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The Efficiency of Zinc-Aspartate Complex on Zinc Uptake in Plasma and Different Organs in Normal SD Rats

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Abstract

Zinc is essential metal and plays a role in a wide variety of physiological and biochemical processes. Prostate gland contains high level of zinc, generally 3-10 folds higher than other organs. Prostatic zinc uptake is resulted from the existence of zinc transporter (ZnT) protein families in membrane. In this study, we investigated the difference of zinc uptake efficiency of zinc-aspartate complex (Zn-Asp) into various organs compared with $ZnSO_4$. We observed that Plasma zinc concentration in both $ZnSO_4$ and Zn-Asp administrated group was increased progressively following administration, and reached a peak level at 2 hr. The increasing pattern of zinc concentration was similar to each groups, however the zinc concentration of Zn-Asp administrated group was higher than that of $ZnSO_4$ administrated group. We found that prostatic zinc level of Zn-Asp administrated group was higher than $ZnSO_4$ administrated group, and was increased approximately ~2.7 fold and ~4.2 fold at 4 and 8 hr after administration. From these observations, we suggest than Zn-Asp has high uptake efficiency of zinc into the prostate gland. Therefore, Zn-Asp is potentially useful treatment of many prostatic diseases.

Keywords: Prostate gland, Zinc, Zinc-Ligand complex, Zinc transporter (ZnT)

Zinc is essential trace element required for numerous enzymes, and involves in number of cellular processes including cell growth, replication, osteogenesis and immunity[1-3]. In the male urinary, zinc has antibacterial function in seminal plasma, and plays a critical role in optimal development and maintains reproductive function[4-7]. Especially, many studied demonstrated that zinc restricts growth of prostatic cancer cells via cell-cycle arrest, apoptosis, and inhibiting mitochondria aconitase activities[3,5]. Normal human prostate gland accumulates high levels of zinc, which is 3-10 times higher than other tissues; however, malignant prostate cells have lost this ability[4,8,9]. It is known that the lost ability to accumulation of zinc is due to the down-regulation of the zinc transporter[10]. Therefore, new strategies for up-regulating zinc transporter are needed to restore and enhance the zinc uptake into the prostate gland.

Total cellular zinc comprise three pools of zinc: (a) tightly bound zinc, (b) loosely bound zinc, (c) free Zn^{2+} ion. Tightly bound zinc, which is an immobile unreactive pool, comprises more than 95% of the total cellular zinc. However, loosely bound zinc, bound to low molecular ligands such as amino acid and citrate, comprise the major mobile reactive pool of zinc[8,11]. The major ligand for zinc is citrate which is accumulated high cellular level in prostate gland. For synthesis of citrate, oxaloacetate is needed and is derived from aspartate. Aspartate is an essential amino acid in specialized prostate cells that is derived from circulation[12-14]. For these reason, we produced zinc-aspartate complex (Zn-Asp) as a new loose bound zinc form.

Intracellular zinc homeostasis is regulated by different proteins involved in zinc uptake, excretion and trafficking, not regulated by simple diffusion. Many transporters that regulate zinc homeostasis have been identified in mammals such as Zrt- and Irt-like prostins (ZIP), Zinc Transporter (ZnT, SLC30) and metallothioneins[5,8,10]. In mammalian, the nine homologous ZnT proteins have been identified ZnT-1 through ZnT-9. ZnT families have six transmembrane-spanning domains and histidine-rich imtracellular loop. ZnT-2 is located in acidic vesicles and regulates vesicular zinc accumulation inside the cell. Many studied demonstrated that ZnT-2 is expressed only in the small intestine, kidney, testis, and prostate gland. ZnT-4 is widely expressed, also involved in zinc uptake in mammary gland and brain[5,15]. Many studies

Table 1. Average weight of body and organs.

Group	Body weight	Organ weight							
		Liver		Lung		Prostate		Testis	
		Weight	Ratio	Weight	Ratio	Weight	Ratio	Weight	Ratio
Control	337.0	12.45	0.036	1.62	0.004	0.61	0.002	2.90	0.008
$ZnSO_4$	333.6	11.13	0.032	1.60	0.005	0.54	0.002	3.21	0.010
Zn-Asp	340.0	11.10	0.033	1.44	0.004	0.67	0.002	3.14	0.009

demonstrated that expression of ZnT mRNA was influenced by zinc deficiency or supplementation *in vivo* and *in vitro*[1,15-17].

