ORIGINAL

Toxicological Study of DTPA as a Drug (VI)

Effects of Intravenously Injected Ca-DTPA, Ca-EDTA, CBMIDA
and Orally Administered Zn-DTPA to Bone
Metabolism in Beagle DogsSatoshi FUKUDA,*,*¹ Haruzo IIDA,**,*¹ Yu Yuan HSEIH*² and Wehzhhi CHEN*²

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Effects of four kinds of chelating agents, Ca-DTPA (calcium diethylenetriaminepentaacetic acid), Ca-EDTA (calcium ethylenediaminetetraacetic acid), CBMIDA [catechol-3,6-bis(methyleiminodiacetic acid)] and Zn-DTPA (zinc DTPA), on bone metabolism were examined in beagle dogs by bone histomorphometry and measurement of serum biochemical constituents related to bone metabolism. Ca-DTPA, Ca-EDTA or CBMIDA (150 μ mol/kg) was injected intravenously to dogs for 1 month, respectively. Three doses (30, 150 and 300 μ mol/kg) of Zn-DTPA were administered orally to dogs for 1 month, respectively. All dogs received twice tetracycline hydrochloride injections at an interval of 7 days before the beginning of administration of chelating agents and also twice calcein injections at the same time schedule prior to sacrifices for analyzing bone dynamics. Bone samples were obtained from ilium and undecalcified bone sections were made. Bone histomorphometry of cancellous bone area of ilium was performed using an image analyzer.

Bone volume and mean trabecular thickness did not change in any of the groups. Osteoid volume in the CBMIDA group increased ($p < 0.05$). Osteoid volume and mean osteoid thickness in the 150 μ mol/kg of Zn-DTPA group decreased ($p < 0.05$ and $p < 0.01$). Mineral apposition rate and bone formation rate did not change in any groups except the CBMIDA and 150 μ mol/kg of Zn-DTPA groups, in which fluorescent bone labeling was absent or obscure, revealing inhibition of bone mineralization.

Serum total calcium levels did not change in any of the groups. Serum phosphorus level decreased significantly in the 30 μ mol/kg dose of Zn-DTPA group ($p < 0.05$). Parathyroid hormone level increased in the 30 μ mol/kg dose of Zn-DTPA ($p < 0.05$), while it decreased in the 150 μ mol/kg dose of Zn-DTPA group ($p < 0.05$).

The results suggest that the protracted therapy using the above four kinds of chelating agents may incur damages of bone such as decrease of bone volume and inhibition of mineralization.

KEY WORDS: Zn-DTPA, Ca-DTPA, Ca-EDTA, CBMIDA, bone metabolism

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I INTRODUCTION

We have examined the toxicity of chelating agent, DTPA (diethylenetriaminepentaacetic acid), for decorporating plutonium and other actinides from the body, in order to investigate the method of applying it safely to accidentally radionuclide contaminated persons.¹⁾ The results obtained up to here show that, as systemic damages, the intra-

venously injected Zn-DTPA induces rapid decrease of calcium level in serum and related to functional abnormality of cardiovascular system^{2,3)} and, as local damages, both Ca-DTPA and Zn-DTPA when administered orally or injected intravenously induce damages of small intestine by the action of enhancement of vascular permeability.⁴⁾ From these findings it is doubted that DTPA induced-toxicity is deeply related to calcium metabolism, although no distinct changes of calcium level in serum were detectable except the case of intravenously injected Zn-DTPA.

In general, it is thought that chelation therapy in humans will be protracted in order to incorporate radionuclides as much as possible or until the risk of radionuclide-induced cancer reduces sufficiently. We have previously reported on the toxicities of intravenously injected Ca-DTPA, Ca-EDTA (ethylenediaminetetraacetic acid), CBMIDA [Catechol-3,6-(methyleiminodiacetic acid)]⁵⁾ and orally administered Zn-DTPA⁶⁾ to various organs except bone in beagle dogs. As described the above, it is considered that the protracted chelation therapy may occur some changes to bone caused by abnormality of calcium metabolism. However, the effects of these chelating agents to bone are not well-known.⁷⁾

A dog such as beagle dog is an useful experimental animal to examine toxicity in human bones, because the pattern of bone metabolism in dogs is similar to that in humans.⁸⁾

The present study is performed to examine the effects of Ca-DTPA, Ca-EDTA, CBMIDA and Zn-DTPA to bone metabolism in beagle dogs by bone histomorphometry and measurement of serum biochemical constituents related to bone.

II MATERIALS AND METHODS

1. Animal

Twenty five beagle dogs of both sexes, aged 3 to 8 yr, were used from our beagle colony. These dogs were divided into four groups.

