ORIGINAL

Effects of zinc supplementation in combination with difructose, an enhancer of intestinal zinc absorption, on serum zinc concentration and ratio of apo-/holo-activities of angiotensin-converting enzyme in patients with refractory taste impairment

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Abstract : Objective : ACE ratio in the serum is a more sensitive indicator of zinc nutrition than serum zinc concentration. In this study, we examined the effects of zinc supplementation in combination with difructose anhydride (DFA) that enhances zinc absorption in the intestine on serum zinc concentration and ACE ratio in patients with refractory taste impairment. Methods : Twenty patients with refractory taste impairment were enrolled. The patients received 34 mg of zinc in combination with 2.0 g of DFA orally every day for three months. Taste impairment was evaluated using visual analogue scale (VAS). Results : After zinc supplementation with DFA (zinc with DFA), ACE ratio was significantly decreased, but serum zinc concentration was not increased in patients with refractory taste impairment. Although VAS score was not changed in the patients after zinc with DFA, we divided the patients into two groups : 14 responders and 6 non-responders. ACE ratio in responders, but not in non-responders was significantly decreased after zinc with DFA. However, serum zinc concentration was not changed in both responders and non-responders after zinc with DFA. Conclusion : The present findings suggest that malabsorption of zinc in the intestine is the primary cause of zinc deficiency in patients with refractory taste impairment. J. Med. Invest. 72 : 156-160, February, 2025

Keywords **:** *taste impairment, zinc supplementation, zinc nutrition, ACE ratio, difructose*

INTRODUCTION

Zinc is an essential trace element in human nutrition (1). Zinc deficiency is associated with growth retardation, taste impairment, dermatitis, and sexual and immune dysfunction (2).

Zinc concentration in the serum has been widely used to evaluate zinc nutrition. However, zinc is mostly bound to albumin, α-macroglobulin, and transferrin in the serum (3) and serum zinc concentration is modified by several factors, such as acute stress and inflammation (4). Since angiotensin-converting enzyme (ACE) is a zinc metalloenzyme that requires zinc for its activity, Kobayashi *et al*. recently reported that the ratio of apo-/holo-activities of ACE (ACE ratio) in the serum is an index of zinc nutrition in humans (5). Subsequently, we showed that ACE ratio in the serum was increased not only in taste impairment patients with low serum zinc concentration, but in those with normal serum zinc concentration (6). We also showed that zinc supplementation improved taste impairment and decreased ACE ratio in the serum of both patients with low and normal serum zinc concentrations (7). These findings suggest that ACE ratio in the serum is a more sensitive indicator of zinc nutrition than serum zinc concentration in patients with taste impairment, and that zinc deficiency is the predominant cause of taste impairment even when serum zinc concentration is within normal range.

Zinc deficiency is usually caused by an inadequate intake or malabsorption of zinc. Previously, we showed that the dietary intake of zinc was not different between patients with taste impairment and age-matched healthy controls, even though ACE ratio in patients was higher than that in healthy controls (8). Therefore, it is likely that patients with taste impairment take adequate dietary zinc. Because zinc is mainly absorbed in the intestine (9, 10), it is possible that intestinal malabsorption of zinc causes zinc deficiency in patients with taste impairments.

In the present study, to investigate the possibility of malabsorption of dietary zinc in patients with taste impairment, we used difructose anhydride (DFA), an indigestible disaccharide that enhances zinc absorption in the small intestine (11) and enrolled patients with refractory taste impairment who did not respond to zinc supplementation alone. We examined the effects of zinc supplementation in combination with DFA on zinc concentration and ACE ratio in the serum of patients with refractory taste impairment.

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PATIENTS AND METHODS

Patients

The present study enrolled 20 patients (8 males and 12 females ; mean age : 55.2 ± 16.9 years) with refractory taste impairment who were referred to the Department of Otolaryngology,

Tokushima University Hospital, because their taste impairment was not improved after zinc supplementation with 150 mg of polaprezinc containing 34 mg of zinc/day for more than two months. They had no other apparent disorders. This study was approved by the Ethical Committee of Tokushima University Hospital (No. 0777). Written informed consent was obtained from each patient before participation in the study.

