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

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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 oral route, Ca-DTPA alone via 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 at 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. In conclusion, the results of this study indicate that D-penicillamine is useful in treating a person contaminated with radiocobalt in an accident.

KEY WORDS: D-penicillamine, Ca-DTPA, removal, radiocobalt, daily recommended human dose

I Introduction

Radiocobalt is a radionuclide that can contaminate persons working in reactors, fuel-reprocessing plants, nuclear waste facilities, hospitals, laboratories and factories with more frequency than other radionuclides.1) A biological half-time of radiocobalt in rodents is estimated to be 9.5 days.1) On the other hand, the greater part of radiocobalt intake in the body decreases with a biological half-time of one day or less. However, the remainder decreases much more slowly with half-times varying from 5 days to 17 years due to chemical and particle size differences.2-6) Therefore, chelation therapy is required for a person accidentally contaminated with a large dose of radiocobalt. Although many kinds of compounds for removing radioactive and stable cobalt have been examined so far, DTPA and D-penicillamine are now recommended.7-13) D-penicillamine was originally known as a drug for removing copper in Wilson's disease14) and was also suggested as an efficient means of removing radiocobalt.1) However, there are few reports on the effects of these...
compounds in removing radiocobalt from the body that are applicable to humans.

The purpose of the present study is to determine the effects of D-penicillamine, Ca-DTPA and their combination on the removal of radiocobalt when these compounds are applied to rats according to a human treatment schedule.

II MATERIALS AND METHODS

1. Radiocobalt

$^{57}$Co$^{++}$ as a Cl₂ solution (150–315 MBq/μg) was purchased from Amersham Pharmacia Biotech UK Ltd. (England).

2. Chelating agents

D-penicillamine (Metalcaptase, Taisho Pharmaceutical Co., Japan) and Ca-DTPA (Heyl Co., Germany) were also purchased. The dose of Ca-DTPA per rat was 30 μmol/kg, which is equivalent to a daily recommended human dose (i.e., 1 g for an adult). The working solution was made by dilution of a Ca-DTPA solution, and the volume was 0.36 ml per rat.

The dose of D-penicillamine per rat was 20 mg/kg, which is equivalent to the maximum daily human dose (1000 mg) for an adult with a body weight of 50 kg. The working solution (10 mg/ml) was made by dilution of D-penicillamine, and the volume was 0.44 ml per rat.

3. Radioactivity measurement

The activities of radiocobalt in the whole body, excreta feces, urine, organs and blood were measured by a Ge detector connected to a multichannel analyzer with appropriate emulation software (Model GMX-30190-P, BG&G Ortec Products, USA).

4. Animals

Thirty male Wistar Mishima (WM) rats, 12 weeks old, were used. The mean±SD of body weight was 221±8 g.

5. Animals and procedures

Rats were pre-injected intraperitoneally with 74,000 Bq of the $^{57}$CoCl₂ solution, and the radioactivity of the whole body was immediately measured. Thereafter, the rats were divided into six groups consisting of five rats per group. Rats in the first and second groups received an intraperitoneal injection of Ca-DTPA with and 1 h after the radiocobalt injection. Rats in the third and forth groups received oral administration of D-penicillamine according to the same schedule as those treated with Ca-DTPA. The fifth group simultaneously received Ca-DTPA and D-penicillamine 1 h after

![Graph](image_url)

Fig. 1 Whole-body retention of radiocobalt after administration of Ca-DTPA and D-penicillamine. Values are mean and bars are standard deviation.
the radiocobalt injection. The sixth group was kept as a control.

The rats were kept in individual metabolic cages. The radioactivity levels of the whole body of the rat, the urine and feces were measured at intervals of 24 h after the radiocobalt injection. On day 4, the liver, kidney, bone (femur), muscles, blood were collected, and the activity was measured.

The retained activity in the organs (relative retained activity) was calculated by means of the following formula:

\[
\frac{\text{Organ activity (Bq)}}{\text{Organ weight (g)}} \times \frac{\text{Whole body activity (Bq)}}{\text{Whole body weight (g)}}
\]

III RESULTS

The whole body radioactivity in all groups decreased rapidly after cobalt injection (Fig. 1). The values in the treated group at 3 days after radiocobalt injection are presented as percentages of the control in Table 1. The whole body activity in the Ca-DTPA-alone groups was reduced to 9.6% of that of the control when the Ca-DTPA was administered with the radiocobalt injection but was 79.0% when the Ca-DTPA was administered 1 h later. That in the D-penicillamine and Ca-DTPA group was reduced to 54.8% of that of the control. Those in the D-penicillamine-alone groups were reduced to 33.6% of that of the control group when the D-penicillamine was administered with the radiocobalt injection and 56.6% when the D-penicillamine was administered 1 h later.

The urinary and feces levels of radiocobalt activity are shown in Figs. 2 and 3. The activities of the liver, kidney, bone (femur), muscle and blood are shown in Table 2. Ca-DTPA was effective in reducing the activity in the liver and kidney when administered immediately after the radiocobalt injection. However, the effects were not observed when the administration was delayed. D-penicillamine was effective in reducing the activity in the liver and kidney when administered immediately

<table>
<thead>
<tr>
<th>Group</th>
<th>Percent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca-DTPA with Co</td>
<td>9.6</td>
</tr>
<tr>
<td>Ca-DTPA 1 h after Co</td>
<td>79.0</td>
</tr>
<tr>
<td>D-penicillamine with Co</td>
<td>33.6</td>
</tr>
<tr>
<td>D-penicillamine 1 h after Co</td>
<td>56.6</td>
</tr>
<tr>
<td>Ca-DTPA + D-penicillamine 1 h after Co</td>
<td>54.8</td>
</tr>
</tbody>
</table>

Fig. 2 Urinary excretion of radiocobalt after administration of Ca-DTPA and D-penicillamine. Values are mean and bars are standard deviation.
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Fig. 3 Feces excretion of radiocobalt after administration of Ca-DTPA and D-penicillamine. Values are mean and bars are standard deviation.

