Effect of Zinc-Containing \(\beta\)-Tricalcium Phosphate Nano Particles Injection on Jawbone Mineral Density and Mechanical Strength of Osteoporosis Model Rats

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Zinc-containing \(\beta\)-tricalcium phosphate (ZnTCP) nano particles were injected into zinc-deficient rats to promote osteogenesis. Sprague-Dawley (SD) rats (4 weeks old, average weight of 70 g) were divided into 4 groups: Normal rats (not ovariectomized (OVX)), Control rats (OVX), and OVX rats injected with a suspension of ZnTCP nano particles or ZnSO\(_4\). The ZnTCP contained 6.17% zinc. The suspensions (0.6 mg as a zinc volume/0.2 ml) were injected around the jaw bone once a week for 12 weeks. Local effects on the bone mineral content (BMC) of jawbone, and systemic effects on body weight, the BMC of both femurs determined by X-ray computed tomography, and bone mechanical strength (BMS) measured by the three-point bending method, were examined. The BMC of jaw bone was significantly higher in the ZnTCP-treated group than un-treated or ZnSO\(_4\)-treated group. Body weight, the BMC of femurs, and BMS were also significantly higher in the ZnTCP-treated group. The zinc-containing \(\beta\)-tricalcium phosphate nano particles were effective at preventing bone loss induced by ovariectomy in rats and have potential uses for treating periodontitis.

Key word  zinc; X-ray computed tomography; osteoporosis; periodontitis; drug delivery system; bone mechanical strength

Periodontosis is caused by smoking, bacterial infections, inflammation, oxidative damage, and aging, and leads to tooth loss. Treatments for periodontosis are generally divided into two types surgical and non-surgical. Surgical treatment involves the use of alveolar bone to support artificial teeth. Non-surgical treatment mainly involves the removal of dental calculus, allowing damaged tissue to heal.

As osteoporosis accelerates the development of periodontosis, drugs used to treat osteoporosis may prevent periodontosis by increasing the density of the jaw bone supporting the teeth. Therefore, many scientists have investigated tissue engineering or transgenic proteins relating to periodontosis, and actual pharmaceutical products are being developed in the United States.

Recently, human recombinant proteins have been developed using novel tissue engineering technology, including a platelet-derived growth factor (PDGF) and bone morphogenetic protein (BMP-2) approved by the Food and Drug Administration (FDA) and in clinical use in the United States. There are many reports concerning bone reproduction using BMP-2 in dentistry. BMP-2 achieves sufficient and reliable bone formation and produces strong bone matrices. However, human recombinant proteins, such as BMP-2, are too expensive for standard treatment.

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, controlling its release is vital for therapeutic use. Bone-promoting effects of Zn-containing \(\beta\)-tricalcium phosphate ceramics have been reported. Stimulatory effects on osteogenesis of injections of Zn-containing calcium phosphate suspension were also reported. Bone was stimulated to form around Zn-containing calcium phosphate ceramics implanted in rabbit femora. 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.

The purpose of the present study is to prevent by using injectable Zn-containing calcium phosphate nano particles and to examine changes in bone mineral content of the jawbone.

MATERIALS AND METHODS

Materials  ZnTCP containing 10 mol% zinc (6.17 w/w% zinc) \([\text{Ca}_{2.7}\text{Zn}_{0.3}(\text{PO}_4)_2]\) was prepared as described elsewhere. Zinc sulfonate 7 hydrate was obtained from Wako Pure Chemical Industries (Osaka, Japan), Corn oil, from Wako Pure Chemical Industries (Osaka, Japan), and sodium pentobarbital, from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). Other reagents were of analytical grade and used without further purification.