2. Chelating agents

Each of $\text{CaNa}_3\text{-DTPA}$ (Ca-DTPA), Ca-EDTA and $\text{ZnNa}_3\text{-DTPA}$ (Zn-DTPA) was dissolved in the isotonic saline solution and adjusted to pH 7.2. CBMIDA was synthesized at Shanghai Institute of Materia Medica and suspended in distilled water and then added Sodium Bicarbonate (NaHCO_3) to adjust to pH 5–6 just before admin-

istration.

Ca-DTPA, Ca-EDTA or CBMIDA ($150 \mu\text{mol/kg}$) was injected intravenously once daily to each three dog for 1 month. Three doses of Zn-DTPA were administered orally once daily to 8 dogs ($30 \mu\text{mol/kg}$), 3 dogs ($150 \mu\text{mol/kg}$) and 5 dogs ($300 \mu\text{mol/kg}$) for 1 month, respectively.

3. Bone histomorphometry

In order to examine the effects of chelating agents on bone metabolism by bone histomorphometry, the following labeling with bone markers (tetracycline and calcein) was performed. Before the administration of chelating agents, all dogs received twice tetracycline hydrochloride (25 mg/kg) injections at an interval of 7 days. Then the right iliac bone was biopsied at the site of 1.5 cm below the anterior iliac spine with a trephine (8 mm internal diameter) 4 days after the second tetracycline injection. Also, prior to sacrifice, they were received twice calcein (8 mg/kg) injections at 18th and 26th day after the beginning of chelating agents administration. All dogs were sacrificed 4 days after the second calcein injection. Thereafter, the left iliac bone at the same position as the right biopsied one was obtained.

The undecalcified bone was stained in Villanueva's bone stain,⁹⁾ dehydrated in ethanol and acetone, and embedded in methylmethacrylate. The block was cut thin with a inner-blade cutter (Mauto Co., Ltd.). Thereafter, fifteen μm thickness section was obtained by grinding with glass plates (Asahi Glass Co., Ltd.). Histomorphometric measurement in the cancellous bone area of ileum was performed by a semiautomatic image analyzer (Kontron 64, Carl Zeiss Co.) using "Osteoplan" software. Histomorphometric parameters measured were shown in Table 1.

4. Serum biochemistry

Blood was withdrawn from the anterior vena brachiocephalica just before beginning of administration of chelating agents and autopsy. Blood was clotted and centrifuged for 15 min at 3,000 r.p.m. Serum sample was separated and frozen at -20°C and then stored for later analyses. Serum total calcium concentration was measured by *o*-Cresolphthalein complexone method. Serum phosphorus concentration was by molybdenum blue method. Serum parathyroid hormone level was by the radioimmunoassay method using a chicken anti-C-terminal human parathyroid hor-

Table 1 Bone histomorphometric parameters.

Parameter and abbreviation	Formula	Unit
Bone volume	Volume of trabecular bone/volume of total bone tissue	%
Mean trabecular thickness	Total trabecular surface/volume of total bone	μm
Osteoid volume	Volume of osteoid/volume of total bone	%
Mean osteoid thickness	Total osteoid volume/total formation surface	μm
Mineral apposition rate	Mean distance of tetracycline or calcein labels/labeling intervals (8 days)	$\mu\text{m/day}$
Bone formation rate	Mean label length $\times$ apposition rate/mean trabecular area	%/yr

mone.

III RESULTS

In the results of bone histomorphometry (Figs. 1–6), bone volume and mean trabecular thickness did not change significantly in all groups. Significant increases of osteoid volume ($p < 0.05$) in the CBMIDA group and of both osteoid volume ($p < 0.05$) and mean osteoid thickness ($p < 0.01$) in the 150 $\mu\text{mol/kg}$ of Zn-DTPA were seen. Mineral apposition rate and bone formation rate did not change significantly in all groups. Histological findings of trabecular bones of ilium in each group under fluorescence microscopy were represented in Figs. 7–14.

In the serum biochemical analyses (Table 2), total calcium levels in serum did not change in all groups. Serum phosphorus level decreased significantly in the 30 $\mu\text{mol/kg}$ dose of Zn-DTPA group ($p < 0.05$). Parathyroid hormone level increased significantly in the 30 $\mu\text{mol/kg}$ dose of Zn-DTPA group ($p < 0.05$), while decreased significantly in the 150 $\mu\text{mol/kg}$ dose of Zn-DTPA group ($p < 0.05$).