Intervention

The patients with refractory taste impairment received 150 mg of polaprezinc containing 34 mg of zinc in combination with 2.0 g of DFA (DFA III, FANCL Co., Japan) orally every day for three months. Zinc concentration and ACE ratio in the serum and visual analogue scale (VAS) score were measured before and after zinc supplementation in combination with DFA for three months. Changes in VSA score was calculated as the final VAS score minus the initial score.

We divided the patients into two groups : the responder group and non-responder group, based on changes in VAS score after zinc supplementation in combination with DFA. Accordingly, responder group included 14 patients (6 males and 8 females ; mean age : 54.4 years old ; mean period of illness : 9.3 months), who showed an improvement in VAS score, while non-responder group included 6 patients (2 males and 4 females ; mean age : 57.2 years old ; mean period of illness : 17.5 months), who showed a deterioration or no change in VAS score.

Measurement

Zinc concentration in the serum was measured using atomic absorption spectrometry (12) by Mitsubishi Kagaku Bio-Chemical Laboratories Inc. (Tokyo, Japan). Normal zinc concentration in the serum ranges from 64 to 111 μg/dl. Among 20 patients enrolled in the present study, zinc concentration in the serum was measured in 18 patients.

ACE activity in the serum was measured spectrophotometrically. ACE Color Kit (Fujirebio Inc., Tokyo Japan) was used, which is based on the colorimetry of the quinoneimine dye product from p-hydroxyhippuril-L-histidyl-L-leucine as the substrate of ACE with hippuricase and peroxydase (13). Because ACE is a zinc metalloenzyme, holo-ACE with zinc shows full ACE activity. After measuring holo-ACE activity in the serum, it was measured again after the addition of zinc (80 μM in phosphate buffer at pH 8.3) to the serum in vitro. The increase in activity over the initial holo-ACE activity was determined as apo-ACE activity in the serum. The ratio of apo-/holo-ACE activities is calculated as follows : ACE ratio (%) = apo-ACE activity/holo-ACE activity × 100 [6,7,8].

Subjective symptoms of taste impairment were assessed using VAS and scored from 0 to 10 (14).

Statistical analysis

Paired t-test was used for statistical analysis. Statistically significant was considered at $p<0.05$.

RESULTS

The mean concentration of zinc in serum was 66.1 ± 12.0 μg/dl (mean ± S.D.) in 18 patients with refractory taste impairment. The serum concentration of zinc was below 63 μg/dl in 7 patients, while it was within the normal range (64-111 μg/dl, Mitsubishi Kagaku Bio-Chemical Laboratories Inc.) in 11 patients. The mean serum concentration of zinc in patients with refractory taste impairment was changed to 69.4 ± 16.3 μg/dl without statistical significant after zinc supplementation in combination with DFA for three months (n=18) (Fig. 1a). On the other hand, the mean ACE ratio in the serum was 13.5 ± 1.9% in 20 patients with refractory taste impairment. The mean ACE ratio in the serum was significantly decreased to 10.4 ± 1.9% after zinc supplementation in combination with DFA for three months ($p<0.01$, n=20) (Fig. 1b).

The mean VAS score in patients with refractory taste impairment was changed from 47.2 ± 24.6 to 62.9 ± 29.3 without statistical significant after zinc supplementation in combination with DFA for three months (n=20). We then divided the patients into two groups : the responder group and non-responder group, based on changes in VAS score. Accordingly, in the responder group of 14 patients, each patient showed an improvement in VAS score after zinc supplementation in combination with DFA (pre : 38.2 ± 21.1, post : 63.8 ± 32.0). In the non-responder group

