Removal of Plutonium by a New Type Calcium and Combination of the Calcium and Ca-DTPA in Rats

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Effects of a new type of calcium product (Seaweed-calcium complex: SWCa) and combination of SWCa and Ca-DTPA on removal of injected $^{239}$Pu were examined in rats. SWCa is a natural product consisting of mixed calcined oyster shell (COS) which is composed of calcium salt probably in the form of calcium oxide and a natural material extracted from seaweed to transport calcium actively throughout the small intestine. In experiment I, a 2% solution of SWCa, COS, CaO, CaCl₂ or CaCO₃ was injected into the small intestinal loops of rats to compare the intestinal absorption rate of each calcium salt. In experiment II, 25 male Wistar rats, 3 months of age, were divided into five groups given; a control diet with 1% CaCO₃ (group A), a SWCa diet with 1% SWCa to replace the 1% CaCO₃ after plutonium injection (group B), a SWCa diet 1 week before plutonium injection until the end of the experiment (group C), a SWCa diet and intraperitoneal injections of a daily dose of 150 $\mu$mol/kg of Ca-DTPA (group D), and a control diet and intraperitoneal injections of the same dose of Ca-DTPA as group D (group E). The rats were injected intravenously with plutonium, $1.85 \times 10^4$ Bq/kg, and killed 14 days later and the femur and liver were excised. All excreta were collected at 24-h intervals during the experimental period. The plutonium concentration was measured by a liquid scintillation spectrometry after a wet ashing treatment.

The levels of serum total and ionic calcium were elevated more rapidly and were higher after the injection of SWCa than after those of COS, CaO and CaCO₃. The percent of plutonium content to administered dose in the skeleton was 69.3, 63.6, 58.8, 10.4 and 19.0%, and that in the liver was 7.74, 6.93, 6.43, 0.22 and 0.35% for groups A, B, C, D and E, respectively. The plutonium contents in the urine and feces were increased during the first 2-3 days after plutonium injection in the SWCa groups. The results indicated that the elevation of serum calcium by the oral administration of SWCa could lower the plutonium content in the skeleton and liver both before and after plutonium injection and the effects were increased by the combination of SWCa and Ca-DTPA. In conclusion, SWCa not only lowered the plutonium content after exposure but also prevented plutonium deposition in the bones and liver.

KEY WORDS: Ca-DTPA, seaweed-calcium complex, plutonium, rat, removal

I INTRODUCTION

Chelating agents such as DTPA (diethylenetriaminepentaacetic acid)\textsuperscript{1-3} and CBMIDA [catechol-3,6-bis(methyleneiminoacetic acid)]\textsuperscript{4,5} can effectively removed plutonium. However, chelation therapy is restricted because of the unavoidable toxic side effects\textsuperscript{6-8} and the difficulty of management for application of such chelating agents. The toxicity of DTPA depends on the dose, administration route, period of use and chemical form such as calcium and zinc salts.\textsuperscript{7} We found that Zn-DTPA is safer than Ca-DTPA with lower possibility of hypocalcemia and subsequent functional damage to the cardiovascular system. Besides, it may be difficult for a contaminated...
person to receive instillation of DTPA immediately after an accident, i.e., within, at the longest, a few hours after exposure when the beneficial effect is highest, because the measurement and assessment of plutonium and special medical handling are necessary. Moreover in the protracted treatment the repeat instillations are accompanied by severe pain and restricted movement of the patient. These problems in chelation therapy using DTPA might not be easily resolved in a short time.

Recently, the bioavailability of so-called natural products to raise or improve various physiological functions has been noticed. The seaweed-calcium complex (SWCa) is a natural product consisting of mixed powdered calcined oyster shell (COS) and a material extracted from seaweed. The COS contains 51.4% calcium which is assumed to be present mainly in the form of calcium oxide. However, the crystalline shape of COS is quite different from that of CaO or CaCO₃. Oral administration of COS in an amount providing the same amount of calcium as calcium carbonate produces a higher serum calcium value and has beneficial effects against bone disorders such as osteoporosis. Previously we found that SWCa mixed with COS and the extracted material from seaweed produced a higher serum calcium level than COS, and the extracted material probably has a role to transport calcium actively from the small intestine. The intestinal absorption rate of calcium carbonate is higher than that of other calcium salts such as calcium acetate, calcium lactate, calcium glucose, calcium citrate and whole milk. Therefore, the bioavailability of SWCa was compared with that of COS, CaO, CaCl₂ and CaCO₃ in this study.

We considered that the bioavailability of SWCa might be useful to reduce plutonium retention particularly in bone because the serum calcium level was increased rapidly and because SWCa, which had a high intestinal absorption rate, was deposited at the mineralizing front on the bone surface faster than plutonium thus inhibiting plutonium incorporation on it.