In this study, we investigated changes in plasma zinc concentration and zinc uptake efficiency after administration of bound zinc-ligand complexes (Zn-Asp) compared with $ZnSO_4$ in various organs including liver, lung, prostate gland, and testis.

Body and Organs Weight

Body weight and various organs weight including liver, lung, prostate and testis are shown in Table 1. There was no difference in body weigh among all experimental groups, and the average body weight is 300 g. Also, we represented that organs weight of each group was similar.

Plasma Zinc Concentration after Zinc Oral Administration

After oral administration of $ZnSO_4$ and Zn-Asp, the change of plasma zinc concentration is shown in Figure 1. Zinc-induced increase plasma zinc concentration was observed at 6 times points tested. Plasma zinc concentration in both $ZnSO_4$ and Zn-Asp administrated group was increased progressively following administration, and reached a peak level at 2 hr. The increasing pattern of zinc concentration was similar to each groups, however the zinc concentration of Zn-Asp administrated group was higher than that of $ZnSO_4$ administrated group. Compared to the baseline of plasma zinc concentration, Zn-Asp administration increased it to a ~3 fold at 2 hr and to ~2.5 fold at 4 hr, respectively.

Zinc Level in Various Organs after Zinc Oral Administration

Zinc levels in the prostate and other tissue are presented in Figure 2. After zinc administration, zinc level in the prostate gland and liver was increased in both of $ZnSO_4$ and Zn-Asp administrated group, but zinc level in testis and lung was nearly same in both groups before administration. Prostatic zinc level of Zn-Asp administrated group was higher than $ZnSO_4$ administrated group, and was increased approximately ~2.7 fold and ~4.2 fold at 4 and 8 hr after administration (Figure 2C). The result demonstrated that Zn-Asp administration resulted in the most significant increase of tissue zinc level in prostate gland compared to other tissues (Figure 3).

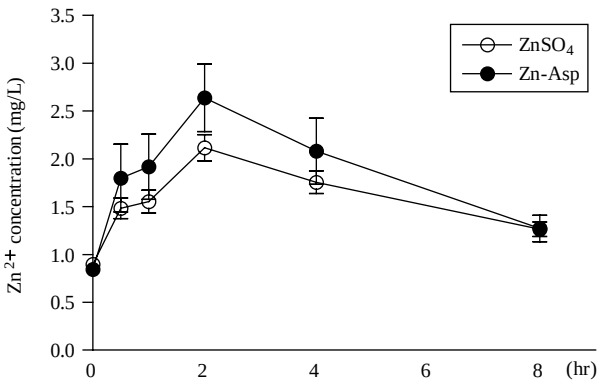

Figure 1. Time-course analysis of plasma zinc concentration. After oral administration of $ZnSO_4$ and Zn-Asp (40 mg/kg), blood was collected at 0, 0.5, 1, 2, 4, and 8 h; and then plasma were incubated with zinquin for 30 min. Fluorescence of tissue suspension was measured in a *Wallac 1420* at excitation wavelength of 370 nm and at emission wavelength at 460 nm.

Discussion

The prostate glands contain heavy metal, such as zinc and cadmium[1]. Especially, the ability of the prostate to accumulate high zinc level contributes to the major prostate function of citrate accumulation and secretion. In contrast, the zinc level in prostate cancer is markedly decreased[18]. Also, it is known that the prostate gland require high level of aspartate, an essential amino acid of prostate cell, from circulation to synthesize citrate[8,12]. Therefore, we selected and produced zinc-aspartate complex (Zn-Asp) as a zinc donor for the prostate gland because its cellular aspartate concentration is high.

