MOLECULAR & CELLULAR TOXICOLOGY, Vol. 3, No. 4, 292-298, December 2007

# Study on Intracellular Zinc Uptake According to Zinc-ligand

**Boo Im Shim[1], Ki Nam Kim[1], Yu-Ri Kim[1], Seung Ho Lee[1], Seung Min Lee[1], Myunggyu Park[2] & Meyoung-kon Kim[1]**

[1]Department of Biochemistry & Molecular biology, Korea University Mrdical College, 126-1, Anam-Dong 5-Ga, Sungbuk-Gu, Seoul 136-705, Korea
[2]MD BioAlpha Co., Ltd, B-1005, Digital Empire Bldg., 980-3, Yeongttong-dong, Yeongtong-gu, Suwon-si, Gyeonggi-do 443-813, Korea
Correspondence and requests for materials should be addressed to M. K. Kim (jerrykim@korea.ac.kr)

Accepted 16 November 2007

## Abstract

**Zinc plays indispensable roles in metabolism, including cell growth, apoptosis, proliferation and differentiation. Kidneys are target organs for various regulators of mineral metabolism, and play a key role in zinc balance. To investigate the zinc uptake efficiency, we examined the zinc uptake and accumulation level *in vivo* and *in vitro* study. Plasma zinc concentration was peaked out at 1 hr after oral zinc administration. The renal zinc level was peaked out at 12 hr after oral zinc administration, and it was the highest in 40 mg/kg Zn-Asp administrated group in comparison with other groups. In addition, the m-RNA expression level of zinc transporter-1 (ZnT-1), zinc transporter-2 (ZnT-2) and high-affinity L-aspartate transporter (EAAT-3) in Zn-Asp administered group were increased compared with control groups and $ZnSO_4$ group. In order to investigate the intracellular zinc uptake mechanism, we performed the *in vitro* study by using human embryonic kidney cell line, HEK 293. Intracellular zinc level was peaked out at 3 hr after zinc treatment. In the same way, the mRNA expression level of ZnT-1 and EAAT-3 were increased compared with control group. This study showed that Zn-Asp is effective the zinc uptake into the kidney by increasing the zinc transporter expression.**

**Keywords:** Kidney, Zinc uptake efficiency, Zinc-aspartate (Zn-Asp) complex, Zinc transporter

Zinc plays indispensable roles in almost all aspects of metabolism, catalysis and regulatory functions. Intracellular homeostasis of zinc is regarded to be important because of the different biological roles that zinc performs. Intracellular zinc concentration is related to cell fate, i.e., differentiation, apoptosis or proliferation, and changes of zinc homeostasis are liked to several pathologies[1,2]. Zinc levels are low in patients with renal disease, liver disease, cardiovascular disease, tumor(s) and some cancers including gastrointestinal and breast cancers[3].

Kidney, intestine and liver play an important part in maintaining zinc homeostasis. To obtain zinc homeostasis under different conditions, cells must adapt the rate of zinc uptake and efflux, binding to intracellular and extracellular proteins or other molecules[4,5]. Intestine and kidney are target organs for various regulators of mineral metabolism[6].

Excess zinc can also be toxic to cells and aberrant levels of zinc have been linked to various disease states thereby making it vital that the level of intracellular zinc is tightly controlled[7]. As zinc is a small hydrophilic charged species, it cannot cross biological membranes by passive diffusion. Therefore, specialized mechanisms are required for both zinc uptake and release[8,9]. A group of proteins called zinc transporters is dedicated to this transport of zinc across biological membranes.

Many transporters regulate zinc homeostasis[6]. Zinc transporter (ZnT) members facilitate its efflux from the cytosol[10]. Zinc transporter 1 (ZnT-1) is expressed ubiquitously, but abundantly in the small intestine, kidney and placenta. Expression of the ZnT-1 gene permitted the cell to grow in the presence of high levels of extracellular zinc by promoting zinc efflux activity. Furthermore, ZnT-1 could offer zinc resistance to some wild-type cell lines[11,12]. ZnT-2 mRNA is detectable in the kidney, small intestine, testis, mammary gland and prostate. The overexpression of ZnT-2 facilitates the accumulation of zinc into the vesicles in high zinc conditions. ZnT-2 functions as a zinc transporter to sequester zinc into endosomes. ZnT-2 expression in the small intestine and kidney is enhanced by supplemental zinc intake[9, 13,14].