Surface Area and Particle Diameter of ZnTCP  The supecific surface area (m\(^2\)/g) of dried ZnTCP powders was measured with a Monosorb single point B.E.T. (Brunauer, Emmet and Teller’s equation) apparatus (Quantachrome Corporation, Boynton Beach, FL, U.S.A.). The dried powders were degased in a vacuum for at least one hour in the glass BET sample cells before measurements. Surface area values was measured three times for each powder. The particle diameter was calculated from the following equations (Eq. 1).

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S_w = \frac{\pi d^2}{6} \frac{\rho}{\rho_s} \left(\frac{d^3}{6}\right)
\]

\(S_w\) = specific particle surface area, \(\rho\) = density and \(d\) = particle diameter.
Animal Experiments Four-week old female Sprague-Dawley (SD) rats (65—75 g) purchased from Japan Clea Inc. (Tokyo, Japan) were used. The rats were maintained in a light (12 h light/dark)- and temperature (25±2 °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. The rats were bilaterally ovarioctomized 5 weeks before the start of injections (age at ovarioctomized (OVX), five weeks), and fed a normal diet for 1 week and the –DCaZn diet for 4 weeks. Normal rats did not under go an ovarioctomy and received a normal diet throughout the experimental period. On commencement of the experiments, the rats in the normal, control, and ZnTCP and ZnSO₄ were treated groups were assigned to receive injections.

ZnSO₄ or ZnTCP powder (0.6 mg as zinc) in 0.2 ml of corn oil was injected around the jawbone once a week for 12 weeks. Blood samples were obtained at predetermined times from the tail artery.

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. The measurement was made using a UV/VIS spectrometer (Type UV4200, Shimadzu, Japan) at 560 nm. The data represent the averages of 4 measurements each.

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.

Computed Tomography (CT) Analysis The rats were anesthetized with an intraperitoneally (i.p.) injection of sodium pentobarbital (50 mg/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; 50 kV, 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 0.164 mm/s using a type 50 kN (Minebea, Kanagawa, Japan). Maximum bending force (N) was used as an index of bone mechanical strength (BMS).

Statistical Analysis All results are expressed as the mean±S.D. for 3 to 6 experiments. The statistical analysis was conducted using Tukey’s multiple statistical tests.

RESULTS Physicochemical Properties of ZnTCP At first, specific surface area of ZnTCP was determined by dynamic flow method for determinations of single-point B.E.T. The particle diameter was calculated from the Eq. 1. the specific surface area and particle diameter were 2.5 m²/g and 756 nm, respectively. ZnTCP powder was observed by scanning electron microscopy (SEM) as reported previously. ZnTCP particles were jagged surface aggregates with fine particles (data not shown). The result of particle size of ZnTCP was consisted with that of SEM in previously.

Changes in Body Weight after the ZnTCP or ZnSO₄ Injections Figure 1 shows changes in body weight, injections of ZnTCP led to significant and continuous increases in body weight. No increase was observed in the rats which received saline.

Effects of ZnTCP or ZnSO₄ on Plasma Zn Levels Plasma zinc concentrations after the ZnSO₄ or ZnTCP injections are shown in Fig. 2. The zinc concentrations in healthy rats did not change over the 12-week period. They did not increase in the untreated or ZnSO₄-injected group either. In the ZnTCP-injected group, however, they increased, peaking 2 h after the injection. Figure 3 shows the area under the concentration vs. time curve for 12 weeks (AUCₒ₋₁₂) calculated using the trapezium method. The healthy rats had the highest, AUC values, followed by the ZnTCP-injected group.
The ZnSO₄-treated and untreated groups had very similar AUC₀—₁₂ values.

Bone Mineral Content of Jaw Bones Figure 4 shows the increase ratio of bone mineral content (BMC) of the jaw bone at the start/end ratio of the experiment. The difference among the groups was not significant at start of the experiment (data not shown). The BMC of jaw bone at 12 weeks after the administration of corn oil (with and without OVX), ZnTCP and ZnSO₄ were 301.1, 431.5, 382.0, and 352.5 mg, respectively. The increase ratio of BMC after the administration of corn oil (with and without OVX), ZnTCP and ZnSO₄ were 2.16, 1.70, 2.22, and 1.81, respectively.