In the present study, we demonstrated that zinc concentration in plasma and in the prostate gland of

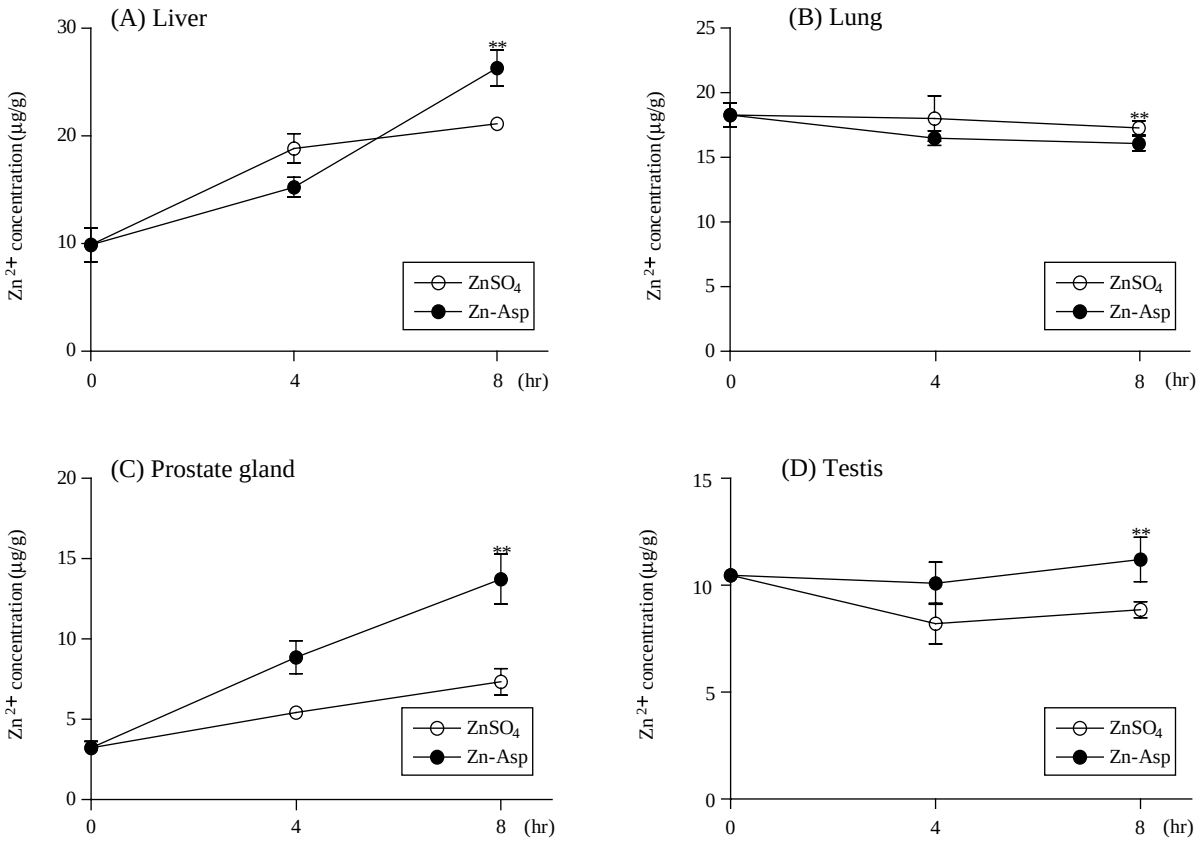

Figure 2. Zinc level in different organs after zinc oral administration. After oral administration of $ZnSO_4$ and Zn-Asp (40 mg/kg), different organs were collected at 4 and 8 h. For measurement of tissue zinc level, homogenated tissues were incubated with zinquin for 30 min. Fluorescence of tissue suspension was measured in a *Wallac 1420* at excitation wavelength of 370 nm and at emission wavelength at 460 nm. (**: $P < 0.01$)

