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CLINICAL STUDY

Effect of zinc supplementation on bone formation in hemodialysis patients with normal or low turnover bone

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Abstract

Zinc (Zn) is an essential trace element, which has been shown to stimulate osteoblastic bone formation and to inhibit osteoclastic bone resorption *in vitro*. In thalassemia, major patients Zn supplementation was reported to increase whole-body bone mineral content and areal bone mineral density. Therefore, we investigated the effect of Zn supplementation on bone formation in hemodialysis (HD) patients. Nine male patients with age of 66 (35–78) years indicated by median (range), HD vintage of 57 (4–97) months and serum intact parathyroid hormone (PTH) of 113 (6–310) pg/mL were supplemented with polaprezinc containing 34 mg Zn/day for 18 months. Doses of vitamin D were not changed during supplementation. Blood was collected at baseline, 3, 6, 12 and 18 months. Serum Zn increased significantly from 58 (52–65) μg/dL to 71 (57–93) μg/dL at three months and remained unchanged until 18 months. No changes were observed in serum intact PTH during supplementation. Although we found no changes in serum bone alkaline phosphatase (BAP) during Zn supplementation analyzed by Friedman test and Scheffe post hoc test, a significant trend of increase in serum BAP was verified by Jonckheere–Terpstra test ($p = 0.0409$). On the contrary, there was no trend in serum TRACP5b by Jonckheere–Terpstra test. Therefore, we suggested the effect of Zn supplementation on promoting bone formation, not affected by the status of PTH and vitamin D, in HD patients with normal or low turnover bone.

Keywords

Bone alkaline phosphatase, hemodialysis, supplementation, tartrate resistant acid phosphatase 5b, zinc

History

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Introduction

Zinc (Zn) is an essential trace element. Most of Zn is bound in metal–protein complexes comprised largely of metalloenzyme-bound Zn, such as alkaline phosphatase (ALP) and metalloprotein-bound Zn such as Zn finger proteins including transcription factor. Therefore, Zn profoundly relates to protein synthesis by activation of DNA polymerase, RNA polymerase and tRNA polymerase synthetase, and to signal transduction by regulation of transcription. Since Zn is the most abundant trace element in bone, it may induce the increase in ALP-related DNA synthesis and stimulate bone growth.[1,2]

Bone ALP (BAP) catalyzes the hydrolysis of pyrophosphate and provides the extracellular phosphate pool, which determines the rate of hydroxyapatite crystal formation in bone. *In vitro*, Zn has been shown to stimulate osteoblastic bone formation by activation of ALP,[3,4] while Zn deficiency reduced bone mineralization by decreasing synthesis of ALP.[5] Zn has been shown to have an inhibitory effect on osteoclastic bone resorption *in vitro*, demonstrated by staining for tartrate-resistant acid phosphatase (TRACP), a marker enzyme of osteoclasts.[6] In healthy human, Zn supplementation with isoflavone resulted in the increase of serum BAP and the decrease of serum TRACP5b,[7] and Zn supplementation was found to increase serum ALP, although bone resorption marker, urine C-terminal collagen peptide, remained unchanged.[8]

HD patients have high mortality risk related to bone fracture and vascular calcification.[9] Therefore, preservation of osteoblast activity and inhibition of osteoclast activity might be important. In addition to osteoporosis, acceleration of bone resorption by secondary hyperparathyroidism and/or disturbance of bone formation by uremic toxins might complexly result in decrease of bone mineral density (BMD) in HD patients. Because serum Zn has been reported to be low in HD patients compared to healthy control,[10] Zn supplementation might be useful for maintaining BMD, but remains to be verified. In thalassemia, major patients with Zn deficiency, Zn supplementation (25 mg Zn/day) for 18 months had reported to increase significantly whole-body bone mineral content and areal BMD.[11] Thus, we investigated the effect of Zn supplementation up to 18 months on bone formation in HD patients.

