Possibility of Alveolar Bone Promoting Enhancement by Using Lipophilic and/or Hydrophilic Zinc Related Compounds in Zinc-Deficient Osteoporosis Rats

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The purpose of this research is improvement of therapeutic treatment for periodontitis by using lipophilic and/or hydrophilic zinc materials. The sample suspension injections were prepared from zinc octanate (C8Zn), zinc stearate (C18Zn), zinc sulfate hepta-hydrate (ZnSO₄) and tricalcium phosphate (ZnTCP) containing 6.17w/w% zinc. After administrating of all injections to around alveolar bone of zinc-deficient osteoporosis rats, plasma Zn concentration, bone mineral content (BMC) of jawbone, BMC and bone mechanical strength (BMS) of femur and permeability tests for hairless rat stripped skin were measured as therapeutic scores. BMC and BMS were measured by using an X-ray computing tomography and the three-point bending method, respectively. The body weight, plasma Zn concentrations and the area under curve (AUC) for Zn of C8Zn, C18Zn and ZnTCP group rats were higher than those of control group, but those of ZnSO₄ group were not changed. BMC of alveolar bone and femur and BMS of femur for C8Zn and C18Zn groups for 12 weeks were significantly higher than those of the control group, but those of ZnSO₄ group were not changed. Stripped rat skin permeability treated by the hydrophilic creams containing C8Zn was 5-times higher than that of ZnTCP.

Key words suspension injection; zinc octanate; zinc stearate; alveolar bone mineral content; bone mechanical strength; plasma zinc concentration

Periodontal disease begins with inflammation cause of bacteria buildup surrounding teeth. When it is deteriorated, the toxin from bacteria stimulates abnormally the osteoclast which plays a role in bone resorption, the alveolar bone supporting teeth begins to dissolve, and teeth may be dropped. Thus, as the lifestyle habit and the relation of many diseases become clearer, the concepts of primary prophylaxis is becoming more important that we need to establish healthy lifestyle and prevents onset of periodontal disease. Additionally, it becomes increasingly clear that treatment of periodontal disease is not only to treat periodontium but also to improve and prevent of onset and progression of systemic disease.

The most important clinical point for periodontal diseases is resorption of the alveolus bone. The mechanism for destruction of the same bone osteoclast participates between alveolar bone retraction in periodontal disease and bone destruction in osteoporosis.^{1,2)} Recently, the treatment for the alveolus bone loss due to periodontal disease is the most necessary treatment. In general, it is thought that plaque microorganism and product material are the leading causes of periodontosis. However, the medical treatment by the antibiotic and an anti-inflammatory agent is only to inhibit partially the progression of periodontitis. Therefore, a treatment by antibiotics, astringent, and gargle is not effective over a long period of time. About bone density, it is known that there are close relations between the bone density of whole teeth, particularly femoral bone density and periodontal condition.

Zinc (Zn), an essential element and a cofactor for more than 200 enzymes, is present in nearly every type of cell in the body. Zn is important for cell proliferation, immunity, spermatogenesis, wound healing, and maintaining the senses of taste

and smell. It also has a very important role in the formation of bone. In fact, the concentration of Zn in bone is higher than that in any other tissue. Zn promotes protein synthesis, and the proliferation and differentiation of osteoblasts. However, as Zn is an important essential metal with very strong bioactivity, and has serious side effects at high concentrations in blood,³⁾ controlling its release is vital for therapeutic use. Recently, a clinical relationship between osteoporosis and Zn deficiency has been established in elderly subjects.^{4,5)} In addition, the utility of the Zn as the essential trace metal has been reviewed.⁶⁾ Bone-promoting effects of Zn-containing β -tricalcium phosphate ceramics have been reported.⁷⁾ Stimulatory effects on osteogenesis of injections of Zn-containing calcium phosphate suspension were also reported.⁸⁾ Bone was stimulated to form around Zn-containing calcium phosphate ceramics implanted in rabbit femora.⁹⁾ Using an animal model of osteoporosis, it was found that the injection of Zn-containing tricalcium phosphate (ZnTCP) was effective in recovering bone mineral density.¹⁰⁾ Particularly, the Zn-containing β -tricalcium phosphate nano particles were administered by injection around alveolar bone and effective at preventing bone loss induced by ovariectomy in rats and have potential uses for treating periodontitis.¹¹⁾ We also have developed the validity of ZnTCP and other zinc related compounds in order to control Zn-release for improvement of various pharmacological effectiveness of Zn using different dosage forms. However, the Zn could not be absorbed well from skin and oral mucosa, because Zn containing calcium phosphates are hydrophilic compounds. When the compounds are applied to improvement of alveolar bone mineral density, an injection or small surgical operation is required. However, the pain by the operations is difficult for the patients. Therefore, the patient friendly Zn administration such as a transdermal therapeutically system is required from the viewpoint of patient quality of life (QOL) as a final purpose. So, as a first step, it is inspected whether