In the present study, the effects of SWCa and combination of SWCa with Ca-DTPA on the removal of plutonium in rats after the intestinal absorption of calcium of SWCa was compared with those of COS, CaO, CaCl₂ and CaCO₃.

II MATERIALS AND METHODS

In experiment I, the intestinal absorption rate of SWCa, COS, CaO, CaCl₂ and CaCO₃ was examined in rats. Twenty four male Wistar rats, 3 months of age, were divided into three groups of eight each. Under the anesthesia of a combination of ketamine hydrochloride and xylazine, the abdominal wall was incised and the proximal duodenum and distal ileum were tied with thread to make an intestinal loop. Five ml of a 2% solution of SWCa, COS, CaO, CaCl₂ or CaCO₃, pH adjusted to 7, was injected into the small intestinal loop. Blood samples of 0.6 ml were collected before injection, 15 and 30 min later from a vena cava posterior to measure the pre-values of total and ionic calcium in serum. Blood was clotted and centrifuged for 15 min at 3,000 r.p.m. The concentration of total serum calcium was determined by the o-cresolphthalein complexone method (Wako Pure Chemical Industries, Ltd.) and ionic serum calcium by an ion electrode method (Sera 250, Horiba Ltd.).

In experiment II, the effect of orally administered SWCa on the removal of injected plutonium was examined in rats. SWCa (1%) was used to replace CaCO₃ (1%) in the control diet (MB-1, Funabashi Co., Ltd.). Twenty five male Wistar rats, 3 months of age, with a mean body weight of 299 g, were divided into the following five groups of five each: group A was given a control diet, group B was switched to a SWCa diet immediately after plutonium injection, group C was given a SWCa diet from 1 week before plutonium injection until the end of the experiment, group D was switched to a SWCa diet immediately after plutonium injection and injected intraperitoneally with a daily dose of 150 μmol/kg of body weight of Ca-DTPA once a day for 14 days, beginning at about 1 h after plutonium injection on the first day of treatment, and group E was given a control diet and injected Ca-DTPA according to the same schedule as group D.

Rats were injected intravenously with plutonium i.e., 5.6 × 10⁵ Bq of ²³⁹Pu, in 0.12 ml of 0.008 M sodium citrate (1.85 × 10⁴ Bq/kg), pH adjusted to 7.2, under anesthesia consisting of a combination of ketamine hydrochloride and xylazine in an airtight box with gloves designed for plutonium administration to a small animal. The plutonium-
injected arts were then put in individual metabolic cages designed to separate urine and feces and placed in an airtight box for small animal breeding. All excreta were collected at 24-h intervals during the experiment.

Rats were killed 14 days after plutonium injection. The femur and liver were excised. Each sample was weighed and treated for the wet ashing. The whole femur, about 0.5 g of liver, 0.5 g of stirred feces, and 0.5 ml of urine were each placed in a glass beaker on a hot plate for wet ashing. Nitric acid was added repeatedly until the color of the residue disappeared completely. Finally, 2 ml of mixed solution (nitric acid: distilled water: hydrofluoric acid = 150:100:0.9) was added to a beaker and 1 ml of the solution was pipetted in a counting vial. The alpha activity of plutonium in a vial was measured by an alpha liquid scintillation counter for 30 min. Recovery and counting efficiency were confirmed by an alpha-spectrometry using an electrodeposited sample which was made of the same plutonium source as that used in this method.

III RESULTS

In experiment I, the levels of both total and ionic serum calcium in the SWCa group were elevated rapidly and were significantly higher than those in the COS, CaO, CaCl₂ and CaCO₃ groups 15 min after the injection (Fig. 1 (a) and (b)).

Figure 2 (a) and (b) show the plutonium contents of the entire skeleton and liver to the administered dose in each group in experiment II. The plutonium content of the entire skeleton was calculated from the amount of plutonium in the femur which is often used as a convenient index of the total skeleton burden, by multiplying the plutonium content of both femurs by 10.¹⁵)

As shown in Fig. 2, the plutonium content to the administered dose in the skeleton was 69.3, 63.6, 58.9, 10.4, 19.0% for groups A, B, C, D and E and that in the liver was 7.74, 6.93, 6.43, 0.22, and 0.35% for groups A, B, C, D and E, respectively. Figure 3 (a), (b) and (d) show the plutonium content in the urine and feces. There were increases in plutonium contents in the urine and feces during the first 2–3 days after plutonium injection.