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Materials and methods

Participants

Figure 1 shows the flowchart for the process of patient selection. We enrolled 67 male HD patients without malignancy, rheumatoid arthritis, liver disease or eating disorder. Female patients were excluded, because serum bone resorption marker, TRACP5b, independently increased after menopause. Figure 2 shows the histogram of serum Zn, indicating that 62.7% of HD patients were below normal range (65–110 μg/dL) obtained in healthy individuals by SRL, Inc. (Tokyo, Japan) Thereafter, we selected 42 patients with serum Zn < 65 μg/dL (below normal range) without taking drugs known to interfere with bone metabolism, such as bisphosphonate, steroid, cinacalcet, warfarin, vitamin K, ipriflavone and without history of parathyroidectomy or renal transplantation. They were considered to have low BMD, because BMDs for the hip, spine and distal wrist were lower in men with the lowest plasma Zn quartile (< 74 μg/dL).[12]

Twelve patients with informed consent were enrolled in the Zn supplementation study. HD was performed three times a week, each for 4–5 hours, designed to achieve Kt/V > 1.2. Dialysate contained 2.75 mEq/L calcium. The primary diseases were diabetes mellitus ($n = 6$), polycystic kidneys ($n = 2$), glomerulonephritis ($n = 1$), nephrosclerosis ($n = 1$), urinary tract malformation ($n = 1$) and unknown origin ($n = 1$). Polaprezinc (produced by Zeria Pharmaceutical Co., Ltd., Tokyo, Japan) 150 mg/day (containing 34 mg Zn) was orally administrated in nine patients up to 18 months. The other three patients were discontinued and were therefore excluded from the study because of the poor medication adherence. Blood samples were collected before dialysis session at baseline and 3, 6, 12 and 18 months after polaprezinc administration. No adverse effect was found during the study. Doses of vitamin D were not changed during Zn supplementation. The study protocol was approved by the local ethical committee.

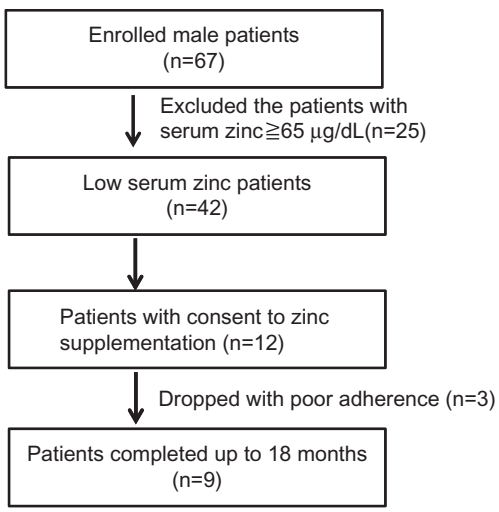

Figure 1. Flowchart for the process of patient selection.

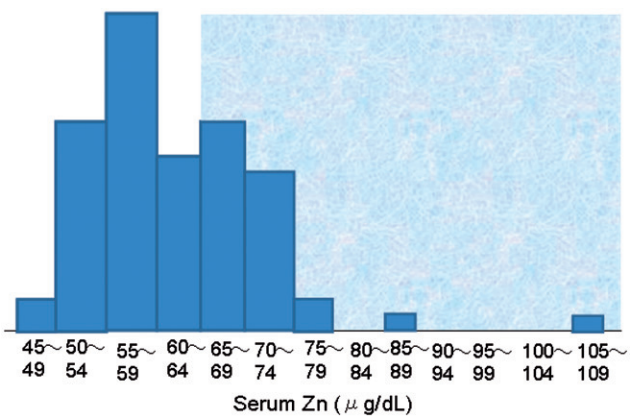

Figure 2. Histogram of serum Zn in 67 male HD patients. Highlighted area shows a normal range (65–110 μg/dL) obtained in healthy individuals.

