Inadequate intake of zinc exacerbates blood pressure and renal function via superoxide radical-induced oxidative stress

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Abstract
Zn-excess intake augmented blood pressure (BP) and reduced renal blood flow (RBF) and inulin clearance. The decline in inulin clearance may be due to a fall in RBF. Treatment with the nitric oxide (NO) synthase inhibitor, L-NAME, further increased BP and markedly decreased RBF in the Zn-excess condition. Inversely, administration of the exogenous superoxide radical scavenger, tempol, significantly decreased BP and substantially increased RBF in the Zn-excess setting. As a result, tempol dramatically restored BP and RBF levels seen in the Zn-excess setting to levels comparable to those observed in the control setting. These observations suggest that both an increment in BP and a decrement in RBF seen in the Zn-excess condition come from a decrement in the action of the vasodilator, NO, via the formation of peroxynitrite based upon the non-enzymatic reaction of NO with increased superoxide radical. Indeed, the activity of the endogenous superoxide radical scavenger, Cu/Zn-superoxide dismutase (SOD), was significantly decreased in the vessel wall of the Zn-excess vs. the control setting. The reduction in the activity of Cu/Zn-SOD in the Zn-excess setting was the result of Cu deficiency secondary to Zn-excess ingestion. With respect to BP, RBF, inulin clearance, L-NAME and tempol treatment and the activity of Cu/Zn-SOD, similar results were observed in the Zn-deficient setting. Thus, inadequate ingestion of Zn leads to the deterioration of BP and renal function through the oxidative stress caused by superoxide radical.

Key words: Zinc, Blood pressure, Renal function, Oxidative stress, Cu/Zn-superoxide dismutase

Introduction
Zinc (Zn), is an essential trace element in humans and animals[1-3]. Zn deficiency causes the disorder of the skin epithelium, the gastrointestinal system, the reproductive system, the neural system, the endocrine system, the ocular system and the immune system[1-3]. Recently, Zn tablets and Zn-supplemented food have been sold in Japan, the USA and Europe in order
to obtain good health and health promotion\cite{3}. In addition, oral Zn therapy has been more recently carried out to treat hypogeusia and decubitus ulcers in adults\cite{3} and hypogonadism and growth retardation from infants to adolescents \cite{4, 5}. Nevertheless, Zn toxicity derived from Zn-excess intake has not been fully understood. Thus, the present literature is focused on the novel clinical aspects, the aggravation of blood pressure (BP) and renal function, caused by the Zn-deficient and Zn-excess settings.

**Physiologic function of zinc**

Zinc serves as the active center of approximately 300 enzymes such as carbonic anhydrase, alkaline phosphatase, Cu/Zn-superoxide dismutase (SOD), etc. (Table 1) \cite{2, 3}. Resultantly, zinc contributes to growth, development, wound healing, immune functions, skin metabolism (particularly collagen synthesis), the maintenance of central nervous system functions, the maintenance of retinal functions (participation in vitamin A metabolism), senses of taste and olfaction, saliva secretion, the production and activity of sperm, the prevention of carcinogenesis and aging (participation in scavenging superoxide radical), the maintenance of gonadal functions and pregnancy (participation in the synthesis and secretion of sex hormones), glucose metabolism (participation in the synthesis and action of insulin), and lipid metabolism \cite{1-3}.

**Cu/Zn-SOD**

The endogenous superoxide radical scavenger, Cu/Zn-SOD, dismutates superoxide radical to hydrogen peroxide\cite{6, 7}. The hydrogen peroxide in turn is converted into water by catalase and glutathione peroxidase\cite{6, 7}. Cu/Zn-SOD is an enzyme requiring both Cu and Zn for exhibiting the function\cite{1, 3}. The activity of Cu/Zn-SOD falls in either a Zn-deficient or a Cu-deficient state, or both\cite{1}. It has been recently accumulated that Cu/Zn-SOD has a protective effect on the oxidative stress induced by superoxide radical through the superoxide radical scavenging ability\cite{3, 8-11}. Thus, Cu/Zn-SOD may play a fundamental role in defending the vessel wall against superoxide radical-induced oxidative stress\cite{12, 13}.