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the candidate lipophilic Zn compounds are effective for new periodontitis treatment. In the present study, new Zn containing materials, such as Zn octanate, stearate and Zn sulfonate 7 hydrate, are used as candidate compounds for therapeutic treating periodontitis. Suspension injections of the compounds were administered for around alveolar bone of Zn-deficient osteoporosis rats, and measured plasma Zn levels, after then, evaluated bone mineral content of alveolar bone, bone mineral density of femur, bone mechanical strength of femur, as therapeutically score. Furthermore, in order to improve the QOL of the patients during therapeutic treatment, we considered that their dosage forms of candidate compounds changed to oral ointment to avoid injection into mouth. Permeability tests of candidate lipophilic Zn related compounds for hairless rat stripped skin were, therefore, performed as basic information to develop orally administrative ointments.

MATERIALS AND METHODS

Materials ZnTCP containing 10mol% zinc (6.17w/w% zinc) $[\text{Ca}_{2.7}\text{Zn}_{0.3}(\text{PO}_4)_2]$ was prepared as described elsewhere.^{12,13} Zinc sulfonate 7 hydrate was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Zinc octanate $[(\text{C}_7\text{COO})_2\text{Zn}, \text{C8Zn}]$ and zinc stearate $[(\text{C}_{17}\text{COO})_2\text{Zn}, \text{C18Zn}]$ was purchase from Mitsuwa Chemicals Co., Ltd. (Osaka, Japan) and Wako Pure Chemical Industries, Ltd., respectively. Corn oil, purchased from Wako Pure Chemical Industries, Ltd., and sodium pentobarbital, from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). Zn-test Wako was obtained from Wako Pure Chemical Industries, Ltd. Other reagents were of analytical grade and used without further purification.

Animal Experiments Four-week old female Sprague-Dawley (SD) rats (average body weight: 65–75 g) purchased from Japan Clea Inc. (Tokyo, Japan) were used. The rats were maintained in a light- (12h light/dark) and temperature- ($25 \pm 2^\circ\text{C}$) controlled barrier facility throughout the study. All animal experiments and maintenance were performed under conditions approved by the animal research committee of Musashino University. The rats were fed a diet deficient in vitamin D, Ca, and Zn (–DCaZn) for 1 week, and Zn-deficient rats with osteoporosis were prepared following the modified protocol reported by Yamaguchi *et al.* and Suda *et al.*^{14,15} The rats were randomly assigned to five groups (six rats per group; control, and ZnTCP and ZnSO_4 , C8Zn and C18Zn), and bilaterally ovariectomized 5 weeks before the start of injections (age at OVX, five weeks), and fed a normal diet for 1 week and the –DCaZn diet for 4 weeks. On commencement of the experiments, the groups of control, and ZnTCP and ZnSO_4 , C8Zn and C18Zn were intramuscularly injected suspension containing no-admiration, ZnSO_4 , ZnTCP, C8Zn and C18Zn (0.6mg as zinc) in 0.2mL of corn oil on around the alveolar bone once a week for 12 weeks, respectively. The group of normal (six rats) did not under go an ovariectomy and received a normal diet throughout the experimental period. After blood samples were obtained at 0, 2, 4, 8, 24, 48 and 72h from the tail artery of the rats, the samples were immediately centrifuged at $4660 \times g$, 5 min (CR13R, Hitachi Koki Co., Ltd., Tokyo, Japan) and a each plasma sample was stored at -30°C .

