Absent Effect of Zinc Deficiency on the Oxidative Stress of Erythrocytes in Chronic Uremic Rats

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Abstract

Both anemia and zinc deficiency are commonly observed in patients with chronic uremia. Oxidative stress of red blood cells (RBC) has been suggested to participate in the development of anemia in these patients with chronic uremia due to reduced life span of RBC. Whether zinc deficiency aggravates the effect of oxidative stress on RBC of chronic uremia is still not understood. We thus performed the study to determine the influence of zinc deficiency on the oxidative stress of RBC in uremic rats. Zinc deficiency was induced by long-term dietary zinc deficiency. Five-sixth nephrectomy (5/6 Nx) was used to produce chronic uremia. Experiment was carried out in the following five groups: normal control (NL), chronic uremia (Nx), chronic uremia + dietary zinc deficiency (Nx-D), Nx-D + zinc supplement (Nx-DZ) and Chronic uremia + pair-fed (Nx-PF). Osmotic fragility and lipid peroxidation of RBC were used to evaluate the oxidative stress of RBC. Five weeks after 5/6 nephrectomy (Nx), 5/6 Nx rats present a syndrome of uremia to elevate the levels of plasma creatinine and urea, and reduce the level of plasma zinc (1.12 ± 0.08 vs 1.35 ± 0.05 ug/ml). But they does not find to produce anemia and to increase osmotic fragility and lipid peroxidation in RBC. Dietary zinc deficiency in Nx-D group produced severe anorexia and reduced plasma zinc and selenium levels and the activity of RBC-GPX. Yet in Nx-D rats, osmotic fragility and susceptibility of lipid peroxidation in red cells did not increase, because of the increase of plasma copper level (1.85 ± 0.3 vs 1.41 ± 0.05 ug/ml) and RBC-SOD activity (1.95 ± 0.27 vs 0.78 ± 0.05 unit/g Hb). Zinc supplement in Nx-D rats (Nx-DZ group) recovered the appetite and normalized the levels of plasma zinc, copper and selenium. Food restriction in 5/6 Nx rats (Nx-PF group) decreased plasma copper level and increased osmotic fragility of RBC and elevated the susceptibility of lipid peroxidation after stressing RBC with H_2O_2. Because Nx-PF rats presented a lower RBC-SOD activity (0.44 ± 0.11 vs 0.78 ± 0.05 unit/g Hb) and a lower plasma copper level. We further found a positive relationship (r=0.802, $p<0.01$) between plasma copper level and RBC-SOD activity in normal and uremic rats. This study suggests that RBC-SOD activity may play an important role in preventing RBC oxidative stress. Plasma copper level may be a marker of RBC-SOD activity. We conclude, in chronic uremia, zinc deficiency doses not result in RBC oxidative stress as plasma copper level is normal, but may affect the absorption of intestinal nutrition.

Key Words: zinc, selenium, antioxidant enzymes, osmotic fragility, lipid peroxidation

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Introduction

Survival of RBC is shortened in patients with advanced renal failure (20,30,45), as a result of RBC oxidative stress (10,11,21,33,55,). It has been evidenced that oxidative stress play an important role in many diseases, including renal and cardiovascular, infection diseases, cancer, diabetes, disorders of peripheral and central nervous system, anemia and accelerated aging (24,26). In uremic patients, however, increased oxidative stress in RBC may result from multifarious factors, such as uremic toxin (46), hemodialysis (HD) (38) or malnutrition (12).

Zinc has shown to be an essential nutrient with a wide range of physiological function including DNA synthesis, cell division and cell immunity (39). It is often found deficient in patients with renal insufficiency (13,47) or uremic patients with HD (35,48), partially because of dietary zinc deficiency owing to protein restriction (3,25). Recent studies indicate that zinc is also an antioxidant (4). It protects against iron-catalyzed free radical damage (39), and plays a biochemical role analogous to that of vitamin E in stabilizing membrane structure and thus reducing peroxidative damage to the cell (5). High level of zinc has been found to inhibit the lipid peroxidation of RBC (14). It is still unknown whether zinc deficiency enhances the oxidative stress of RBC in patients with chronic renal failure. Dietary zinc deficiency is able to affect the lipid composition of RBC membrane (22) and decreases the level of plasma vitamin E (6). Thus, the aim of the present study was to evaluate the effect of dietary zinc deficiency on the susceptibility of RBC membrane lipid peroxidation and the osmotic fragility as well as the activities of endogenous antioxidant enzymes, including SOD, GPX and catalase (CAT) in red cells of uremic rats.