There was a significant difference between the ZnTCP-administered group and control (OVX/corn oil) group, but not between the ZnSO₄-administered group and the controls.

Bone Mineral Content (BMC) and Bone Mechanical Strength (BMS) of Femurs Figure 5a shows the BMC of femurs at 12 weeks. Values after the administration of corn oil (with and without OVX), ZnTCP and ZnSO₄ were 38.3, 77.4, 61.5, and 34.1 mg, respectively. There was a significant difference between the control group and the ZnTCP-administered group, but not ZnSO₄-administered group.

Figure 5b shows the effect of ZnTCP and ZnSO₄ injections on the BMS of the right and left femurs. BMS in the control group was 80.9 N. That in the groups administered ZnTCP and ZnSO₄ was 157.0 and 98.8 N, respectively. The ZnTCP-administered group had a significantly higher value than the control group, but the ZnSO₄-administered group did not.

DISCUSSION

Here, the effect of injectable zinc-containing calcium phosphate (ZnTCP) nano particles on the mineral content of jawbone was investigated in an animal model of osteoporosis.

As reported previously, in vitro Zn release of ZnTCP was inhibited by the precipitated hydroxyapatite layer on the particle surface in simulated body fluid (SBF) containing 10 mg/100ml calcium (SBF/H), since SBF/H was supersaturated with respect to the solubility of hydroxyapatite as similar as healthy condition. Conversely, in calcium free SBF (SBF/−) unsaturated with calcium and phosphate, TCP matrices dissolved, accelerating Zn release as similar as osteoporosis condition. Therefore, in vitro Zn release rates depended on calcium levels in body fluid. Since ZnTCP had self drug release regulating ability in body fluid, responsive to a physiological dynamic parameter like plasma calcium
concentration, in vivo Zn release from ZnTCP was slower than that from ZnSO₄, indicated that it may have an advantage on therapeutical effect for osteoporosis.

Vehicle (corn oil), and ZnSO₄ and ZnTCP suspensions were injected weekly around the jaw bone of Zn-deficient rats. After 12 weeks, BMC was significantly higher in the ZnTCP-administered group than control group. The ZnSO₄-administered group, however, did not differ form the controls (Fig. 4). Body weight (Fig. 1), plasma concentrations of zinc (Figs. 2 and 3), BMC of femurs, and BMS of femurs (Fig. 5b), were measured as indexes of systemic effects on osteoporosis. These indexes were significantly higher in the ZnTCP-administered group than control and ZnSO₄-administered groups.

The bioavailability of pharmaceutical products in target tissues affects therapeutical scores based on pharmacokinetics. In general, the bioavailability of a pharmaceutical product is significantly affected by the rate of drug release from the dosage form. The results of dissolution tests indicated that the release of Zn from ZnTCP preparations in vitro depended on plasma calcium concentrations and was sufficiently slow in body fluid. Since ZnTCP is only slightly soluble, the release of Zn from the calcium phosphate crystalline device was well controlled and significantly slow, and the ZnTCP suspension induced sufficient bone formation in rats with experimental osteoporosis. In contrast, the ZnTCP suspension dissolved in pure water within 1 min (data not shown), since ZnSO₄ is soluble in water. It is therefore thought that the injected ZnSO₄ particles were immediately dissolved, diffused to tissues, absorbed, and distributed throughout the body, and the plasma Zn concentration might not have reached a therapeutically effective level. The physicochemical properties of the administered materials affected the therapeutical scores for osteoporosis. From the results, ZnSO₄ did not significantly affect osteogenesis, but ZnTCP was highly effective in promoting the formation of bone.

The results suggested that ZnTCP can control the release of zinc. Zinc-containing compounds may influence the formation of bone. The crystallinity and solubility of zinc-containing compounds should be examined further.

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