**Nitric oxide and superoxide radical**

The vasodilatory gas, nitric oxide (NO), is predominantly generated by endothelial nitric oxide synthase (NOS) from the terminal guanidino nitrogen of L-arginine in vascular endothelial cells\cite{14, 15}. NO takes part in the regulation of vascular tone in the vessel wall through the soluble guanylate cyclase-mediated signaling pathway\cite{14}. As a consequence, NO contributes to the regulation of systemic BP and RBF via the modulation of vascular tone \cite{9, 16}. Evidence of interest \cite{9, 11, 16-19} has been recently accumulated that NO non-enzymatically reacts with superoxide radical (OO\textsuperscript{-}) in the vessel wall, consequently resulting in

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\text{NO}^\cdot + \text{OO}^- \rightarrow \text{ONOO}^-
\]

*Fig. 1* Non-enzymatic reaction of nitric oxide with superoxide radical, resultantly generating peroxynitrite.

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**Table 1** Zinc enzymes and their Functions

<table>
<thead>
<tr>
<th>Zinc enzyme</th>
<th>Function</th>
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<tbody>
<tr>
<td>carbonic anhydrase, peptidase, alcohol dehydrogenase, alkaline phosphatase,</td>
<td>cell division, nucleic acid</td>
</tr>
<tr>
<td>polymerase, Cu/Zn-superoxide dismutase, angiotensin-converting enzyme,</td>
<td>metabolism, enzymes</td>
</tr>
<tr>
<td>collagenase, ( \delta )-aminolevulinic acid anhydrase, protein kinase C,</td>
<td></td>
</tr>
<tr>
<td>phospholipase C, aspartate transcarbamylase, nucleotide phosphorylase</td>
<td></td>
</tr>
<tr>
<td>(5'-nucleotidase), RNase, etc.</td>
<td></td>
</tr>
</tbody>
</table>

References [2, 3].
a decrease in the vasodilatory action of NO via the formation of peroxynitrite (ONOO−) (Fig. 1).

Effects of Zn-excess intake on blood pressure and renal function

Rats given 22 g/day of 0.05% Zn-excess diet (11 mgZn/day) and 0.2% Zn-excess diet (44 mgZn/day) for 4 weeks showed a dose-dependent increase in basal systolic BP, diastolic BP and mean BP levels and a dose-dependent decrease in basal renal blood flow (RBF) and inulin clearance levels when compared to rats fed a control diet containing 0.005% Zn (1.1 mgZn/day) for 4 weeks (Fig. 2). These findings indicate an increase in basal systemic BP levels [19, 20] and a fall in basal renal function derived from Zn-excess ingestion [20]. The fall in inulin clearance in rats fed two Zn-excess diets may be the result of a decrease in RBF because hematoxylin-eosin (H-E) staining exhibited no significant morphologic changes in the kidneys from rats fed a 0.2% Zn-excess diet (20).

Intravenous injection of the NOS inhibitor, N^−nito-L-arginine methyl ester (L-NAME), increased

Fig. 2 Basal blood pressure (mmHg) levels (A) and basal renal blood flow (arbitrary units) and inulin clearance (ml/min/g kidney weight) levels (B) obtained from rats fed a control diet containing 0.005% Zn or two Zn-excess diets containing 0.05% Zn or 0.2% Zn for 4 weeks. Blood pressure, renal blood flow and inulin clearance were measured at 4 weeks after the initiation of dietary manipulation. Data reported represent means ± SD of the values obtained from seven rats in each group. Statistical analysis was based upon one-way analysis of variance. Reference [20]
mean BP levels and decreased RBF levels in rats fed a control diet and two Zn-excess diets (Fig. 3). The mean BP levels increased and the RBF levels decreased were comparable among the three groups of rats. These observations suggest the involvement of NO in the regulation of systemic BP and RBF in the three groups of rats. Therefore, the vasodilatory gas, NO, may have a central role in suppressing an elevation in systemic BP and a fall in renal function in the Zn-excess setting.