Measurement of Zn Concentrations in Plasma Samples The concentration of Zn in plasma was determined based

on the 5-Br-PAPS method, which uses the reagent 2-(5-bromo-2-pyridylazo)-5-(*N*-*n*-propyl-*N*-3-sulfopropylamino)phenol (Zn-test Wako, Wako Pure Chemical Industries, Ltd., Tokyo, Japan).¹⁶ The measurement was made using a UV/VIS spectrometer (Type UV4200, Shimadzu, Kyoto, Japan) at 560nm. The data represent the averages of 4 measurements each. Area under the concentration vs. time curve for 72h (AUC_{0-72}) calculated using the trapezium method.

Diets for Rats A diet deficient in vitamin D and Zn (–DZn) was used. The –DCaZn diet and normal diet were prepared and supplied by Clea Co., Ltd. (Tokyo, Japan). The –DZn diet was prepared by supplementing the –DCaZn diet with calcium carbonate at 1.5%. The composition of all the diets was given in a previous paper.¹⁷

X-Ray Computed Tomography (CT) Analysis The rats were anesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbital (50mg/kg) before each treatment. Bone mineral content (BMC) was also measured using an X-ray CT for animals scanner (LCT-100A, ALOKA, Tokyo, Japan) once a week under anesthesia (conditions of X-ray CT; pixel size; 480×480 , fan beam; tube voltage; 50kV, tube current; 1 mA, resolution; 0.25 mm).

Bone Mechanical Strength (BMS) After 12 weeks, all the rats were sacrificed with an overdose of sodium pentobarbital. The three-point bending strength of excised right and left femurs was measured as described with a bending speed of 0.164mm/s using a type 50kN (Minebea, Kanagawa, Japan).¹⁸ Maximum bending force (N) was used as an index of bone mechanical strength (BMS).

Skin Permeation Study Abdominal full-thickness skin of hairless rats (HWY stain, 7-weeks-old, male) was excised under anesthesia by i.p. injection of sodium pentobarbital (50mg/kg). Excised skin and stratum corneum stripped skin which excess subcutaneous fat was carefully eliminated was mounted in a modified Franz-type diffusion cell having an effective diffusion area of 1.13cm^2 . To obtain stratum corneum (SC)-stripped skin, adhesive tape was applied to hairless mouse skin with uniform pressure and then removed. This procedure was repeated 20 times. The receiver solution was 6.0mL of acetate-buffered solution, pH 4.0, which was maintained at 32°C . The donor compartment contained Zn-TCP, ZnSO_4 , C8Zn and C18Zn in acetate buffer as $3000\mu\text{g}$ zinc. The skin permeation of zinc concentration was determined in all samples by Inductively Coupled Plasma-Atomic Emission Spectrometer, ICPS-7500 (Shimadzu, Kyoto, Japan).

Statistical Analysis All results are expressed as the mean $\pm$ S.D. for 3 to 6 experiments. The statistical analysis was conducted using Tukey's multiple statistical tests (SAS ver. 9.2).

RESULTS

Changes in Body Weight after the Zn-Containing Compounds Suspension Injections Figure 1 shows changes in body weight of the rats, after injections of suspension of ZnTCP, C8Zn and, C18Zn. After injections containing ZnTCP, C8Zn and, C18Zn, body weights of the rats increased significantly as the same as that of the healthy rats, and were around 200% after 12 weeks, but no increase was observed in the rats which received corn oil (control) and ZnSO_4 injections.

Effects of Zn-Related Compound Suspension Injections

Fig. 1. Changes in Body Weight after Injections around the Alveolar Bone in Rats

The symbols represent the average and standard deviation ($n=4-6$), respectively. Symbols: Normal rat (●), Control (Osteoporosis rat); (○), ZnSO₄ (◇), C8Zn (■), C18Zn (▲), ZnTCP (◆).

on Plasma Zn and AUC of Zn Levels Figure 2A shows plasma Zn level profiles after the first injection of ZnSO₄, ZnTCP, C8Zn and C18Zn suspensions. Plasma Zn levels of normal healthy group rats had almost the constant values at 1.0–1.5 $\mu\text{g/mL}$ for over 72 h period, but those of diseased control had constant lower values at around 0.5 $\mu\text{g/mL}$. In contrast, C8Zn, ZnTCP and C18 Zn-injected group had a maximum concentration, 2.68, 1.49 and 1.27 $\mu\text{g/mL}$ at 2 h, and after then it decreased, respectively (Table 1). The order of maximum plasma levels was C8Zn>ZnTCP>C18Zn>ZnSO₄. Plasma Zn levels of ZnSO₄-injected group did not increase as the same as that of the control group.