Laboratory assay

The following serum biochemical parameters were measured as follows. Serum Zn was measured using polarized Zeeman atomic absorption spectrophotometer Z-6100 (Hitachi High-Technologies, Tokyo, Japan). Serum Alb, ALP and iP were measured using a multichannel biochemical analyzer (BM8060, JEOL, Tokyo, Japan). Ca was measured by colorimetric method and corrected Ca (cCa) was calculated by Payne's formula.[13] Serum intact PTH was measured by chemiluminescent immunoassay (Architect-PTH, Abbott Japan, Tokyo, Japan). Serum BAP was measured by chemiluminescent enzyme immunoassay (Access Ostase, Beckman Coulter, Tokyo, Japan; normal range: 3.7–20.9 μg/L). Serum TRACP5b was measured by fragment absorbed immunocapture enzyme assay (Osteolinks TRAP-5b, DS Pharma Biomedical, Osaka, Japan; normal range: 170–590 mU/dL). BAP and TRACP5b were adopted as bone formation marker and bone resorption marker, not affected by renal dysfunction.[14]

Statistical analyses

As some of continuous variables were not normally distributed, all values were expressed as median (range) and were analyzed using non-parametric methods. Demographic and laboratory characteristics were compared using Mann–Whitney test and Fisher's exact probability test between participants for Zn supplementation study ($n = 9$) and total patients with serum Zn < 65 μg/dL ($n = 42$). Serum Zn, BAP, ALP, TRACP5b, intact PTH, iP, cCa and Alb were compared using Friedman test between baseline, 3 months, 6 months, 12 months and 18 months' time points. Comparisons between groups were performed by Scheffe post hoc test when a significant difference was indicated. Trend analysis for the change of serum BAP, TRACP5b, intact PTH, iP, cCa and Alb during Zn supplementation was performed using Jonckheere–Terpstra test. Correlations between serum Zn and either intact PTH, iP or cCa were analyzed using Spearman correlation test. A p value of < 0.05 was considered as statistically significant. Statistical analyses were performed using Ekuseru-Toukei 2012 version 1.20 (SSRI, Tokyo, Japan) for Windows statistical software.

Results

No differences were observed in the demographic and laboratory characteristics related to nutrition and bone

metabolism between participants for Zn supplementation study ($n = 9$) and total patients with serum Zn < 65 μg/dL ($n = 42$) (Table 1). Serum Zn of participants ($n = 9$) was 58 (52–65) μg/dL, and mineral bone metabolism was fairly regulated in accordance with the guideline by Japanese Society for Dialysis Therapy.

Table 2 indicates the changes of laboratory data during Zn supplementation for 18 months in nine participants. Serum Zn increased significantly from 58 (52–65) μg/dL at baseline to 71 (57–93) μg/dL at three months and remained unchanged until 18 months except 6 months. We also found a significant increase between baseline and three months using Paired-T test ($p < 0.01$). Although we found no changes in serum BAP during Zn supplementation analyzed by Friedman test and Scheffe post hoc test, a significant trend of increase in serum BAP was shown by Jonckheere–Terpstra test ($p = 0.0409$) (Figure 3). Although a significant increase of serum TRACP5b from 203 (84–321) mU/dL at 6 months to 368 (161–807) mU/dL at 12 months was found by Friedman test and Scheffe *post hoc* test, we found no changes in serum TRACP5b during Zn supplementation by Jonckheere–Terpstra test. We found no changes in serum intact PTH. Although serum iP increased significantly from 4.9 (3.3–5.6) at 6 months to 5.5 (4.2–7.1) at 18 months, we found no changes in serum iP during Zn supplementation by Jonckheere–Terpstra test. No changes were observed in serum cCa, intact PTH and Alb. No significant correlations were found between serum Zn and either serum intact PTH, serum iP or serum cCa at baseline, 3 months, 6 months, 12 months and 18 months.

Table 1. Comparison of demographic and laboratory characteristics in patients with serum Zn < 65 μg/dL between participants for zinc supplementation study and the other patients.