IV DISCUSSION

The finding obtained in experiment I that the levels of total ionic serum calcium were elevated more rapidly and were higher by SWCa administration than by COS, CaO, CaCl₂ and CaCO₃, indicated that the material extracted from seaweed in the SWCa transported actively the calcium of COS throughout the small intestine of the rats. This material is under further examination. The serum calcium value after oral administration of SWCa was higher than that after administration of CaCO₃ in beagle dogs.¹³)

In experiment II, the plutonium content in the skeleton in groups B, C and E administered SWCa was lower than those in groups A and D administered CaCO₃. The difference in plutonium content in the skeleton was 5.7% between group A and group B, and 8.6% between group D and group E. These findings indicated that the plutonium contents was lowered by oral administration of SWCa after plutonium injec-

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**Fig. 1** Changes in total (a) and ionic (b) serum calcium concentrations after injection of SWCa, COS (calcined oyster shell), CaO, CaCl₂ and CaCO₃ solution into the intestinal loop of rats. Each value shows the percent to the pre-injection level. Each mark and vertical bar represents the mean±SD for eight rats.
The difference between group A and group C was 10.4% and higher than that between groups A and B, or between groups D and E, which indicated that preventive administration of SWCa was more effective to reduce the plutonium content in the skeleton than the post-plutonium administration.

The amount of plutonium in the skeleton lowered by SWCa administration was less than that by Ca-DTPA. Plutonium was distributed initially both on the resting and active bone surfaces. The percentage of newly formed bone (active bone surface × mineral apposition rate) to total trabecular bone measured by bone histo-

Fig. 2 Plutonium contents in the skeleton (a) and liver (b) in each group. Each value represents the percent to the injected plutonium dose. Each column and bar represents the mean ± SD for five rats.

Fig. 3 Plutonium excretions in the daily urine (a), (b) and feces (c), (d) of each group. Each mark and vertical bar represents the mean ± SD for five rats.
morphometry in 3-month-old male rats of the same strain used in this study during this experiment was calculated to be about 12% from the findings reported previously.\(^1\)\(^6\) Therefore, the rates (5.7 and 8.6\%) of reduction in plutonium content in the skeleton in the SWCa administered groups might be comparable to that in the CaCO\(_3\) administered groups. This finding supports our view that excessive serum calcium deposits on the mineralizing surface and inhibits the incorporation of plutonium. The difference (8.7\%) between group D and group E administered Ca-DTPA was larger than that (5.7\%) between group A and group B. The action of Ca-DTPA to lower the plutonium content was independent of the content of calcium. The amount of incorporated plutonium was lower in group D than in group E probably because the Ca-DTPA compounded with plutonium which could not deposit on the mineralizing front by the inhibition of calcium-deposition (Fig. 4). A possible explanation for the reduction in the plutonium content in the skeleton by the elevation of serum calcium is that the excessive calcium induced by the rapidly and highly elevated serum calcium level at least within 15 min after SWCa administration reached the bone and was deposited in the mineralizing front of the bone surface earlier than the injected plutonium and inhibited the incorporation of plutonium (Fig. 4), because about half of the intravenously injected plutonium was deposited on the endosteal bone surface 2–24 h later and the plutonium was buried gradually into the bone matrix accompanied by slow bone turnover in rats.\(^1\)\(^5\),\(^1\)\(^7\)

Bone metabolism is produced by an equal balance between the osteoclast activity resolving an old bone and osteoblast activity forming a new bone. As the increase in serum calcium rapidly destroyed the osteoclasts and resulted in the decrease of temporary bone formation and mineralization by osteoblasts, plutonium could not deposit. The decrease in the number of osteoclasts may also contribute to the reduction of plutonium incorporation by phagocytosis.\(^1\)\(^3\)

SWCa administration had a beneficial effect to lower the plutonium contents in the liver for an unknown reason. The mechanism of the reduction of plutonium incorporation in the liver remains to be elucidated.

The removal of plutonium by bioavailability of natural products might help resolve troublesome problems such as the difficulty of practical use and the avoidable toxicity of chelating agents. In the chelation therapy, DTPA application to humans is recommended within 30 min after exposure. However, plutonium-contaminated persons may not be able to receive chelation therapy easily or promptly because the assessment of plutonium intake and special medical handling by medical doctors will take many hours.\(^9\) However, the contaminated person can take oral SWCa immediately after exposure and also continue to take it during the protracted treatment, because SWCa is present in a powder form which can be taken orally.

An interesting finding is that the effect to lower the plutonium contents in the skeleton and liver was increased by the preventive administration of SWCa as compared with the administration after plutonium injection in this study. Also a combination of SWCa and DTPA may be expected to decrease the dose and toxicity of DTPA.

**V CONCLUSION**

SWCa was demonstrated to be effective to lower the plutonium contents in the skeleton and liver by oral administration both before and after plutonium exposure. The effect was increased by the pre-administration of SWCa and by the combination of SWCa and Ca-DTPA.

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