum in the LIHOPO group were significantly lower than those of the control group. The plutonium concentration of muscle and serum in the DTPP group and of liver and femur in the Ca-DTPA group were significantly lower than those of the control group (Table 2).

**Table 2** Concentrations of plutonium in the organs and serum (percent of Pu injected dose)

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Femur</th>
<th>Muscle</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.794±0.099</td>
<td>0.060±0.034</td>
<td>0.017±0.014</td>
<td>0.154±0.027</td>
<td>10.207±4.884</td>
<td>0.020±0.005</td>
</tr>
<tr>
<td>DTPP</td>
<td>0.439±0.355</td>
<td>0.031±0.029</td>
<td>0.007±0.007</td>
<td>0.056±0.032</td>
<td>8.701±7.168*</td>
<td>0.007±0.006*</td>
</tr>
<tr>
<td>CAP</td>
<td>0.802±0.142</td>
<td>0.054±0.030</td>
<td>0.010±0.003</td>
<td>0.143±0.010</td>
<td>16.091±3.149</td>
<td>0.021±0.004</td>
</tr>
<tr>
<td>LIHOPO</td>
<td>0.394±0.066*</td>
<td>0.042±0.022</td>
<td>0.007±0.002</td>
<td>0.069±0.008*</td>
<td>10.030±6.701</td>
<td>0.007±0.008*</td>
</tr>
<tr>
<td>Ca-DTPA</td>
<td>0.522±0.127*</td>
<td>0.045±0.029</td>
<td>0.110±0.002</td>
<td>0.090±0.050*</td>
<td>13.327±5.125</td>
<td>0.021±0.005</td>
</tr>
</tbody>
</table>

* Significant difference from the control value (*p<0.05)

4. Discussion

The result of measuring total amounts of plutonium in the excreta for 3 days indicated that LIHOPO is superior for removing plutonium, even to Ca-DTPA, which is similar to the results of a previous study [1]. The route of plutonium excretion in the LIHOPO group was different from that in the Ca-DTPA group. The former was by feces and the latter by urine. The rate of plutonium excretion is generally low, and it is changeable in response to the pH of the solution and the administration route. The combined treatment of LIHOPO and Ca-DTPA may be more effective than the use of either one of these agents alone in accelerating plutonium excretion, although additional toxicological study is necessary [1,2]. The application of LIHOPO during early treatment may be worthwhile, either in combination with Ca-DTPA or by itself.

The results indicated that the effect of DTPP was similar to that of Ca-DTPA. For example, the urinary excretion rate of plutonium following the first administration was similar to that following administration of Ca-DTPA, and the concentrations of plutonium in all organs and serum tended to be lower than those following Ca-DTPA administration. In particular, the low plutonium concentration in the muscles suggests the positive effects of DTPP administration. If the side effects of using DTPP are lower than those of using Ca-DTPA, greater beneficial effects may be expected with a large dose of DTPP compared to the effects of Ca-DTPA.
References