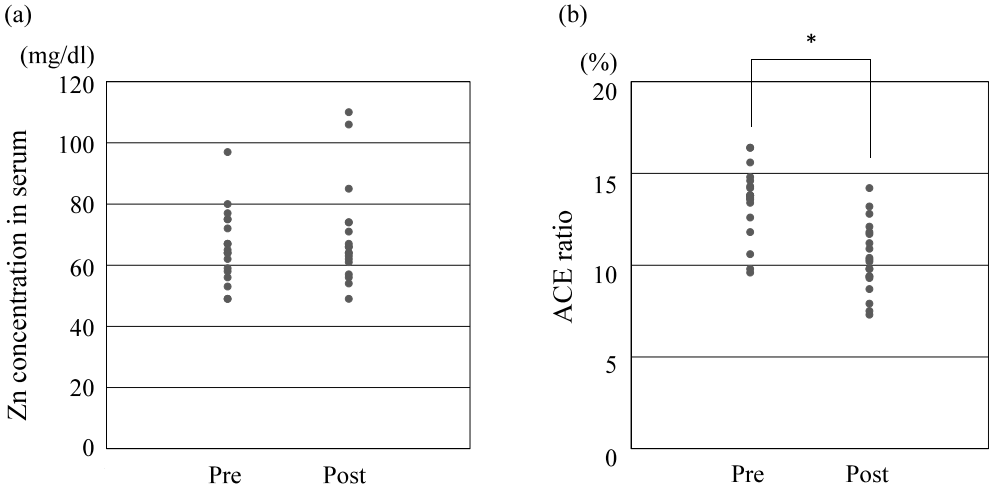

Fig. 1. Changes in zinc concentration and ACE ratio in the serum after zinc supplementation in combination with difructose anhydride (DFA) in patients with refractory taste impairment. (a) zinc concentration (n=18) and (b) ACE ratio (n=20). *$p<0.01$.

of 6 patients, each patient showed a deterioration or no change in VAS score after zinc supplementation in combination with DFA (pre : 68.3 ± 19.1, post 60.8 ± 24.2).

The mean ACE ratio in the serum was 13.3 ± 1.7% in the responder group and was significantly decreased to 9.8 ± 1.5 % after zinc supplementation in combination with DFA (n=14. $p<0.01$) (Fig. 2a). However, the mean ACE ratio in the serum in the non-responder group was changed from 14.1 ± 2.3% to 11.4 ± 1.6% without statistical significant after zinc supplementation in combination with DFA (n=6) (Fig. 2b). On the other hand, after zinc supplementation in combination with DFA, the mean serum concentrations of zinc in the responder and non-responder groups were changed from 67.7 ± 12.5 μg/dl (n=5) and 61.8 ± 10.6 μg/dl (n=13) to 71.4 ± 17.3 μg/dl and 64.2 ± 13.4 μg/dl without statistical significant, respectively (Fig. 3a, b).

There were no differences in the sex ratio, mean age, or period of illness between the responder and non-responder groups.

DISCUSSION

The present study enrolled patients with refractory taste impairment, who reported no improvement of taste impairment after zinc supplementation for more than two months. The concentration of zinc in serum was still below the lower limit of the normal range in some patients, while it was within the normal range in others. Previously, we reported that ACE ratio in the serum was a more sensitive indicator of zinc nutrition than serum zinc concentration in patients with taste impairment, and that zinc deficiency is the predominant cause of taste impairment (6, 7). ACE ratio in the serum was 1.10 ± 0.6% in healthy volunteers (6). In the present study, the mean ACE ratio was 13.5 ± 1.9% (9.6-16.4%) in patients with refractory taste impairment, suggesting that their taste impairment was still attribute to zinc deficiency even after zinc supplementation.