Participants ($n = 9$)	Others ($n = 42$)	p^a	p^b
Zn (μg/dL)	58 (52–65)	57 (47–65)	0.2102
Age (years)	66 (35–78)	67 (35–91)	0.7385
HD vintage (months)	57 (4–97)	69 (4–336)	0.1448
DM: yes/no	5/4	17/25	0.4740
Dry weight (Kg)	60.0 (49.3–74.5)	61.9 (42.0–110.0)	0.8918
VD pulse: yes/no	5/4	16/26	0.4601
Alb (g/dL)	3.6 (3.2–4.4)	3.6 (3.2–5.1)	0.5086
CRP (mg/dL)	0.03 (0.01–0.17)	0.09 (0.01–1.85)	0.0474
ALP (IU/L)	142 (129–387)	225 (111–435)	0.0813
iP (mg/dL)	5.8 (3.8–6.6)	5.2 (2.9–6.7)	0.3042
cCa mg/dL)	9.2 (7.5–10.1)	9.2 (6.8–10.5)	0.7282
Intact PTH (pg/mL)	113 (6–310)	158 (6–804)	0.2256
BAP (μg/L)	8.5 (6.7–18.5)	11.0 (5.1–18.5)	0.4659
TRACP5b (mU/dL)	200 (98–684)	272 (98–1100)	0.5597

Notes: All values are expressed as median (range).
In others, $n = 30$ except for intact PTH ($n = 33$), BAP ($n = 31$) and TRACP5b ($n = 31$).
[a]Mann–Whitney test.
[b]Fisher's exact probability test.

Discussion

Serum Zn was below normal range (65–110 μg/dL) in 62.7% of HD patients, which is similar (58%) to the previous report with the cut-off point of 70 μg/dL.[15] In this study, serum Zn increased significantly at three months and remained unchanged thereafter (Table 2). Serum Zn might have reached at the physiologically maximal level after three months of Zn supplementation due to the autoregulation of intestinal absorption, because excess Zn is sequestered in enterocyte by metallothionein and lost into feces with desquamation[1] or lost through epithelium by regulation of Zn transporters.[16]

Participants in this study having intact PTH of 113 (6–310) pg/mL at baseline might be mostly composed of patients with low bone turnover, because more than one-third of the HD patients with serum intact PTH within the normal or high kidney disease outcomes quality initiative (K/DOQI) guideline range (150–300 pg/mL) was defined as low bone turnover by bone biopsy.[17] In addition, the baseline BAP in this study of 8.5 (6.7–18.5) μg/L (normal range: 3.7–20.9 μg/L) is similar to the BAP (10.8 ± 4.2 ng/mL, normal range: 4–25 ng/mL) in the HD patients defined as normal or low turnover bone disease by

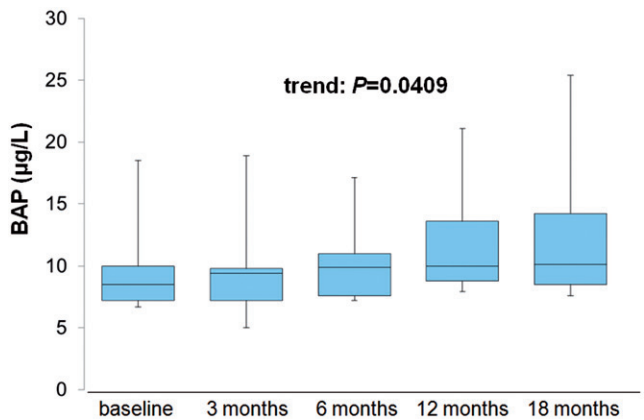

Figure 3. Changes of serum BAP up to 18 months during zinc supplementation. $p = 0.0409$: Jonckheere–Terpstra test, $n = 9$.

Table 2. Effects of zinc supplementation on serum Zn, bone metabolic markers and Alb from baseline up to 18 months.