Materials and Methods

Animals and Diets

Experiments were performed on 40 adult male Sprague-Dawley rats, weighing 180-200g. These animals were kept at 25 °C in a 12-hour light/dark cycle and were randomly assigned to the following five groups: normal control (NL), chronic uremia (Nx), chronic uremia + dietary zinc deficiency (Nx-D), Nx-D + zinc supplement (Nx-DZ) and chronic uremia + pair-fed (Nx-PF). All animals were fed standard rat chow containing 23% protein, 0.004% zinc and 5% other minerals and drank deionized distilled water except Nx-D and Nx-DZ groups in free access to food and water. Nx-D and Nx-DZ groups were given a zinc-deficient rat chow (Purina 5888C, Purina Mills, Inc, test diets) containing 21% protein, 0.0001% zinc and 5% other mineral. Nx-D group drank deionized distilled water, but Nx-DZ was given a 120 mg/l zinc sulfate solution. Zinc content in the diet was measured by atomic absorption spectrophotometry as described earlier (41). Five-sixths nephrectomy (5/6 Nx) was used to induce chronic uremia of rats. The procedure of 5/6 Nx has been described in detail in our previous report (15). Pair feeding was carried out in each rats of PF group by restricting daily food intake to that amount of Nx-D rat in 24-h food intake, aiming to evaluate the difference between similar caloric intake dietary zinc deficiency and dietary zinc non-deficiency. Because the restriction of caloric intake has been suggested to lower steady-state levels of oxidative stress and damage in cells (49).

Rats were weighed weekly and food was weighed twice a week. After 5 weeks of feeding, finally after 24-hour urine collection and 12-hour fasting in a Nalgene plastic metabolic cage, animals were weighed and anesthetized with sodium pentobarbital 40 mg/kg. Blood samples were collected from the abdominal aorta. Heparinized whole blood samples were used to measure RBC osmotic fragility. Automated hematology analyzer (SYSMEX SE. 9000 Japan) was used to measure hemoglobin and hematocrit. The remaining heparinized blood samples were immediately centrifuged in 4 °C 8000 g for 10 min to obtain plasma for analysis of zinc, ceruloplasmin and plasma biochemistry. Urine samples were assayed for creatinine, total protein and zinc. Plasma creatinine, blood urea nitrogen (BUN) and renal creatinine clearance (Ccr) were determined to monitor the development of chronic uremia. The Ccr was used as an indicator of glomerular filtration rate (GFR).

RBC Samples

A 5ml blood sample was collected into a polypropylene tube containing 0.5 ml of 3.8% sodium citrate. The samples were centrifuged at 4 °C 4000 g for 20 min, and the plasma and buffy coat were removed by aspiration. The RBC was washed three times with isotonic saline solution and centrifuged at 4 °C 4000 g

for 20 min to yield packed RBC. 1 ml packed RBC, collected from the bottom of tubes to minimize contamination of leukocytes, was added to 1.5 ml 0.9% NaCl solution to achieve an RBC suspension about 30% hematocrit. Three volumes of ice-cold deionized water containing 5 ml/l triton X-100 was added into one volume of RBC suspension to lysis RBC. After 10 min, the lysate is centrifuged at 8000 g for 10 min. The pellet is discarded and the supernatant was used to determine the activities of GPX, Zn-Cu SOD, and CAT. For assay of Zn-Cu SOD activity, hemoglobin was removed from the lysed RBC by adding an equal volume of chloroform/ethanol (3/5, v/v).

Enzyme Assays

The activity of RBC Zn-Cu SOD was measured by monitoring the autoxidation of pyrogallol as we previously described (15). The activity of RBC Zn-Cu SOD was expressed as unit/mg Hb.

The activity of glutathione peroxidase (GPX) was measured with the method of Paglia and Valentine (42) with modification as described by Ceballos et al. (16) using tert-butyl hydroperoxide (t-BHP) as a substrate, and in detail in our previous publication (15). The activity of RBC-GPX was expressed as unit/mg Hb.