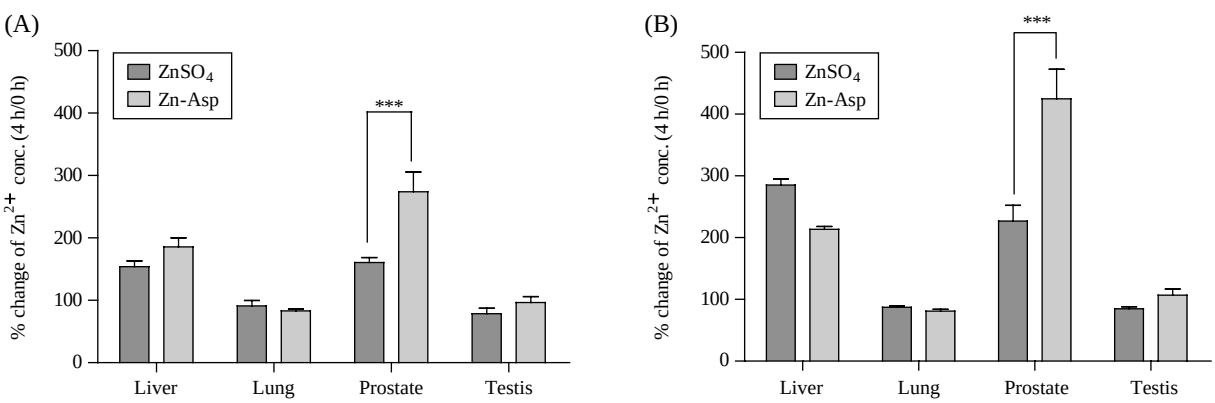

Figure 3. Effect of zinc on zinc uptake efficiency into different organs. After zinc administration, zinc levels in different organs were compared to baseline of zinc level in each tissue. (***: $P < 0.001$)

Zn-Asp administrated group was higher than that of $ZnSO_4$ administrated group. Our time-course study analysis revealed that pattern of zinc concentration was similar to each groups, and plasma zinc concentration peaked out at 2 hr after zinc administration. In addition, we found that Zn-Asp administration result-

ed in the most significant increase of tissue zinc level in prostate gland compared to other tissues. Recently, kinetic study of many zinc-ligand complex such as zinc-citrate complex (Zn-Cit), zinc-histidine (Zn-His), and zinc-cysteine (Zn-Cys) as well as Zn-Asp demonstrated that these zinc-ligand complexes was served as effective zinc donors for zinc transporter in prostate cells. Moreover, Z. Guan *et al.* demonstrated that zinc uptake into the prostate cell from zinc-ligand complexes resulted from uptake of the undissociated zinc-ligand complexes. From our observations and those of others, we can suggest that Zn-Asp is effective against zinc uptake into the prostate gland[8].

Z. Guan *et al.* reported that zinc derived from zinc-ligand complex was directly transferred to the transporter, which provides the mechanism for zinc uptake in the prostate gland[8]. Many transporters that participate in zinc trafficking across membranes have been identified in mammals, and decreased expression of these zinc transporter result in lost ability to accumulate zinc[15,19]. Zinc transporter (ZnT) family has been identified as one of the families of mammalian zinc transporter, and currently contains nine members (ZnT-1 through ZnT-9)[16,19]. In particular, ZnT-2 is involved in zinc uptake in vesicle and is highly expressed in the lateral and dorsal prostate[5].

In conclusion, we observed that administration of zinc increased the zinc concentration in plasma and in the prostate gland. Particularly, compare with $ZnSO_4$, zinc-aspartate complex is more effective against increase prostatic zinc level. From these results, we suggest that zinc-aspartate complex has high uptake efficiency of zinc in the prostate gland. Therefore, zinc-aspartate complex acts as the prostate gland specific and is potentially useful treatment of many prostatic diseases.

Methods

Animals

Male Sprague-Dawley (SD) rats, 8 weeks old, were housed in an environmentally controlled room (25˚C, 12 hr light: 12 hr dark cycle) and fed with standard laboratory chow pellets. Animals were fasted for 14 hr prior to experiments but allowed free access to water.

Experimental Design and Administration of Animals

Twenty two animals were randomly divided into three groups: Group I, intact rats (control: $n=4$), Group II, oral administration of zinc sulfate (40 mg/kg $ZnSO_4$: $n=9$), and Group III, oral administration of zinc-aspartate complex (40 mg/kg Zn-Asp: $n=9$). After oral administration of $ZnSO_4$ and Zn-Asp, blood was collected at 0, 0.5, 1, 2, 4, and 8 hr. The animals were sacrificed by ether 4 hr and 8 hr after oral administration of $ZnSO_4$ and Zn-Asp, then organs including liver, lung, prostate gland, testis, and spleen were collected. Blood and organs were immediately frozen and stored at −70˚C.

Determination of Zinc Concentration

Whole blood was centrifuged at 3,500 rpm, room temperature for 10 min, and then serum was collected. Tissues from zinc administrated rats were excised, rinsed in 1X PBS and homogenized in 9 vol. of autoclaved distilled water. The homogenates were centrifuged at 3,500 rpm, 4˚C for 10 min. Serum and supernatants were incubated with 30 μL of 24 μM zinquin for 30 min at 37˚C. Then samples read on a *Wallac 1420* multi label counter at excitation and emission wavelength at 370 nm and 460 nm respectively.

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