ORIGINAL**Effects of CBMIDA [Catechol-3,6-bis(methyleiminodiacetic acid)] on Removal of Plutonium in Rats**Satoshi FUKUDA,^{*,*1} Haruzo IIDA,^{**, *1} Yu Yuan HSEIH^{*2}
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Effects of CBMIDA [catechol-3,6-bis(methyleiminodiacetic acid)] on injected ^{239}Pu were examined in rats; in our previous studies, CBMIDA had almost the same toxicity as those of DTPA and EDTA. Rats were injected intravenously with ^{239}Pu (1.85×10^4 Bq/kg) and divided into four groups of five rats each. Three groups were each injected intraperitoneally with a daily dose of $150 \mu\text{mol/kg}$ of CBMIDA, Ca-DTPA or Zn-DTPA for 2 weeks, beginning at about 1 h after plutonium injection. Rats were sacrificed 14 days later and the femur and liver were removed. All excreta were collected at 24-h intervals during the experimental period. Plutonium contents of the femur, liver, feces and urine treated by a wet ashing method were measured by a liquid scintillation spectrometry.

The plutonium contents in the skeleton of administered dose were 11.5% for CBMIDA group, 19.0% for Ca-DTPA group, and 26.9% for Zn-DTPA group, whereas that was 63.9% for the control; those in the liver were 0.62% for CBMIDA group, 0.35% for Ca-DTPA group and 0.54% for Zn-DTPA group, whereas that was 7.74% for the control. The data obtained indicate that CBMIDA is superior to Ca-DTPA and Zn-DTPA on removing plutonium from bone, and also has almost the same effectiveness on removing it from liver as those of both Ca-DTPA and Zn-DTPA. The higher effectiveness of CBMIDA on removal of plutonium from the skeleton might be, besides the stronger chelating action, due to the inhibition of plutonium deposition into bone, judging from the results of our previous study wherein the effects of CBMIDA on bone metabolism were examined in beagle dogs.

In conclusion, taking together the results obtained in this study and those from our previous studies on the toxicity, it was demonstrated that CBMIDA could be utilized, as a new chelating agent, to remove plutonium from human body.

KEY WORDS: CBMIDA, ^{239}Pu , rat, chelating agent

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I INTRODUCTION

At present, probably DTPA (diethylenetriamine-pentaacetic acid) is the only chelating agent to be utilized for the removal of plutonium and other actinides from human body, because the efficacy is superior to other chelating agents examined so far^{1,2)} and the toxicity is lower^{1,3)} However,

there remain some reluctant problems in that the applications to human are restricted because of the toxicity⁴⁻⁷⁾ and the efficacy; for example, the beneficial effects are limited at the longest within a few days after exposure.^{8,9)} Therefore, it is necessary to develop new, more effective and lower toxic agents, besides DTPA.

It is advisable that, for applying a new chelating agent to the treatment of human exposure, the detailed examination in experimental animals should be performed to clarify both the toxicity and efficacy with therapeutic schedules.¹⁰⁾ Our previous studies demonstrated that CBMIDA, which was developed as a new chelating agent for removal of plutonium, had almost the similar degrees of toxicity as those of DTPA and EDTA (ethylenediaminetetraacetic acid) in soft tissues¹¹⁾ and bone,¹²⁾ and also indicated that it had the possibility to be able to remove more plutonium, particularly from bone, because the significant increase of osteoid volume on bone surface and the absence of a bone marker at the mineralizing front, showing the inhibition of mineral and metal depositions, was observed in the beagle dogs that received successive intravenous injections of CBMIDA for 1 month.¹²⁾

The present study is performed to examine the effect of CBMIDA on removal of injected plutonium and to compare it to those of Ca-DTPA and Zn-DTPA in rats.

II MATERIALS AND METHODS

Twenty male Wistar rats, 3 months of age, with a mean body weight of 296 g were injected intravenously with plutonium, *i.e.*, 5.6×10^3 Bq of ²³⁹Pu, in 0.12 ml of 0.008 M sodium citrate solution (1.85×10^4 Bq/kg body weight), pH adjusted to 7.2, under the anesthesia of a combination with ketamine hydrochloride and xylazine in the airtight box with gloves for plutonium administration to small animals. Plutonium-injected rats were each then placed in an individual metabolic cage designed to separate feces and urine in the airtight box with gloves for small animal breedings. They were divided into four groups of five rats each. Three groups were each daily given intraperitoneal injections of a dose of 150 μ mol/kg CBMIDA, Ca-DTPA, or Zn-DTPA for 2 weeks, beginning at about 1 h after plutonium injection on the first day of treatment. The remaining one group

was for plutonium control and received no therapy at all. Animals were sacrificed 14 days later. The femur and liver were removed for the measurement of plutonium content. All excreta, separated feces and urine, were collected from the individual rats at 24-h intervals during the experiment.