Catalase: A lysed RBC-ethanol solution was achieved by adding 10 µl 95% ethanol solution to 1 ml lysed RBC. Ethanol served to decompose any compound II (a reversible inactive complex of CAT) (17). The catalase activity in lysed RBC-ethanol solution was assayed at 30 °C using the method of Aebi (1). The method of assay was described in our previous reports (15) and expressed in unit/mg Hb.

The recycling method of Tietze (56) was employed to determine the total glutathione (GSH) and oxidative glutathione (GSSG) concentrations in RBC. The mechanism of assay is based on the reduction of Ellman reagent. Briefly, 0.1 ml RBC suspension was added to 0.4 ml 1 mmol/l disodium EDTA. After about 1 min, the lysed RBC were immediately deproteinized with 0.5 ml 5% trichloroacetic acid (TCA) and centrifuged at 4 °C 5000 g for 20 min to achieve a clear supernatant. The supernatant was used to measure total GSH content according to our previous description (15).

Oxidized glutathione. 0.5 ml RBC suspension was incubated with 0.1 ml 0.25 M N-ethylmaleimide (NEM) for 60 min and 5% TCA was then added to stop the reaction and centrifugation at 4 °C 8000 g for 20 min to achieve a clear supernatant. The supernatant used ether to remove the excess NEM for 5 times. After ether extraction, the supernatant was used to determine the level of GSSG using total GSH assay.

Ceruloplasmin activity was measured by oxidation of o-dianisidine dihydrochloride. The enzymatic activity of ceruloplasmin was designated as change in absorbance at 540 nm. Unit was expressed in international unit (50).

Biochemical Analyses

The determinations of Plasma creatinine, BUN, albumin, calcium and inorganic phosphate as well as urine protein and creatinine were performed in a clinical chemistry antoanalyzer (Express Plus, Ciba-Corning Diagnostics Corp, MA, USA). These assay kits were prepared by Chiron diagnostics S.A. except urine protein. We used CPT diagnostics urine protein assay kit to measure the concentration of urine protein, which is described by Orsonneau et al (36).

Susceptibility of RBC Lipid Peroxidation

RBC Susceptibility to lipid peroxidation was based on the assay of malondialdehyde (MDA), using the addition technique of Stocks and Dormandy (51). Briefly, 0.1 ml RBC suspension was incubated for 60 min in 1 ml phosphate buffer saline (pH=7.4) with 2 mM sodium azide and 0.03% H_2O_2. An equal volume of 5% TCA solution was added to precipitate protein to stop the reaction and the mixture was then centrifuged to achieve clear supernatant. A 0.3 ml supernatant was added to a reaction mixture consisting 1 ml 1% phosphoric acid, 0.5 ml 0.6% thiobarbituric acid and 0.1 ml 8.1% lauryl sulfate. A solution of 0.05 ml 1% butylated hydroxytoluene was added to inhibit autoxidation of lipid during reaction. This solution was placed in a dry head bath kept at 95 °C for 40 min. After removal from the heat bath, the solution was added with 1.5 ml n-butanol, agitated and subsequently centrifuged at 8000 g for 10 min. The upper organic layer was pipetted off, and absorbance of this fraction was read at 532 nm in a Beckman DU-40 spectrophotometer. A calibration curve was constructed using 1,1,3,3-tetramethoxypropane (Sigma). MDA value was expressed as nmol/g Hb.

Analysis of Zinc, Copper and Selenium Levels in

Table 1. Effects of Zinc Deficiency on Plasma Biochemistry and Renal Function in Rats with Chronic Uremia

	Control (8)	Nx (8)	Nx-D (8)	Nx-Dz (8)	Nx-PF (8)
Cr mg/dl	0.51 ± 0.02	1.04 ± 0.05[a]	1.28 ± 0.04[a]	1.14 ± 0.03[a]	0.98 ± 0.03[a]
BUN mg/dl	18.5 ± 1.2	37.5 ± 0.8[a]	48.9 ± 1.3[a]	42.3 ± 1.5[a]	32.1 ± 0.8[a]
Ca mg/dl	10.5 ± 0.2	9.8 ± 0.1	10.2 ± 0.3	9.7 ± 0.2	10.2 ± 0.4
P mg/dl	6.98 ± 0.13	7.21 ± 0.25	7.51 ± 0.20	6.94 ± 0.22	7.35 ± 0.30
Alb g/dl	3.08 ± 0.05	2.81 ± 0.06	2.74 ± 0.04[a]	2.77 ± 0.09[a]	2.78 ± 0.08[a]
TG mg/dl	44.3 ± 1.5	47.6 ± 3.2	26.8 ± 2.6[a]	42.8 ± 1.8	44.5 ± 2.4
Ccr ml/min	1.62 ± 0.05	0.82 ± 0.10[a]	0.68 ± 0.08[a]	0.85 ± 0.09[a]	0.88 ± 0.05[a]
UP μg/min	4.6 ± 0.5	9.1 ± 0.5[a]	8.1 ± 0.8[a]	7.8 ± 0.5[a]	8.5 ± 0.4[a]
Hb g/dl	18.3 ± 0.4	17.8 ± 0.6	18.2 ± 0.7	18.2 ± 0.5	17.2 ± 0.4