Figure 2B shows the area under the Zn concentration vs. time curve for 72 h (AUC) calculated using the trapezoid method. The healthy rats group and C8Zn group had the highest, AUC values, followed by the C18Zn and ZnTCP-injected group. The ZnSO₄-treated and untreated groups had very similar lower AUC values.

Bone Mineral Content of Alveolar Bones Figure 3 shows the increase ratio of bone mineral content (BMC) of the alveolar bone. The BMC after the administration of corn oil (with and without OVX), ZnSO₄, C8Zn, C18Zn and ZnTCP were 166.2, 75.8, 112.7, 166.9, 162.0, and 133.8, respectively. There were significant differences between the C8Zn, C18Zn and ZnTCP-administered groups with the control (OVX+corn oil) group, respectively. There was no difference between the ZnSO₄-administered group and the controls.

Bone mineral content (BMC) of right femur and bone mechanical strength (BMS) of right femur as indexes of

Fig. 2. Plasma Zn Levels (a) and AUC of Zn (b) after Injections around the Alveolar Bone

The symbols and bars represent the average and standard deviation ($n=4-6$), respectively. Symbols: Normal rat (●), Control (osteoporosis rat); (○), ZnSO₄ (◇), C8Zn (■), C18Zn (▲), ZnTCP (◆). ** $p<0.01$, compared to the control group (Tukey's multiple comparison tests).

therapeutic scores of osteoporosis rats.

Figure 4A shows the BMC of right femur at the experiment. BMC in the control group (OVX, corn oil) was 19.1 mg, and those in the groups administered ZnSO₄, C8Zn, C18Zn and ZnTCP were 14.8, 35.8, 40.8, and 44.0 mg, respectively. There was a significant difference between the control group and the C8Zn, C18Zn and ZnTCP-administered, but not ZnSO₄-administered group.

Figure 4B shows the effect of Zn-containing compounds injections on the BMS of the right and left femurs. BMS in the control group (OVX, corn oil) was 80.9 N, but those in the

Table 1. Plasma Zinc Concentration ($\mu\text{g/mL}$) after Injections of Zinc Compounds around the Alveolar Bone

	0h	2h	6h	10h	24h	48h	72h
Normal	1.04	1.04	1.19	1.41	1.10	1.01	1.11
Control	0.43	0.58	0.50	0.63	0.27	0.39	0.27
ZnSO ₄	0.29	0.27	0.41	0.62	0.43	0.37	0.38
C8Zn	0.52	2.68** ^{##}	1.83** ^{##}	1.68** ^{##}	1.28** ^{##}	0.74** ^{##}	0.49
C18Zn	0.45	1.27	1.00* ^{##}	1.19	1.15** ^{##}	0.66* [#]	0.55
ZnTCP	0.36	1.49	0.81	0.75	0.54	0.38	0.38

* $p<0.05$, ** $p<0.01$, compared to the control group, [#] $p<0.05$, ^{##} $p<0.01$, compared to the ZnSO₄ (hydrophilic zinc compound) group (Tukey's multiple comparison tests).

Fig. 3. Effect of Injection of Zn Related Materials on BMC of Alveolar Bone

The bars represent the average and standard deviation ($n=4-6$), respectively. ** $p<0.01$, compared to the control group (Tukey's multiple comparison tests).

Fig. 4. Effect of Injection of Zn Related Materials on BMC (a) and BMS of Femur (b)

The bars represent the average and standard deviation ($n=4-6$), respectively. Symbols: Normal rat (●), Control (osteoporosis rat; ○), ZnSO₄ (◇), C8Zn (■), C18Zn (▲), ZnTCP (◆). ** $p<0.01$, compared to the control group (Tukey's multiple comparison tests).

groups administered ZnSO₄, C8Zn, C18Zn and ZnTCP were 98.8, 130.8, 136.9, and 157.0N, respectively. The ZnTCP-administered group was had a significantly higher value than the control group, but the ZnSO₄-administered group did not. The

Fig. 5. Relationship between BMC and BMS of Femur (A), BMS of Femur and BMC of Alveolar Bone (B) in All Groups

The bars represent the average and standard deviation ($n=4-6$), respectively. Symbols: Normal rat (●), Control (osteoporosis rat; ○), ZnSO₄ (◇), C8Zn (■), C18Zn (▲), ZnTCP (◆).

relationship between the BMS and BMC of the right femur (Fig. 5A) and alveolar bone (Fig. 5B) in all groups. This result indicated that increase ratio of BMC was increased by the injection of Zn-containing compounds in corn oil, and BMS increased with BMC. Since all the samples were injected in around alveolar bone, and the BMC and BMS of the right femur increased, the effect appears to be greater on bone closer to the injection site.