IV DISCUSSION

Bone metabolism is producing by the osteoclast resorbing old bones and osteoclast forming new bone. In the normal state, as the activities of osteoclast and osteoblast are normally balanced, bone histomorphological changes do not occur, also levels of serum constituents related to bone are always controlled within the normal ranges.

In the Ca-DTPA group, no significant changes of serum biochemical and bone histomorphometric values were seen. However, the bone histomorphometric data show the tendency that

Fig. 1 Bone volume in the cancellous bone area of ilium in each group.

Fig. 2 Mean trabecular thickness in the cancellous bone area of ilium in each group.

Fig. 3 Osteoid volume in the cancellous bone area of ilium in each group. *Significantly different from the pre values ($p < 0.05$).

Fig. 4 Mean osteoid thickness in the cancellous bone area of ilium in each group. **Significantly different from the pre value ($p < 0.01$).

Fig. 5 Mineral apposition rate in the cancellous bone area of ilium in each group. The value is not shown because the bone labeling line of calcein is absent or obscure in all samples of CBMIDA group of one sample in the 150 μmol/kg of Zn-DTPA group.

Fig. 6 Bone formation rate in the cancellous bone area of ilium in each group. The value is of one sample in the 150 μmol/kg of Zn-DTPA group.

bone volume, mean trabecular thickness and bone formation rate decrease while osteoid volume and mineral apposition rate increase. These may appear that the bone metabolism become high turnover state which bone resorption is at advantage over bone formation and calcium is released from bone, or that the calcium deposition in osteoid is inhibited. Because there is some doubt that $\text{CaNa}_3\text{-DTPA}$ can compound with Ca ion in bones or serum ($\text{CaNa}_3\text{-DTPA} + \text{Ca} \longleftrightarrow$

$\text{Ca}_2\text{Na-DTPA}$).

In the Ca-EDTA group, all data showed no significant changes. But there are tendencies to decrease bone volume while to increase mean trabecular thickness, osteoid volume and mean osteoid thickness. These may suggest that Ca-

Table 2 Measured values of serum biochemical constituents.

Group	Total calcium (mg/dl)		Phosphorus (mg/dl)		Parathyroid hormone (ng/ml)	
	Pre	Post	Pre	Post	Pre	Post
Ca-DTPA (150 μ mol/kg)	10.4 $\pm$ 0.4	11.7 $\pm$ 1.0	2.9 $\pm$ 0.7	3.2 $\pm$ 0.8	0.67 $\pm$ 0.31	1.03 $\pm$ 0.15
Ca-EDTA (150 μ mol/kg)	11.6 $\pm$ 1.6	11.6 $\pm$ 2.8	2.7 $\pm$ 0.7	3.2 $\pm$ 0.5	1.17 $\pm$ 0.81	1.05 $\pm$ 0.35
CBMIDA (150 μ mol/kg)	10.4 $\pm$ 0.4	10.4 $\pm$ 0.4	3.3 $\pm$ 0.8	3.8 $\pm$ 0.4	1.33 $\pm$ 0.06	1.43 $\pm$ 0.15
Zn-DTPA (30 μ mol/kg)	10.8 $\pm$ 1.1	11.8 $\pm$ 1.2	4.1 $\pm$ 0.4	3.4 $\pm$ 0.7*	0.57 $\pm$ 0.13	0.86 $\pm$ 0.20*
(150 μ mol/kg)	12.0 $\pm$ 1.2	11.1 $\pm$ 1.2	6.2 $\pm$ 0.9	5.7 $\pm$ 0.5	0.90 $\pm$ 0	0.67 $\pm$ 0.12*
(300 μ mol/kg)	10.3 $\pm$ 0.3	9.9 $\pm$ 0.3	3.2 $\pm$ 0.4	3.7 $\pm$ 0.3	1.28 $\pm$ 0.99	1.10 $\pm$ 0.47

* Significantly different from the pre values ($p < 0.05$)

EDTA inhibits mineralization in bones. As one of the reasons, it is considered that Ca-EDTA promotes to excrete calcium in urine.¹⁰⁾

In the CBMIDA group, the remarkable osteoid accumulation on bone surface due to the increase of osteoid volume and mean osteoid thickness, and the absent or obscure lines of calcein labeling in the histological observation was noted (Figs. 3, 4 and 11). On the other hand, levels of all serum constituents did not change. These findings show that CBMIDA may inhibit directly and strongly calcium deposition to mineralizing surface of bone. Conversely speaking, this result suggests the possibility that CBMIDA can prevent deposition of plutonium in bone. Therefore, it is expectable that CBMIDA will be adopted as a drug to reduce the risk of plutonium.