	Baseline	3 months	6 months	12 months	18 months	p
Zn (μg/dL)	58 (52–65)	71 (57–93)*	72 (58–82)	73 (68–87)*	73 (62–120)*	0.0013
BAP (μg/L)	8.5 (6.7–18.5)	9.4 (5.0–18.9)	9.9 (7.2–17.1)	10.0 (7.9–21.1)	10.1 (7.6–25.4)	0.0118
TRACP5b (mU/dL)	200 (98–684)	225 (115–595)	203 (84–321)	368 (161–807)**	208 (88–536)	0.0020
Intact PTH (pg/mL)	113 (6–310)	128 (15–288)	129 (6–167)	126 (17–298)	82 (8–265)	0.2042
iP (mg/dL)	5.1 (3.4–7.1)	5.3 (4.2–6.0)	4.9 (3.3–5.6)	5.8 (3.8–7.2)	5.5 (4.2–7.1)**	0.0165
cCa (mg/dL)	9.8 (7.5–10.3)	9.1 (7.4–9.9)	9.2 (8.3–10.6)	9.1 (7.8–10.2)	9.2 (8.5–10.1)	0.2610
Alb (g/dL)	3.6 (3.2–4.4)	3.7 (3.2–4.3)	3.6 (3.3–4.1)	3.8 (3.3–4.4)	3.6 (3.3–4.1)	0.2407

Notes: All values are expressed as median (range); $n = 9$.
p: Friedman test.
Comparisons between groups were performed by Scheffe post hoc test:
*$p < 0.05$ versus baseline.
**$p < 0.05$ versus 6 months.

bone biopsy.[18] Thus, HD patients in this study might have been in the state of normal or low turnover bone disease.

The present Zn supplementation study verified the trend of increase in serum BAP analyzed by Jonckheere–Terpstra test. Zn could promote bone formation by the mechanism not affected by the potent regulation of PTH and vitamin D, because PTH level and the dose of vitamin D were kept unchanged during 18 months of Zn supplementation. We found no changes in serum intact PTH during Zn supplementation in this study, and Dashti-Khavidaki et al.[15] found no correlation between serum PTH and Zn in HD patients, although Navarro-Alarcon et al.[19] reported linear correlation, requiring further investigation. It seems to be elucidated why the long-term Zn supplementation was required for the increase of serum BAP in this study. Because the diverse transcription factors and the signals that regulate their activity form a highly interconnected, cooperative network in osteoblasts,[1] the effects of Zn might be the sum of opposing actions which offset each other, resulting in the slow promotion of bone formation and a time-consuming increase in serum BAP. We measured serum BAP by mass concentration, but not by enzyme activity. Peretz et al.[8] has reported that the increase in serum BAP measured by mass concentration required longer period of time than enzyme activity.

We found no changes in serum TRACP5b during Zn supplementation by Jonckheere–Terpstra test. Serum TRACP5b increased transiently to 368 (161–807) at 12 months (Table 2), although no change in serum intact PTH was observed in this study. The mechanism of increase in TRACP5b at 12 months was unknown, but serum TRACP5b at 12 months was comparable with the previous report[20] indicating that annual cortical bone loss in the distal third of radius was not observed except for the highest tertile of HD patients including female with TRACP5b of 490 ± 320 mU/dL, and Zn administration has been shown to decrease osteoclastogenesis in Zn adequate rats.[21] Therefore, it is suggested that Zn supplementation might not cause bone mineral reduction in spite of the transient increase in serum TRACP5 at 12 months.

Although PTH is the key regulator of bone metabolism, we found no correlation between serum Zn and either serum intact PTH, iP or cCa. Thus, we suggested that Zn supplementation (34 mg Zn/day) can increase BMD by promoting bone formation directly, although this study has the limitation of the small number of patients and the single-center setting.

In conclusion, we found the effect of Zn supplementation on bone formation independent of PTH and vitamin D in HD patients with normal or low turnover bone.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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