Intravenous administration of the membrane-permeable SOD mimetic compound, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (tempol), led to a reduction in mean BP levels and an increase in RBF levels in rats fed a control diet and two Zn-excess diets (Fig. 4). These observations indicate

**Fig. 3**  Effects of L-NAME administration on mean blood pressure (mmHg) levels (A) and renal blood flow (arbitrary units) levels (B) observed in rats fed a control diet containing 0.005% Zn or two Zn-excess diets containing 0.05% Zn or 0.2% Zn for 4 weeks. Data reported represent means ± SD of the values obtained from seven rats in each group. Statistical analysis was based upon two-way analysis of variance. White bars; basal condition groups. Black bars; L-NAME administration groups. Reference [20]

**Fig. 4**  Effects of tempol administration on mean blood pressure (mmHg) levels (A) and renal blood flow (arbitrary units) levels (B) observed in rats fed a control diet containing 0.005% Zn or two Zn-excess diets containing 0.05% Zn or 0.2% Zn for 4 weeks. Data reported represent means ± SD of the values obtained from eight rats in each group. Statistical analysis was based upon two-way analysis of variance. White bars; basal condition groups. Black bars; tempol administration groups. Reference [20]
that superoxide radical may be a modifier of systemic BP and renal function via a fall in the action of the vasodilatory gas, NO, based on the formation of peroxynitrite in rats fed two Zn-excess diets. Thus, the mechanisms responsible for an elevation in systemic BP and the deterioration of renal function in rats fed two Zn-excess diets may be due to an increase in the action of superoxide radical in the vessel wall. In fact, a dose-dependent increment in 8-hydroxy-2′-deoxyguanosine formation caused by enhanced superoxide radical production was observed in rats fed two Zn-excess diets when compared to rats fed a control diet (Table 2). In addition, the activity of the intrinsic superoxide radical scavenger, Cu/Zn-SOD, in the thoracic aorta was significantly decreased in rats fed two Zn-excess diets relative to rats fed a control diet in a dose-dependent manner (Table 3). This fall in the activity of Cu/Zn-SOD may be the effect of Cu deficiency secondary to Zn-excess ingestion, contributing to an increment in the action of superoxide radical in the vessel wall of rats fed two Zn-excess diets.

There were no significant differences in mean BP and RBF levels after L-NAME or tempol treatment among rats fed a control diet and two Zn-excess diets (Fig. 3, 4). Mean BP levels obtained from rats fed two Zn-excess diets were essentially comparable to those obtained from rats fed a control diet. However, RBF levels obtained from rats fed two Zn-excess diets were somewhat different from those obtained from rats fed a control diet. There is a possibility that in addition to both NO and superoxide radical, some vasoconstrictive factor participates in the modulation of RBF levels in the Zn-excess settings. We have recently found a significant and dose-dependent increase in the potent vasoconstrictor, angiotensin II, in the kidneys of rats fed two Zn-excess diets when compared to those of rats fed a control diet (21). This increased angiotensin II may be in part related to an alteration in RBF levels observed in Zn-excess ingestion.

### Lowest observed adverse effect level of Zn

Values for both systemic BP and renal RBF observed in rats fed a 0.05% Zn-excess diet (11 mgZn/day) were at the borderline in statistical significance when compared to those obtained from rats fed a control diet (1.1 mgZn/day). This suggests that the lowest observed adverse effect level of Zn is approximately 11 mg/day in rats (Fig. 2).

### Effects of zinc-deficient intake on blood pressure and renal function

Spontaneously hypertensive rats (SHR) fed a Zn-deficient diet with no addition of Zn for 4 weeks had a significant increase in systolic BP levels at 2 and 4 weeks after the start of dietary manipulation when compared to SHR fed a control diet for 4 weeks (11). Intravenous administration of L-NAME significantly augmented mean BP levels in SHR fed a control or a Zn-deficient diet for 4 weeks. Levels of mean BP after L-NAME treatment were comparable in the two

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**Table 2** Levels of 8-OHdG in the kidneys of rats fed a control diet or two Zn-excess diets for 4 weeks

<table>
<thead>
<tr>
<th>Diet</th>
<th>Levels of 8-OHdG (ng/μg DNA)</th>
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<tbody>
<tr>
<td>Control diet</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>0.05% Zn diet</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>0.2% Zn diet</td>
<td>4.8 ± 0.6*</td>
</tr>
</tbody>
</table>

Levels of 8-OHdG (ng/μg DNA) in the kidneys were measured at the end of dietary treatment for 4 weeks. Data reported are means ± SD of the values obtained from eight rats in each group. Intergroup comparisons were based upon one-way analysis of variance. (*) P<0.005 compared to the value obtained from the control diet group. Abbreviations used: 8-OHdG, 8-hydroxy-2′-deoxyguanosine. Reference [20].