There is other transporter, which associated to zinc uptake. The high-affinity transporter for anionic amino acids, originally called EAAC1, and now mostly referred to as EAAT3, is expressed in the intestine and kidney, as well as in the brain[15]. EAAT-3 functions as the high-affinity L-aspartate transporter in prostate cells that is responsible for the uptake and accumulation of aspartate[16].

**Table 1.** Sequences of oligonucleotide primers for PCR.

| Zinc transporter | | Primer | Size bp |
|---|---|---|---|
| ZnT-1 | F | 5′-GGAGTGGAGGAAGTCCATGA-3′ | 897 |
| | R | 5′-GGCACGTAACTACCCCTCAA-3′ | |
| ZnT-2 | F | 5′-TCCATCAGTCTGGACATGGA-3′ | 266 |
| | R | 5′-CCCTTCCATGAGAACCAAGA-3′ | |
| EAAT-3 | F | 5′-GGCTTGCAATCCACTCCATT-3′ | 160 |
| | R | 5′- AGAAGAGCCTGAGCCATTCC-3′ | |
| G3PDH | F | 5′-ATGACTCTACCCACGGCAAG-3′ | 388 |
| | R | 5′-ACTGTGGTCATGAGCCCTTC-3′ | |

F: Forward / R: Reverse

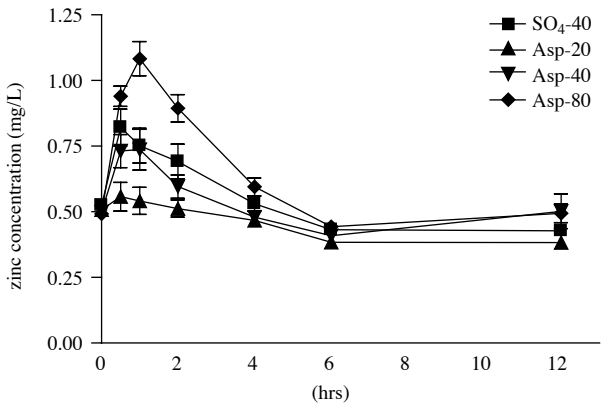


**Figure 1.** Time-course analysis of plasma zinc concentration. After oral administration of $ZnSO_4$ (40 mg/kg) and Zn-Asp (20, 40, 80 mg/kg), blood was collected at 0, 0.5, 1, 2, 4, 6 and 12 hrs, and then plasma were incubated with zinquin for 30 min. Fluorescence was measured in a *Wallac 1420* at excitation wavelength of 370 nm and at emission wavelength at 460 nm.

In the present study, we investigated change in plasma zinc concentration and renal zinc uptake efficiency after oral administration of zinc-aspartate complexes (Zn-Asp) compared with $ZnSO_4$. Also, we examined the expression of transporters to clarify the intracellular zinc uptake mechanism(s). Elucidation of the zinc retention system in the kidney will be useful for investigating kidney diseases.

### *In vivo* study

**Body and Organs Weight.** Body and various organs weight including kidney, brain, liver, prostate gland, thymus and testis are shown in Table 1. The average body weight of each group 40 mg/kg $ZnSO_4$ administrated group, and 20 mg/kg, 40 mg/kg and 80 mg/kg Zn-Asp administrated group were 235.2 kg, 235.3 kg, 236.3 kg and 236.2 kg, respectively.

**Plasma Zinc Concentration after Zinc Oral Administration.** As shown in Figure 1, plasma zinc concentration was peaked out at 1 hr, and its concentration reached minimum level at 6 hrs after oral administration. The level of plasma zinc returned to the basal level at 12 hrs after oral administration. We observed that the plasma zinc level was increased in response to zinc oral administration in dose-dependent manner. The plasma zinc level of 80 mg/kg Zn-Asp administered group reached up to the highest level among these groups. The plasma zinc level was dose-dependent.