Table 2 Relative retained activity in organs 3 day after the administration of D-penicillamine and Ca-DTPA.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>DTPA with Co</th>
<th>DTPA 1 h after Co</th>
<th>D-penicillamine with Co</th>
<th>D-penicillamine 1 h after Co</th>
<th>DTPA + D-penicillamine 1 h after Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>16.90</td>
<td>8.60*</td>
<td>14.08</td>
<td>7.52*</td>
<td>15.48</td>
<td>11.95</td>
</tr>
<tr>
<td>Kidney</td>
<td>15.75</td>
<td>5.03*</td>
<td>11.58</td>
<td>8.16*</td>
<td>10.83*</td>
<td>11.29*</td>
</tr>
<tr>
<td>Skeleton</td>
<td>0.42</td>
<td>0.42</td>
<td>0.30</td>
<td>0.41</td>
<td>0.48</td>
<td>0.47</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.44</td>
<td>0.42</td>
<td>0.29</td>
<td>0.42</td>
<td>0.48</td>
<td>0.47</td>
</tr>
<tr>
<td>Blood</td>
<td>0.11</td>
<td>0.10</td>
<td>0.08</td>
<td>0.10</td>
<td>0.11</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*Significantly different from the values of control group by Student's t test ($p < 0.05$)

IV DISCUSSION

The data obtained in this study indicated that both Ca-DTPA and D-penicillamine were effective in removing radiocobalt from the body. However, the effects depended on the time of administration. When the Ca-DTPA was injected just after the radiocobalt injection, the activity was reduced to 9.6%, but the effects decreased to 79.0% when the timing of administration was delayed 1 h after the radiocobalt injection. In a previous study, even high doses of Ca-DTPA (equivalent to 33 and 85 times 30 $\mu$mol/kg) administered 6 and 168 h after radiocobalt injection, respectively, were not effective (reduction to 90 and 91% of the control, respectively).[8] The results indicated that Ca-DTPA has efficacy when administered just after an accident. However, as the injection of Ca-DTPA must be carried out by a medical doctor, it may actually be difficult to obtain significant effects of Ca-DTPA after an accident.

On the other hand, when D-penicillamine was
administered to rats immediately after the radiocobalt injection, the whole body activity was reduced to 33.6% of that of the control group and when the timing of the administration was delayed 1 h after the radiocobalt injection, the effect was 56.6%. These results indicate that D-penicillamine is more effective than Ca-DTPA when the treatment is delayed. In an actual accident, D-penicillamine has the advantage that a person can administer it to himself immediately after the accident. Also, the side effects of D-penicillamine should not generally be a cause for fear because it has been used for patients with Wilson’s disease.

The effect of the combination of D-penicillamine and Ca-DTPA was 54.8% of that of the control and nearly 56.6% of that of D-penicillamine alone when it was administered 1 h after the radiocobalt injection. This result indicated that D-penicillamine was more effective than Ca-DTPA. The present data was superior to the results in a previous study indicating that radiocobalt activity was reduced to 86% of the control by using a combination of 360 μmol/kg Ca-DTPA and 0.24 mg/kg D-penicillamine, and to 96% by using a dose of 360 μmol/kg Ca-DTPA alone.1)

In a previous study comparing the effects of eleven chelating agents including DTPA and D-penicillamine on the removal of radiocobalt from organs, D-penicillamine was the most effective for reducing the radiocobalt, followed DTPA which was superior to the remaining agents. That is, the radiocobalt relative retained activity in the liver and kidney was 11.9% and 18.1% of that of the control at 48 h after a lower dose (15 mg/kg) of D-penicillamine was orally administered immediately after radiocobalt injection. That data is superior to the present data of 44.5% in the liver and 51.8% in the kidney although a dose (20 mg/kg) was administered immediately after radiocobalt injection (Table 2). On the other hand, the effects were lowered when a large dose (150 mg/kg) of D-penicillamine was injected intraperitoneally immediately after radiocobalt injection. In the data of the previous study, DTPA was not effective for the kidney but was effective in reducing the activity of the liver (37.7%). These data also indicated that D-penicillamine is useful for removing radiocobalt from the organs as well as in the case of whole body retention.

In previous studies, the efficacy of Co-DTPA has been pronounced greater than that of Ca-DTPA.1,7,8,10) However, there is no evidence concerning the safety of Co-DTPA for humans or the methods of application of this substance in humans such as administration route and dose, which have been established for Ca-DTPA and Zn-DTPA.

In conclusion, D-penicillamine is useful for removing radiocobalt from the body, and Ca-DTPA may not be useful except the administration just after an accident. Therefore, D-penicillamine should be provided in facilities or sites dealing with radiocobalt. In addition, as a large amount of radiocobalt intake will be eliminated with short biological half-time and long-term retention of the remainder, D-penicillamine should be used for a person contaminated with a large dose of radiocobalt.

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