**Table 3** Activities of Cu/Zn-superoxide dismutase in the thoracic aorta of rats fed a control diet or two Zn-excess diets for 4 weeks

<table>
<thead>
<tr>
<th>Diet</th>
<th>Activities of Cu/Zn-SOD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td>32.6 ± 2.9</td>
</tr>
<tr>
<td>0.05% Zn diet</td>
<td>24.2 ± 1.8*</td>
</tr>
<tr>
<td>0.2% Zn diet</td>
<td>8.3 ± 0.7*</td>
</tr>
</tbody>
</table>

Activities of Cu/Zn-superoxide dismutase (U/mg protein) in the thoracic aorta were measured at the end of dietary treatment for 4 weeks. Data reported are means ± SD of the values obtained from eight rats in each group. Intergroup comparisons were based upon one-way analysis of variance. (*) P<0.005 compared to the value obtained from the control diet group. Reference [20].
groups of rats (11), demonstrating the involvement of the vasodilator, NO in the regulation of BP. On the other hand, tempol treatment significantly reduced mean BP levels in SHR fed a control or a Zn-deficient diet for 4 weeks (11). Resultantly, intravenous tempol treatment dramatically restored levels of mean BP observed in SHR fed a Zn-deficient diet for 4 weeks to levels comparable to those seen in SHR fed a control diet for 4 weeks (11), indicating the participation of superoxide radical in the regulation of BP. As with rats fed a Zn-excess diet for 4 weeks (19-21), these findings demonstrate that an elevation in BP levels seen SHR fed a Zn-deficient diet for 4 weeks may be due to a fall in the action of the vasodilator, NO, based on the formation of peroxynitrite derived from the non-enzymatic reaction of NO with increased levels of superoxide radical. Indeed, the activity of Cu/Zn-SOD scavenging superoxide radical was significantly reduced in rats fed a Zn-deficient diet for 4 weeks than in rats fed a control diet for 4 weeks (11).

Normotensive rats (NMR) fed a Zn-deficient diet for 4 weeks exhibited no significant increases in mean BP levels during the dietary conditioning when compared to NMR fed a normal diet for 4 weeks (9,22). However, NMR fed a Zn-deficient diet for 4 weeks, when compared to NMR fed a normal diet for 4 weeks, had a significant decrease in RBF levels and a significant increase in renal vascular resistance (RVR) levels, resulting in a fall in inulin clearance (9). Intravenous administration of L-NAME significantly increased mean BP and RVR levels and significantly decreased RBF levels in rats fed a control or a Zn-deficient diet for 4 weeks (9). Contrary to L-NAME administration, intravenous treatment with tempol significantly reduced mean BP and RVR levels in rats fed a control or a Zn-deficient diet for 4 weeks, although there are no significant differences in RBF levels between the two groups of rats (9). These observations are similar to those observed in the Zn-excess setting (19-21). Thus, the mechanisms responsible for the deterioration of renal function seen in rats fed a Zn-deficient diet for 4 weeks may be caused by a decrease in the action of the vasodilator, NO, as a consequence of an increase in the action of superoxide radical (9). In fact, the activity of the intrinsic superoxide radical scavenger, Cu/Zn-SOD, was significantly decreased in the kidneys of rats fed a Zn-deficient diet for 4 weeks relative to rats fed a control diet for 4 weeks (9).

In summary
Zn participates in the regulation of BP and renal function. Both Zn-deficient and Zn-excess ingestion alter BP levels and renal function through superoxide radical-induced oxidative stress. The mechanisms may be due to a reduction in the action of the vasodilator, NO, resulting from the formation of peroxynitrite generated by the non-enzymatic reaction of NO with
superoxide radical. Therefore, adequate intake of Zn is essential to maintain BP and renal function (Fig. 5).

References