Alpha counting in a sample was performed by a liquid scintillation method. Each sample was weighed and treated for wet ashing. The whole femur, about 0.5 g of liver and stirred feces, and 0.5 ml of urine were each placed in a glass beaker on a hot plate. Nitric acid was added repeatedly until the color of residue disappeared completely. Finally, 2 ml of the mixed solution (nitric acid–distilled water–hydrofluoric acid = 150:100:0.9) was added into a beaker and 1 ml out of 2 ml 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 the alpha spectrometry using the electrodeposited sample which was made of the same plutonium source as that used in this method.

III RESULTS

The plutonium contents of the entire skeleton and liver in each group are shown in **Table 1**. The content of entire skeleton was calculated based on the method that the amount of plutonium in the femur is often used as a convenient index of the total skeletal burden, by multiplying the plutonium content of both femurs by 10.¹⁸⁾ The plutonium content of skeleton was significantly ($p < 0.001$) reduced to 11.5% for CBMIDA group, 19.0% for Ca-DTPA group, and 26.9% for Zn-DTPA group of the injected amount, whereas that was 63.9% for the control. There was significant difference ($p < 0.01$) between CBMIDA and Zn-DTPA groups. In **Fig. 1**, these mean values of the skeleton and liver in each group represented as percentages of the corresponding control were 18, 30 and 42% in the CBMIDA, Ca-DTPA and Zn-DTPA groups, respectively.

The plutonium content of liver was significantly ($p < 0.001$) reduced to 0.62% for CBMIDA group, 0.35% for Ca-DTPA group, 0.54% for Zn-DTPA group of the injected amount, whereas that was 7.74% for the control. In **Fig. 1**, these mean values represented as percentages of the

Table 1 Plutonium contents of skeleton and liver in the CBMIDA, Ca-DTPA, Zn-DTPA and control groups on day 14 after plutonium injection.

Group	Entire skeleton % administered dose* ¹	Liver % administered dose
Control	63.9 ± 8.9%	7.74 ± 1.62%
CBMIDA	11.5 ± 6.1* ^{a,b}	0.62 ± 0.25* ^a
Ca-DTPA	19.0 ± 8.5* ^a	0.35 ± 0.15* ^a
Zn-DTPA	26.9 ± 4.5* ^a	0.54 ± 0.13* ^a

Each value indicates the mean ± SD for 5 rats.

*¹ These values were obtained by multiplying the plutonium content of femurs by 10.

*² Significant difference from the control ($p < 0.001$).

*³ Significant difference from the Zn-DTPA ($p < 0.01$).

Fig. 1 Plutonium contents of the skeleton and liver as the percentages of control in the groups treated with various chelating agents shown in Table 1.

corresponding control were 8, 5 and 7% in the CBMIDA, Ca-DTPA and Zn-DTPA groups, respectively.

The excretions of plutonium up to 14 days in daily urine were always higher in the CBMIDA, Ca-DTPA and Zn-DTPA groups—reaching the peak at the 1st day in the CBMIDA and Ca-DTPA groups and at the 2nd day in the Zn-DTPA group—than that of the control for 2 weeks, except for the values of CBMIDA on day 14 (Fig. 2). The excretions of plutonium in daily feces were higher in the CBMIDA, Ca-DTPA and Zn-DTPA groups than that of the control for the

initial 4 days, reaching the peak at the 2nd day after treatment, but thereafter those decreased and were rather lower than that of the control, except for the values of the 5th and 6th day in the Ca-DTPA group (Fig. 3).