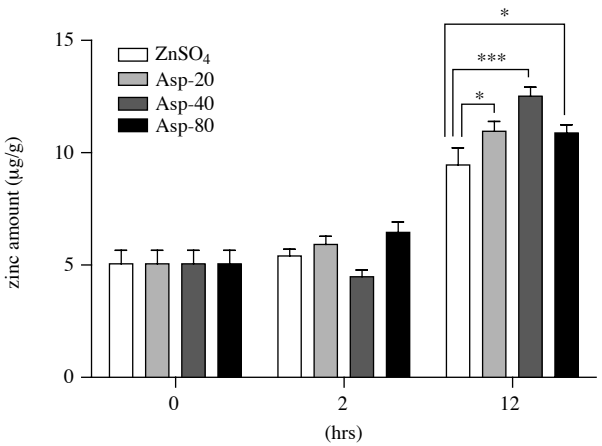


**Figure 2.** Zinc concentration in kidney after zinc oral administration. After oral administration of $ZnSO_4$ and Zn-Asp, kidney was collected at 0, 2 and 12 hrs. For measurement of renal zinc level, homogenated tissue was incubated with zinquin for 30 min. Fluorescence of tissue suspension was measured in a *Wallac 1420* at excitation wavelength of 370 nm and at emission wavelength at 460 nm. *indicates statistically significant differences ($P < 0.05$) ***indicates statistically significant differences ($P < 0.001$)

**Zinc Level in Kidney after Oral Zinc Administration.** As shown in Figure 2, the renal zinc level was no change at 2 hrs after oral zinc administration. The zinc levels were peaked out in all groups at 12 hrs. The zinc concentration of 40 mg/kg Zn-Asp administered group was the highest level among others after oral zinc administration. The renal zinc level was increased in response to zinc oral administration in time-dependent manner.

**The Effect of Zinc-ligand Complexes on mRNA Expression of Transporters.** Figure 3 shows that mRNA expression of each ZnT genes including ZnT-1, ZnT-2 and EAAT-3 in kidney. ZnT-1and ZnT-2 mRNA expression were significantly increased in Zn -Asp administered group. ZnT-1 mRNA expression level in Zn-Asp administered group was increased approximately 2-fold compared to control and $ZnSO_4$

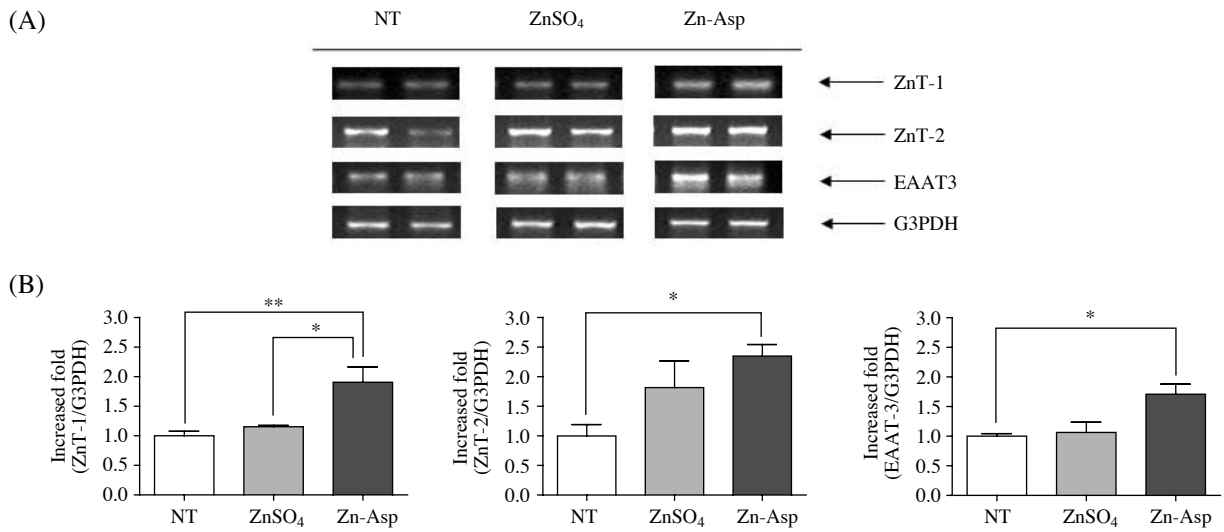


**Figure 3.** The effect of $ZnSO_4$ and Zn-Asp on mRNA expression zinc transporters *in vivo* study by RT-PCR. Relative mRNA expression of ZnT-1, ZnT-2 and EAAT-3 in kidney in response to zinc oral administration. (A) ZnT-1, ZnT-2, EAAT-3 mRNA and G3PDH (used as a RNA loading control). (B) show relative abundance of ZnT-1, ZnT-2 and EAAT-3 mRNA determined by RT-PCR analysis of total RNA samples(5 μg) from individual rats as measured by scanning densitometry. G3PDH was used as an RNA loading control. *indicates statistically significant differences ($P<0.05$) **indicates statistically significant differences ($P<0.01$).