Stripped Skin Permeation of Zinc by Zinc-Containing Compounds The stripped skin permeation of Zn in hydrophilic ointment was shown in Fig. 6. Because the influence of the injection was higher than other compounds, the only C8Zn and ZnTCP were examined. The cumulative amount of Zn from its hydrophilic ointment of C8Zn and ZnTCP was 15.06 and 3.91%, respectively. Stripped skin permeation of C8Zn was significant higher than that of ZnTCP ($p<0.01$).

DISCUSSION

Effects of Zn Related Material Suspension Injections on Plasma Zn Levels of the Zn Deficient Rat The plasma Zn levels of C8Zn, C18Zn and ZnTCP group rats increased after administrations of Zn related material suspension injections (Fig. 2A), and after 2h, their levels decreased with elapse of the time, respectively. In contrast, in ZnSO₄ and control groups, their plasma Zn levels were not changed in all experimental period. In general, plasma Zn levels in the rats were maintained to keep at homeostatic constant values by supporting from endogenous and exogenous Zn resources. Plasma Zn levels of the normal rat group were kept at a constant value by mainly Zn supplying from the daily foods, but the levels of the diseased control rats were supposing from limited endogenic resource caused by dissolution of bone matrices under current animal experimental condition (Zn deficiency).¹⁹⁾ Plasma Zn levels of the other group rats (C8Zn, C18Zn, ZnTCP and ZnSO₄) were supported from both of external Zn administrations and endogenous Zn release (Fig. 2A).

Plasma Zn levels of ZnSO₄ group rats did not increase after the administration, since it may be that ZnSO₄ was water-soluble material, and so, those dissolved, metabolized and eliminated very fast reflecting their solubility in a body fluid, before reaching an equilibrium Zn concentration in plasma. In contrast, C8Zn, ZnTCP and C18Zn groups had higher plasma Zn profiles, those had significantly different pharmacokinetics properties in a body, since all of the materials had slower dissolution rate in a body fluid.

AUC of C8Zn, C18Zn and ZnTCP administered group (Fig. 2B) were significant increased compare with that of the control group, reflecting of their plasma Zn profiles. C8Zn, C18Zn and ZnTCP have lower solubility against to water, however, their *in-vivo* dissolution rate might be appropriate increasing of plasma Zn level in a blood circulation system. In contrast, since ZnSO₄ has high water solubility, it might be too quickly eliminated after the injection. Therefore, plasma Zn level did not increase in plasma in a blood circulation system. So, *AUC* of ZnSO₄ administered group did not increase as compared with that of the control group.

Since plasma Zn level was depended on a balance of Zn release and elimination rates, solubility of their materials affected on their profiles. These results suggested that the Zn pharmacokinetics of the lipophilic zinc compounds (C8Zn and C18Zn) were remarkably different from ZnSO₄ (water-soluble zinc compound) and ZnTCP (water-insolubility).

Therapeutic Effects of C8Zn and C18Zn Injections on BMC and BMS of Alveolar Bone in the Zn Deficient Rat Suspension injections containing hydrophilic and/or lipophilic Zn related compounds were administered for Zn-deficient rats around alveolar bone at once a week for 12 weeks. After the administrations, BMC of alveolar bone (Fig. 3), BMC and BMS of femur bone (Fig. 4) were measured and evaluated as local and systemic therapeutic effects. The body weight of C8Zn, C18Zn and ZnTCP group rats (Fig. 1) increased continuously and reached the same level as that of the normal group after 12 weeks. In contrast, the body weights of ZnSO₄ and the control groups were unchanged. Since Zn promotes protein synthesis, and the proliferation and differentiation of all types of body cells,³⁾ it is well known that Zn induced to increasing of body weight. Current result suggested that the

pharmacological effects of C8Zn and C18Zn were almost the equal to that of ZnTCP, respectively.