There are two possible explanations for why zinc-deficiency

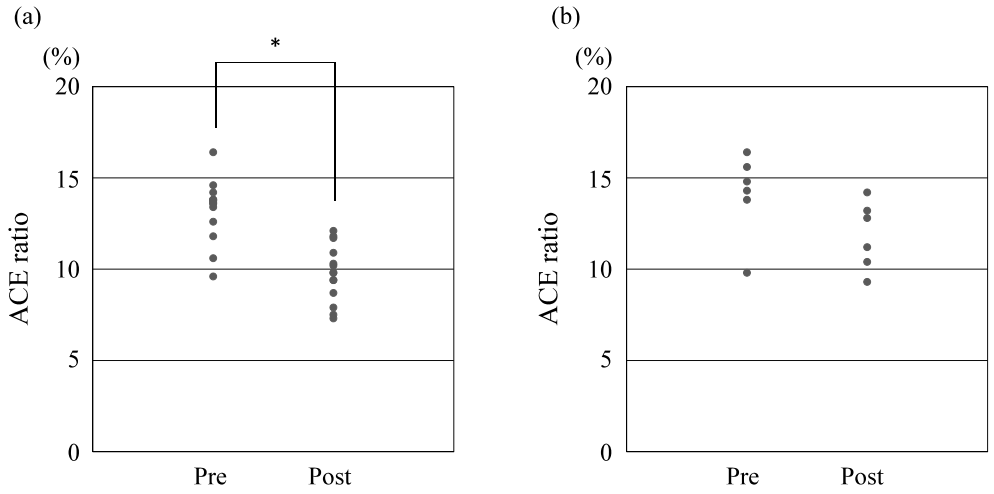

Fig. 2. Changes in ACE ratio in the serum after zinc supplementation in combination with DFA in responder and non-responder groups with refractory taste impairment. (a) responder group (n=14) and (b) non-responder group (n=6). *p<0.01.

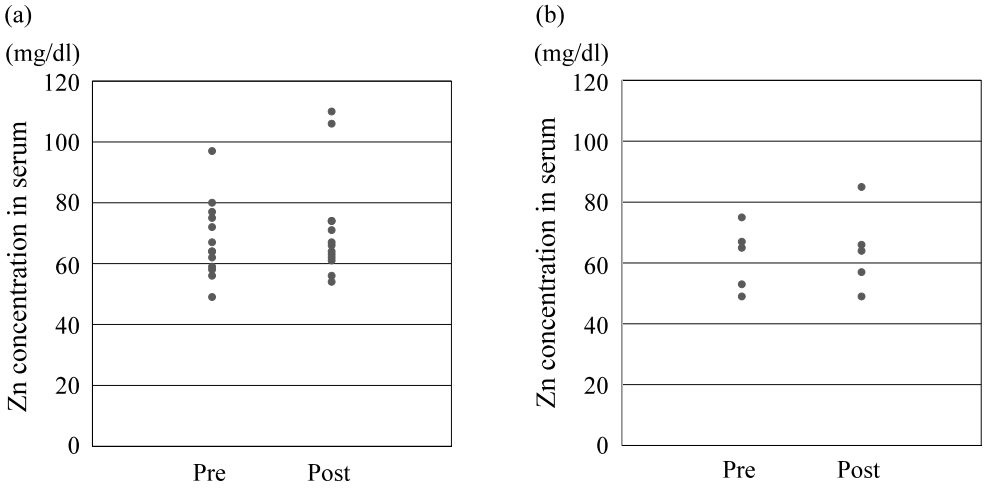

Fig. 3. Changes in zinc concentration in the serum after zinc supplementation in combination with DFA in responder and non-responder groups with refractory taste impairment. (a) responder group (n=13) and (b) non-responder group (n=5). *p<0.01.

was not improved after zinc supplementation in patients with refractory taste impairment : the habitual inadequate dietary intake of zinc and malabsorption of dietary zinc. The former is unlikely because our previous study showed that dietary zinc intake was not different between patients with taste impairment and age-matched healthy controls (8). Moreover, our previous randomized controlled trial showed that the efficacy of zinc supplementation with polaprezinc at a double dose of 300 mg/day was not different from that at a standard dose of 150 mg/day in patients with taste impairment, suggesting that malabsorption of dietary zinc, rather than inadequate dietary intake of zinc, is responsible for taste impairment (15). Therefore, to clarify the possibility of malabsorption of dietary zinc, the present study examined the effects of zinc supplementation in combination with DFA that enhances zinc absorption in the small intestine (11) on zinc concentration and ACE ratio in the serum in patients with refractory taste impairment.