In the 30 μ mol/kg of Zn-DTPA group, serum phosphorus value decreased significantly and parathyroid hormone level increased significantly. Serum calcium level trended to increase. Though bone histomorphometric values did not significantly change, bone volume, mean trabecular thickness, osteoid volume, mean osteoid thickness and mineral apposition rate trended to decrease. These suggest that bone metabolism is in the state of low turnover which bone formation falls while bone resorption is accelerated by increase of parathyroid hormone action, though bone formation rate has a tendency to increase. Finally, it is considered that the bone volume decreases when this dose of Zn-DTPA extends over a long period of time.

In the 150 μ mol/kg dose of Zn-DTPA group, parathyroid hormone level decreased significantly and also both serum calcium and phosphorus

levels trended to decrease. Mean osteoid thickness decreased significantly, though other parameters in bone histomorphometry did not change. These may appear that mineralization in bone inhibits slightly.

In the 300 μ mol/kg dose of Zn-DTPA group, levels of all serum constituents and bone morphometric values did not change significantly. However, the increase of bone volume and mean trabecular thickness due to osteoid volume and mean osteoid thickness were noted. In histological observation, the wide osteoid thickness is ascertained (Fig. 14). It is considered that high dose of Zn-DTPA may occur inhibition of bone mineralization.

In short, the results in Zn-DTPA groups show that the 30 μ mol/kg dose induce the decrease of bone volume by low turnover, and the doses of more than 150 μ mol/kg induce inhibition of mineralization and accumulate osteoid, in any case some damages of bone after Zn-DTPA oral administration may occur.

It is said that a daily recommended human dose of DTPA is 30 μ mol/kg.¹¹⁾ In the results of present study, it is suggested that the 30 μ mol/kg dose of DTPA may occur some damages of bone due to inconspicuous changes of calcium metabolism in humans. However, as it is thought that this is the maximum dose to induce almost no side effects from our own judgements based on the results of our previous studies, probably a practical daily dose for humans will be further low. Therefore a small amount of four kinds of chelating agents may not occur severe damages of bones in a short period of time. However, the data send out a warning that some abnormalities

in bone metabolism will occur, when the administration of these chelating agents is protracted.

V CONCLUSION

The results obtained in this study suggest that short-term administration of chelating agents such as Ca-DTPA, Ca-EDTA, CBMIDA and Zn-DTPA will induce almost no abnormal bone metabolisms, however the protracted therapy using these agents may occur some damages of bone.

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Fig. 7 Fluorescence micrograph of undecalcified trabecular bone of ilium obtained by the biopsy before administration of chelating agents. MB: mineralized trabecular bone, BM: bone marrow, AS: active surface on trabecular bone labeled with lines of tetracycline. Villanueva bone stain, $\times 100$.

Fig. 8 High power fluorescence micrograph of undecalcified trabecular bone before administration of chelating agents. The double light bands (arrows) are labels of tetracycline hydrochloride when they appear under fluorescence microscopy. T_1 : first tetracycline labeled line on active surface which a new bone is forming, T_2 : second tetracycline labeled line, OS: osteoid. Villanueva bone stain, $\times 200$.

Fig. 9 Fluorescence micrograph showing the trabecular bone in a dog of Ca-DTPA intravenous injection group. Wide resorbing surface (RS) is seen. Villanueva bone stain, $\times 100$.

Fig. 10 Fluorescence micrograph showing the trabecular bone in a dog of Ca-EDTA intravenous injection group. A slight wide osteoid (OS) is seen. Villanueva bone stain, $\times 100$.

Fig. 11 Fluorescence micrograph showing the trabecular bone in a dog of CBMIDA intravenous injection group. Remarkable wide osteoid (OS) accumulated on mineralized bone surface is noted. Line of calcein is absent. Villanueva bone stain, $\times 100$.

Fig. 12 Micrograph under fluorescence microscopy showing the trabecular bone in a dog of Zn-DTPA (30 $\mu\text{mol/kg}$ dose) oral administration group. A narrowing of trabecular bone width and osteoid (OS) accumulation are seen. Villanueva bone stain, $\times 100$.

Fig. 13 Micrograph under fluorescence microscopy showing the trabecular bone in a dog of Zn-DTPA (150 $\mu\text{mol/kg}$ dose) oral administration group. A narrowing of trabecular bone width is seen. Villanueva bone stain, $\times 100$.

Fig. 14 Micrograph under fluorescence microscopy showing the trabecular bone in a dog of Zn-DTPA (300 $\mu\text{mol/kg}$ dose) oral administration group. A wide osteoid (OS) accumulated on mineralized bone surface is seen. Villanueva bone stain, $\times 100$.