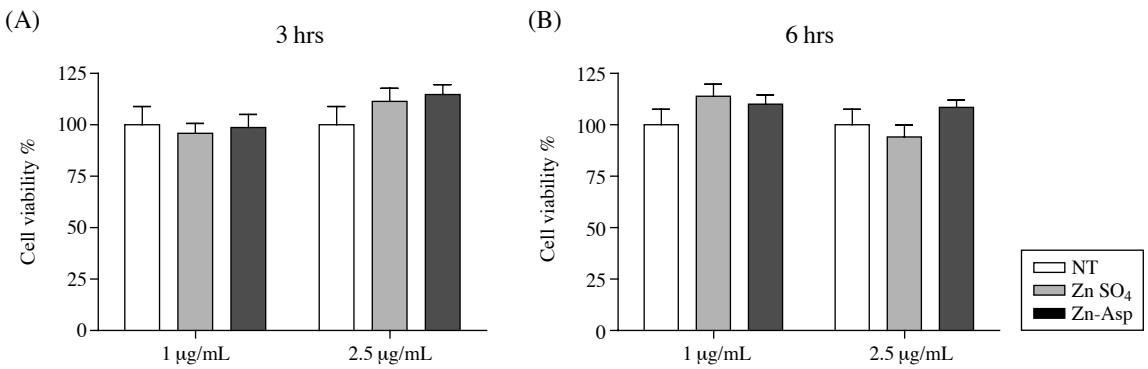


**Figure 4.** The effect of $ZnSO_4$ and Zn-Asp on cell viability. Cell viability was determined by MTT assay. Result was expressed as percentage viability, with 100% viability for non treated cells. HEK 293 cell were incubated with the concentration of zinc in the medium for 3 hrs and 6 hrs.

administered groups. Also, ZnT-2 mRNA expression in Zn-Asp administered group was increased 2.4-fold in comparison with control group. The m-RNA expression of EAAT-3 was elevated 1.7-fold comparison with control and $ZnSO_4$ groups.

### *In vitro* study

**The Effect of Zinc-ligand Complexes on Human Embryonic Kidney Cell Viability.** HEK293 cells were treated with different concentration and time of zinc. Cell viability was maintained in $ZnSO_4$ and Zn-Asp treated medium for 6 hrs, but decreased at 24 hrs. Therefore, we decided that 2.5 μg/mL zinc-ligand complexes was optimal at 6 hrs.

**Intracellular Zinc Concentration in HEK293 Cell.** As shown in Figure 6, intracellular zinc concentration was peaked out at 3 hrs in both of $ZnSO_4$ and Zn-Asp treated group after zinc treatment. Intracellular zinc concentration was increased approximately 1.8 and 2.2-fold in comparison with control group at 3 hrs after $ZnSO_4$ and Zn-Asp treatment, respectively. Its level was increased about 1.4 and 1.7-fold compared to control group at 6 hrs after $ZnSO_4$ and Zn-Asp treatment, respectively.

**The Effect of Zinc-ligand Complexes on mRNA Expression of Transporters in HEK293 Cell Lines.** To investigate the effect of zinc-ligand complexes on the transcription level of transporters such as ZnT-1 and EAAT-3, we treated HEK293 cells with 2.5 μg/mL $ZnSO_4$ and Zn-Asp for 3 hrs and 6 hrs. The mRNA expression of ZnT-1 in $ZnSO_4$ and Zn-Asp treated group was elevated 3.8 and 4.2-fold at 3 hrs, and was increased 2.9 and 3.2-fold in comparison with control group at 6 hr, respectively. The mRNA expression of EAAT-3 in $ZnSO_4$ and Zn-Asp groups was increased 2-fold in comparison with control group at 6 hrs.

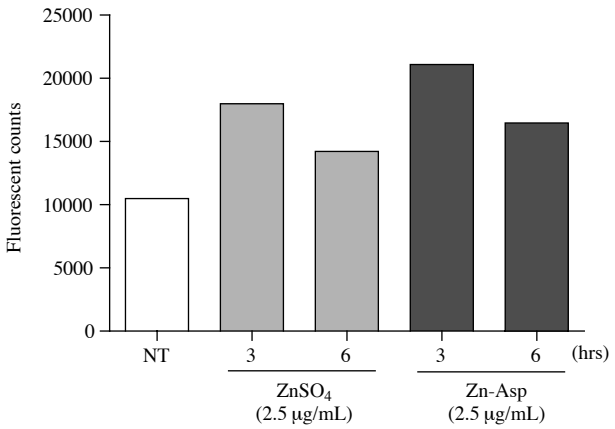


**Figure 5.** Intracellular zinc level in HEK 293 cell line. Cells were harvested at 3 hrs and 6 hrs after treatment of 2.5 μg/mL $ZnSO_4$ and Zn-Asp. For measurement of intracellular zinc level, cells were incubated with zinquin for 30 min. Fluorescence of cell suspension was measured in a *Wallac 1420* at excitation wavelength of 370 nm and at emission wavelength at 460 nm (NT: Not Treatment).