Zinc is absorbed in the small intestine in humans (9, 10). The absorption of mineral ions from the intestine involves two mechanisms : an active transcellular pathway in the duodenum, and a passive paracellular pathway throughout the small intestine (16). The transcellular pathway is mediated by zinc transporters, such as ZRT, IRT-like protein and cation diffusion facilitator (17). The paracellular pathway involves zinc absorption through tight junctions that regulate the permeability of ions, nutrients and water (18). The dysfunction of zinc transporters or tight junctions may disturb the absorption of dietary zinc from the small intestine in patients with taste impairment.

Recently, it was reported that indigestible disaccharides, such as DFA, open tight junctions and enhance zinc absorption in the intestinal epithelium (19). In the present study, zinc supplementation in combination with DFA for three months decreased ACE ratio, but did not increase zinc concentration in the serum of patients with refractory taste impairment. These findings suggest that DFA increased zinc absorption in the small intestine and improved zinc deficiency in patients with refractory taste impairment.

Although VAS score was not changed in patients with refractory taste impairment after zinc supplementation in combination with DFA, it was improved in 14 responders, but was not in 6 non-responders. In responder group, taste impairment was improved and ACE ratio in the serum was decreased. These findings suggest that zinc supplementation in combination with DFA improved zinc deficiency, resulting in an improvement of refractory taste impairment in most patients. It is also suggested that malabsorption of zinc in the small intestine due to dysfunction of tight junctions causes zinc malnutrition in patients with taste impairment.

However, in some non-responders, neither taste impairment nor ACE ratio in the serum were improved after zinc supplementation in combination with DFA. Therefore, longer-term zinc supplementation in combination with DFA, higher doses of DFA or other agents that act on zinc transporters to facilitate intestinal absorption of zinc is needed to increase the efficacy in the treatment of refractory taste impairment.

LIMITATIONS

In the present study, taste impairment was assessed using subjective visual analog scale, but not objective taste tests. Taste test with filter paper disc had been used as a quantitative evaluation of the cognitive threshold of taste in Japan. Unfortunately, Taste Disc Kit has not been commercially available. Moreover, taste test with filter paper disc failed to show the efficacy of polaprezinc at a dose of 150 mg containing 34 mg of zinc on taste impairment in our previous randomized controlled trial (15), although the dose of polaprezinc is clinically approved off-label use for the treatment of taste impairment in Japan and its efficacy was demonstrated using subjective symptom scores in the randomized controlled trial (15). Accordingly, it is suggested that subjective evaluation of taste impairment is more sensitive than taste test with filter paper disc. Taste strips, quantitative tests for taste function in Europe (20), may be used in the next study.

CONCLUSION

Our previous study showed that zinc deficiency has a major role in the underlying mechanism of taste impairment and that ACE ratio is a more reliable index of zinc nutrition than serum zinc concentration. Zinc supplementation was effective to improve zinc deficiency, resulting in an improvement of taste impairment. However, some patients still complained of taste impairment after zinc supplementation. The present study enrolled the patients with refractory taste impairment and demonstrated that their zinc nutrition was sill insufficient. In the present study, zinc supplementation in combination with DFA that enhances zinc absorption in the small intestine improved zinc deficiency, resulting in an improvement of taste impairment in most, but not all patients with refractory taste impairment. Taken together with our previous findings that dietary zinc intake was adequate in patients with taste impairment, the present findings suggest that zinc malnutrition is caused by zinc malabsorption in the small intestine of patients with taste impairment. Zinc supplementation in combination with DFA may be a promising therapy for patients with refractory taste impairment.

CONFLICT OF INTEREST

The authors declare no conflicts of interest in this study.

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