Next, change of BMC of alveolar bones was investigated (Fig. 3). Before injection, BMC of alveolar bone was not change in Zn-deficient bilaterally ovariectomized rats. The systemic effects of Zn-compounds injection were further supported by increases in the BMC and BMS (Figs. 3, 4). The BMC and BMS of the ZnSO₄ administrated group were almost comparable as those of the control group. On the other hand, BMC and BMS of C8Zn and C18Zn for 12 weeks were significantly ($p<0.01$) higher than those of the control group, respectively. Their increasings in BMC and BMS of femur were considered as a systemic whole body effect, since the C8Zn and C18Zn suspensions were injected in far local sites around nearby alveolar bones. In addition, it was revealed that relationship between BMC and BMS of femur by applications of the Zn related compounds (Fig. 5A). Furthermore, the BMS of femur and BMC of alveolar bone were correlated (Fig. 5B). Since it has been observed that there is a correlation between the BMS of femur and a BMC of alveolar bone, it is presumed that in alveolar bones with increasing BMC, strength also increases.

In these results, ZnSO₄ administered group, all of the therapeutic scores, such as the plasma Zn concentration, *AUC* of Zn, BMC of alveolar bones, body weight and BMC and BMS of femurs are lower as the same as those of the control. It is considered that administration of ZnSO₄ solution had lower therapeutic effect for improvement of bone remodeling, since the compound had fast *in-vivo* distribution, metabolism and elimination rate, reflecting high water solubility. In contrast, since C8Zn, C18Zn, and ZnTCP have relatively high lipid solubility, the plasma Zn concentration, *AUC* of Zn, body weight, the BMC and BMS of femurs and BMC of alveolar bone significantly increased, respectively. With all these results, applying comparatively high lipid solubility compound may lead to remain in tissues and could sustain to relief Zn and show high therapeutic effect. As for the pharmacologic effect on the alveolar bone promoting enhancement, lipophilic compounds of zinc (C8Zn and C18Zn) and ZnTCP are more than it in equivalence.

It is assumed that Zn release patterns from the dosage forms and their amount of the doses of Zn appear to play a role in efficacy deeply, therefore, further study will be needed.

Therapeutic Opportunity of C8Zn and C18Zn to Apply *trans*-Mucosal Delivery System for Improvement of BMC and BMS of Alveolar Bone in the Zn Deficient Rat On the other hand, considering improvement in the patient QOL, it is thought that an application by the intraoral ointment and toothpaste is better than an injection. Furthermore, when clinical use of these compounds, compounds were required consideration of membrane permeability. In this section, stratum corneum stripped rat skin was used as mimic mucosal membrane. Cutaneous permeability of stripped rat skin and porcine oral mucosal membrane permeability were similar (data not shown). As the administration of C8Zn and ZnTCP suspension injections had high therapeutically effective results on improvement of alveolar bones mineral content in the above section, the both materials were used as the test bulk materials to prepare for hydrophilic cream. The creams were tested hairless rat percutaneous absorption drug delivery. Stripped rat skin permeability of C8Zn group was shown higher than

Fig. 6. Cumulative Amount of Zn after Hydrophilic Cream Containing Zn Related Materials Applied to Stripped Hairless Rat Skin

ZnTCP (▲), C8Zn (■). The bars represent the average and standard deviation ($n=4-6$), respectively. ** $p<0.01$ (Tukey's multiple comparison tests).

5-times compared with ZnTCP (Fig. 6). ZnTCP is a compound based on tricalcium phosphate, and distribution of the skin may be comparatively low compared with C8Zn with high lipophilic compounds. Moreover, ZnTCP is relatively stable in neutral solution and Zn releases from ZnTCP in acidic region, the difference by release with pH could be affected stripped rat skin permeability.

In these results, the plasma Zn concentration, AUC of Zn, BMC of alveolar bone, BMC and BMS of femur, and cutaneous permeability. Since C8Zn had comparatively high lipid solubility, it was a compound with high utility as candidate of the bulk drug for formulation of cream preparations. In further study, we will apply the formulation containing the compound to the membrane of a model rat, and determine therapeutic effects, such as bone quantity changes.

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