**The Effect of Zinc-ligand Complexes on Protein Level of Zinc Transporter-1 in HEK293 Cell Lines.** Figure 7 shows that relative protein expression of ZnT-1 in HEK293 cell in response to different zinc treatment. We treated HEK293 cells with 2.5 μg/mL $ZnSO_4$ and Zn-Asp for 3 hrs and 6 hrs. The protein expression of ZnT-1 in $ZnSO_4$ and Zn-Asp treated group was elevated 1.4 and 1.8-fold at 3 hrs, and was increased 2.4 and 2.3-fold in comparison with control group at 6 hrs, respectively.

## Discussion

Kidney plays indispensable roles in homeostasis of many minerals including zinc, iron and manganese. Especially, the portion of zinc that enters the glomerular filtrate is efficiently reabsorbed along the nephron through a mechanism yet to be identified[17]. It is reported that patients with renal diseases had low zinc levels in kidney[18]. Therefore, zinc homeostasis through zinc uptake and release is effective against supplementation of zinc loss and prevention of zinc accumulation for patients with renal disease.

In the present study, we first examined the effect of Zn-Asp on renal zinc uptake efficiency *in vivo* study. We found that the renal zinc level was higher in Zn-Asp administrated group than control and $ZnSO_4$ administered group. In addition, we showed that Zn-Asp administration increased mRNA expression of zinc transporters and aspartate transporter in kidney. Our time course study revealed that the plasma zinc concentration was peaked out at 1 hr, and its concentration reached minimum level at 6 hrs after oral zinc

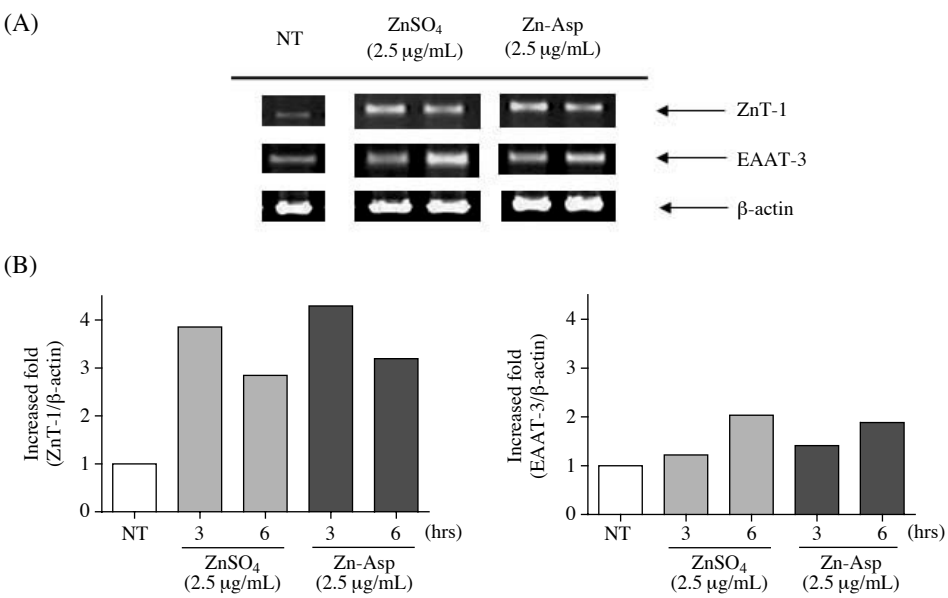


**Figure 6.** Relative mRNA expression of zinc transporter ZnT-1 and EAAT-3 mRNAs in HEK293 cell in response to different zinc treatment. (A) ZnT-1, EAAT-3 mRNA and β-actin (used as an RNA loading control). (B) show relative abundance of ZnT-1 and EAAT-3 mRNA determined by RT-PCR analysis of total RNA samples (5 μg) by scanning densitometry.