IV DISCUSSION

Our data indicated that the plutonium content of liver in the CBMIDA group was at a level similar to those in the Ca-DTPA and Zn-DTPA groups and the highest excretions of plutonium in the feces and urine, reaching the peak at the 1st to 2nd day after the administrations, were observed as well as those in the Ca-DTPA and Zn-DTPA groups, as shown in Figs. 2 and 3. These results showed that CBMIDA could strongly compound with plutonium being in the blood, prior to deposit in bone, as well as DTPA, because the highest excretion of plutonium by DTPA was exceeded when more injected plutonium was remaining in blood. Conversely speaking, as the effectiveness of CBMIDA on removal of plutonium decreased rapidly after plutonium exposure, the beneficial effects of CBMIDA such as the reduction of risk of plutonium-induced cancer and the prolongation of the shortened life span might be obtained if CBMIDA is promptly utilized in the treatment, for example, within 0.5 to 1 h after plutonium exposure when over a half of injected plutonium remains in blood and does not deposit in bone yet.¹³⁾ In short, the sooner the administration of CBMIDA is initiated after exposure, the more the plutonium can be removed effectively, as well as in the case of DTPA.

Judging from our results on the excretion of plutonium in feces and urine (Figs. 2 and 3), the main excretion route of CBMIDA-plutonium compound was determined in urine throughout kidneys from blood, though the excretion in feces was seen during a few days after plutonium administration, as well as those of Ca-DTPA and Zn-DTPA.

It was noticeable that the plutonium content of bone, a target organ of plutonium, in the CBMIDA group had a tendency to be lower than that of the Ca-DTPA group and decreased significantly as compared to that of the Zn-DTPA group. This could be explained by the results in our previous study: a remarkable increase of

Fig. 2 Plutonium excretions of injected plutonium in the daily urine of each group. Each mark and vertical bar represents the mean $\pm$ SD for five rats.

Fig. 3 Plutonium excretions of injected plutonium in the daily feces of each group. Each mark and vertical bar represents the mean $\pm$ SD for five rats.

osteoid volume on bone surface and the absence or obscure labeling of a bone marker, Calcein, which was labeled at the mineralizing front of trabecular bone, were observed in the histological examination and histomorphometric measurement using undecalcified bone sections of beagle dogs which were injected intravenously with the same dose ($150 \mu\text{mol/kg/day}$) of CBMIDA for 1 month as that used in this study.¹²⁾ In a word, the lower plutonium content of skeleton in the CBMIDA group might be, besides the stronger chelating action, due to the addition of inhibition of plutonium deposition in bone and the subsequent removal of plutonium which was prevented from depositing in bone.

The excretion of plutonium in urine and feces by CBMIDA, however, was not significantly increased as compared to that of Ca-DTPA and Zn-DTPA (Figs. 2 and 3). Our unpublished data obtained by a histomorphometric measurement on the tibial trabecular bone in normal rats of the same strain, sex and age as those used in this study showed that the bone volume (volume of trabecular bone/volume of total bone tissue) was 15%, the mineral apposition rate was about $1.00 \mu\text{m/day}$ and osteoid volume (volume of osteoid/volume of total bone) was only 0.5%. Also, our previous study using beagle dogs indicated that the osteoid volume in the CBMIDA group increased as compared to those of Ca-DTPA and Zn-DTPA

groups, suggesting that CBMIDA can inhibit the plutonium deposition in bone. As described above, the quality of osteoid volume occupied against the bone volume and the accumulated rate of osteoid in a day are very slight. Therefore, the distinct differences of plutonium excretion in the daily urine and feces between CBMIDA and both DTPA groups might be not observed.

In order to apply a chelating agent to the practical use in a chelation therapy, as the essential conditions, firstly it must be able to remove completely or reduce efficiently plutonium from human body till the risk of plutonium-induced cancer disappears. Secondly, it should have no or very lower toxicity as a higher dose could be applied successively in the protracted treatment without the anxiety about toxic side effects. Although a matter for regret is that the toxic side effects of CBMIDA might be not lower than those of Ca-DTPA and Zn-DTPA, our data obtained in this study demonstrated that the effect on removal of plutonium from bone, a target organ of plutonium (besides that from liver, which was equivalent to Ca-DTPA and Zn-DTPA) was higher than those of Ca-DTPA and Zn-DTPA which is, at present, regarded to be the most effective in the chelation therapy for human exposure, showing the possibility to reduce earlier the risk of plutonium-induced osteosarcoma, a final aim for the chelation therapy, as compared to Ca-DTPA and Zn-DTPA. Such beneficial effects of CBMIDA might be confirmed in further study.

V CONCLUSION

It was demonstrated that the effects of CBMIDA on removal of plutonium especially from bone are superior to those of Ca-DTPA and Zn-DTPA which are regarded to be the most effective on removal of plutonium; taking together the results obtained in this study and those on the toxicities obtained in our previous studies, CBMIDA can be practically utilized, as a new chelating agent, for the chelation therapy of plutonium-contaminated persons.

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