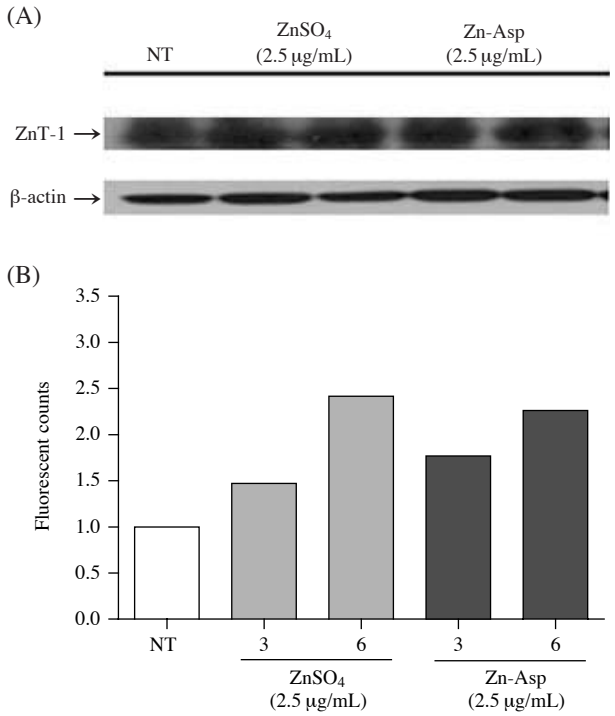


**Figure 7.** Relative protein expression of ZnT-1 in HEK293 cell in response to different zinc treatment. HEK 293 cells were treated with 2.5 μg/mL zinc for 3 or 6 hrs, after which their protein extracts (30 μg) were size-separated on a 15% SDS-PAGE gel, transferred onto NC membranes (0.45 μm) and immunoblotted with an anti-ZnT-1 antibody. (A) A representative Western blot showing ZnT-1 protein levels in the zinc-ligand complexes treatment for 3 and 6 hrs. (B) Quantitative analysis of the ZnT-1 protein levels in the gel shown in (A) (NT: not treated).

administration. Also plasma zinc levels were restored to the basal levels until 12 hrs. We observed that zinc concentration in kidney was steadily increased until 12 hrs after zinc administration. It suggested that administrated zinc was started to absorb into kidney from plasma after 1 hr.

As shown in Figure 3, zinc uptake into kidney were higher in Zn-Asp administered group than in $ZnSO_4$ administrated group. As zinc is a small hydrophilic charged species, it cannot cross biological membranes by passive diffusion, but can cross the biological membrane freely through their transporters. We speculated that increase of renal zinc level was related with the expression of zinc transporters that regulate the zinc homeostasis in kidney. We examined the mRNA expression of zinc transporters including ZnT-1, ZnT-2 and EAAT-3. Juan P. Liuzzi *et al.* reported that ZnT-1 and ZnT-2 mRNA levels were increased in kidney when zinc was provided as a 70 mg/kg body oral dose. We showed that Zn-Asp was resulted to increase mRNA expression of ZnT-1, ZnT-2 and EAAT-3 (Figure 3, 4). Therefore, we can guess that increase of zinc uptake into kidney was related to mRNA expression of zinc transporters such as ZnT-1 and ZnT-2.

We attempt to investigate the optimal concentration and exposing time of zinc-ligand complexes for subsequent experiments. Cell viability was maintained 2.5 μg/mL $ZnSO_4$ and Zn-Asp treated medium for 6 hrs, but it decreased at 24 hrs. Therefore, we determined that 2.5 μg/mL zinc-ligand complexes was optimal at 6 hrs. Our time course *in vitro* study revealed that intracellular zinc level was peaked out at 3 hrs in after zinc treatment. The mRNA expression level of ZnT-1 was peaked out at 3 hrs, but EAAT-3 was peaked out at 6 hrs after zinc treatment. The protein expression of ZnT-1 in $ZnSO_4$ and Zn-Asp treated group was elevated 2.4 and 2.3-fold in comparison with control group at 6 hrs, respectively.

In conclusion, this study showed that Zn-Asp is effective the zinc uptake into the kidney by increasing the zinc transporter expression. Therefore, zinc homeostasis through zinc uptake and release is effective against supplementation of zinc loss and prevention of zinc accumulation for patients with renal disease.

## Methods

### *In vivo* Study

**Experimental Animal.** Male Sprague-Dawley Rats, 8 weeks old, were housed in an environmentally controlled room (25°C, 12L : 12D cycle) and fed standard chow pellets and water. Animal were starved for 14 hrs prior to experiments but allowed free access to water. Eighty-one SD rats were divided into three groups: control group (n=9), $ZnSO_4$ (n=18) and Zn-Asp (n=54). After oral administration of zinc substrate 20 mg/kg, 40 mg/kg, 80 mg/kg, we collected the blood at 0, 0.5, 1, 2, 4, 6 and 12 hrs. The animals were sacrificed by ether at 2 hrs and 12 hrs, and collected kidney.

**Determination of Zinc Concentration.** Whole blood was centrifuged at 3,500 rpm for 10 min, and then plasma was collected. Tissue and autoclaved distilled water was in the ratio of nine to one, and then was homogenized. The homogenate was centrifuged at 3,500 rpm, 4°C for 10 min. Plasma 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.

**Table 2.** Average weight of body and organs.

| Treatment group | Body weight | Weight | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Kidney | | Brain | | Liver | | Prostate | | Testis | | Thymus | |
| | | Weight (g) | Ratio (%) | Weight (g) | Ratio (%) | Weight (g) | Ratio (%) | Weight (g) | Ratio (%) | Weight (g) | Ratio (%) | Weight (g) | Ratio (%) |
| $ZnSO_4$-40 | 235.2 | 1.94 | 0.008 | 1.58 | 0.007 | 9.18 | 0.039 | 0.21 | 0.001 | 2.78 | 0.012 | 0.61 | 0.003 |
| Zn-Asp-20 | 235.3 | 1.87 | 0.008 | 1.59 | 0.007 | 8.62 | 0.037 | 0.23 | 0.001 | 2.72 | 0.012 | 0.53 | 0.002 |
| Zn-Asp-40 | 236.3 | 1.86 | 0.007 | 1.58 | 0.007 | 8.96 | 0.038 | 0.22 | 0.001 | 2.75 | 0.012 | 0.52 | 0.002 |
| Zn-Asp-80 | 236.2 | 1.91 | 0.007 | 1.57 | 0.007 | 9.18 | 0.039 | 0.21 | 0.001 | 2.75 | 0.012 | 0.53 | 0.002 |

*ratio=organ weight/ body weight

### *In vitro* Study

**Cell Lines and Culture Conditions.** Human embryonic kidney cell line (HEK293) were routinely cultured in RPMI1640 plus 10% fetal bovine serum and 1% antibiotic-antimycotic (WelGENE) at 37°C with 5% $CO_2$.

**MTT Assay.** Cells were plated at a density of $1 \times 10^4$ in 10 mm dishes. After incubation for 48 hrs, the cells were treated with 1 μg/mL and 2.5 μg/mL $ZnSO_4$ and Zn-Asp for 3 hrs and 6 hrs. Cellular viability in the presence or absence of experimental agents was determined using the Mosmans's MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-dimethyl tetrazolium bromide; Sigma) assay. Briefly, following $ZnSO_4$ and Zn-Asp treatment, 20 μL of a 5 mg/mL MTT solution were added in each well, and the plate was incubated for 3 hrs at 37°C. The medium was then removed, and the colored reaction product was solubilized in 200 μL DMSO (Sigma). Absorbance was measured at 540 nm using a *MicroPlate Reader*.

**Quantitative Reverse Transcriptase-Polymerase Chain Reaction Analysis.** Total cellular RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Extracted RNA was dissolved in diethylpyro-carbonate (DEPC)-treated water (Invitrogen) and quantified by measuring the absorbance at 260 nm. Aliquots of 2.5 μg (*In vitro*) and 5 μg (*In vivo*) of total RNA were used to synthesize the first-strand complementary DNA (cDNA) with MMLV RT-ase (Invitrogen) and subjected to PCR amplification with the oligonucleotide primers listed in Table 2 using a thermal cycler. The optimal PCR conditions were determined as the amount of amplification product in proportion to that of input RNA. G3PDH, β-actin served as an internal RNA control to allow comparison of RNA levels among different specimens. After PCR, the reaction products were resolved on 2.0% agarose gels and visualized with ethidium bromide.

**Western Blot.** Cell were treated with 2.5 μg/mL for 3 and 6 hrs and harvested, washed with ice-cold PBS, treated with lysis buffer and centrifuged to collect the protein sample. Diluted samples containing equal amounts of protein were mixed with 6X SDS sample buffer and separated on a 15% SDS-polyacrylamide gel, followed by electrophoretic transfer to 0.45 μm nitrocellulose membranes. The membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.05% Tween 20 (TBS-T) and incubated overnight with antibody against zinc transporter-1 (ZnT-1) at 4°C. Detection was performed with a horseradish peroxidase-conjugated rabbit anti-goat antibody and then enhanced with Western Blotting Luminol Reagent (Santa Cruz Biotechnology, Inc.).

## Acknowledgements

We thank Dr. Yoon S. Cho-Chung (Cellular Biochemistry Section, BasicResearch Laboratory, CCR, NCI, NIH, Bethesda, MD) and Dr Kevin G. Becker (DNA Array Unit, NIA, NIH, Baltimore, MD) for valuable advices on cDNA microarray. This study was partially supported by grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, R. O. K (Hmp-00 GN-01-0002 & 03-PJ10-PG13-GD01-0002). This work was partially supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea Government (MOST) (No. R13-2003-016-03002-0 & No. M20502240001-06L0224-00111).

## References

1. Devergnas, S. *et al*. Differential regulation of zinc efflux transporters ZnT-1, ZnT-5 and ZnT-7 gene expression by zinc levels: a real-time RT-PCR study. *Biochem Pharmacol* **68**:699-709 (2004).
2. McMahon, R. J. & Cousins, R. J. Regulation of the

zinc transporter ZnT-1 by dietary zinc. *Proc Natl Acad Sci* **95**:4841-4846 (1998).
3. Boonsiri, P. *et al*. Serum vitamin A and zinc levels of healthy people in northeast Thailand. *Clin Chim Acta* **373**:132-138 (2006).
4. Liuzzi, J. P., Blanchard, R. K. & Cousins, R. J. Differential regulation of zinc transporter 1, 2, and 4 mRNA expression by dietary zinc in rats. *J Nutr* **131**: 46-52 (2001).
5. Kaler, P. & Prasad, R. Molecular cloning and functional characterization of novel zinc transporter rZip10 (Slc39a10) involved in zinc uptake across rat renal brush-border membrane. *Am J Physiol Renal Physiol* **292**:F217-229 (2007).
6. Prasad, R., Kumar, V., Kumar, R. & Singh, K. P. Thyroid hormones modulate zinc transport activity of rat intestinal and renal brush-border membrane. *Am J Physiol* **276**:E774-782 (1999).
7. Taylor, K. M. & Nicholson, R. I. The LZT proteins; the LIV-1 subfamily of zinc transporters. *Biochim Biophys Acta* **1611**:16-30 (2003).
8. Cousins, R. J. & McMahon, R. J. Integrative aspects of zinc transporters. *J Nutr* **130**(5S Suppl):1384S-1387S (2000).
9. Kumar, R. & Prasad, R. Functional characterization of purified zinc transporter from renal brush border membrane of rat. *Biochim Biophys Acta* **1509**:429-439 (2000).
10. Krebs, N. E. & Hambidge, K. M. Zinc metabolism and homeostasis: the application of tracer techniques to human zinc physiology. *Biometals* **14**:397-412 (2001).
11. Iguchi, K. *et al*. High-level expression of zinc transporter-2 in the rat lateral and dorsal prostate. *J Androl* **23**:819-824 (2002).
12. Kambe, T., Yamaguchi-Iwai, Y., Sasaki, R. & Nagao, M. Overview of mammalian zinc transporters. *Cell Mol Life Sci* **61**:49-68 (2004).
13. Cousins, R. J. *et al*. Regulation of zinc metabolism and genomic outcomes. *J Nutr* **133**:1521S-1526S (2006).
14. Pawan, K. *et al*. Upregulation of Slc39a10 gene expression in response to thyroid hormones in intestine and kidney. *Biochim Biophys Acta* **1769**:117-123 (2007).
15. François, Verrey. *et al* Novel renal amino acid transporters. *Annu Rev Physiol* **67**:557-572 (2005).
16. Franklin, R. B., Zou, J., Yu, Z. & Costello, L. C. EAAC1 is expressed in rat and human prostate epithelial cells; functions as a high-affinity L-aspartate transporter; and is regulated by prolactin and testosterone. *BMC Biochem* **27**:7-10 (2006).
17. Ranaldi, G. *et al*. Intracellular distribution of labile Zn(II) and zinc transporter expression in kidney and MDCK cells. *Am J Physiol Renal Physiol* **283**:F1365-1375 (2002).
18. Palmiter, R. D. Protection against zinc toxicity by metallothionein and zinc transporter 1. *Proc Natl Acad Sci* **101**:4918-4923 (2004).