Values are expressed as mean ± SEM. Animal number is in paretheses. Cr = creatinine; BUN = plasma urea nitrogen; Alb = albumin; TG = triglyceride; Ccr = creatinine clearance; UP = urine protein; Hb = hemoglobin; Ca = calcium; P = inorganic phosporus. [a]$p<0.05$ when compared with control group.

Table 2. Body Weight and Daily Food Intake in the Experimental Rats

	n	Body weight (g) initial	Body weight (g) final	Food intake (g/rat/day)
Control	8	190.5 ± 10.2	358.8 ± 18.9[a]	23.8 ± 1.1[a]
Nx	8	190.4 ± 9.4	342.1 ± 15.6[a]	24.1 ± 1.8[a]
Nx-D	8	190.3 ± 8.3	206.8 ± 11.5[b]	10.2 ± 0.8[b]
Nx-DZ	8	191.3 ± 6.8	361.4 ± 12.9[a]	24.5 ± 1.5[a]
Nx-PF	8	192.2 ± 10.3	213.6 ± 12.8[b]	10.8 ± 0.3[b]

Nx = chronic uremia, Nx-D = chronic uremia + dietary zinc deficiency, Nx-DZ = Nx-D + zinc supplement, Nx-PF = Chronic uremia + pair-fed. All values are expressed as mean ± SEM. Values in a row with different superscripts are statistically different ($p<0.05$), using one-way ANOVA and Student's t-test to analyze. The initial-final period was 5 weeks.

Plasma, Urine and RBC

The zinc, copper and selenium levels were measured by atomic absorption spectrophotometer, using a Perkin Elmer 3110 atomic absorption spectrophotometer (AAS) to assay the levels of zinc and copper and using a Perkin Elmer 4100ZL AAS to assay the level of plasma selenium. The plasma samples were diluted 5 times with 0.2N nitric acid to determine plasma zinc. 0.1 ml RBC suspension was digested in a dry heat bath with 0.5 ml 2 N HNO_3 and was then diluted to 1 ml with deionized distilled water to determine RBC zinc. Undiluted urine samples were used to determine urine zinc. In the assay of plasma copper, samples were diluted 5 times with 0.5% triton-100 solution.

The osmotic fragility of RBC was evaluated by the method of Bettger et al. (7), using one concentration of 0.38% NaCl solution. Briefly, 20 ul heparinized whole blood was incubated in 1 ml 0.9%, 0.38% and 0% NaCl solution for 30 min. Subsequently, the RBC were centrifuged, and the absorption of the supernatant was measured against water at 540 nm in a Beckman DU-40 spectrophotometer. The osmotic fragility was calculated by the following equation.

Osmotic fragility of RBC (%)=

$$\frac{OD_{0.38\%} - OD_{0.9\%}}{OD_{0\%}} X100\%$$

Table 3. The Concentrations of Copper and Zinc in Plasma, RBC and Urine 5 Weeks after Start of Treatment

	n	Plasma Se μg/mL	Plasma Cu μg/mL	Plasma Zn μg/mL	RBC Zn μg/g Hb	Urine Zn μg/24 h
Control	8	0.56 ± 0.03	1.41 ± 0.05[a]	1.35 ± 0.05[a]	22.8 ± 0.4	2.5 ± 1.2[a]
Nx	8	0.48 ± 0.04	1.32 ± 0.11[a]	1.12 ± 0.08[b]	21.9 ± 0.5	5.2 ± 1.9[b]
Nx-D	8	0.35 ± 0.05	1.85 ± 0.3[b]	0.82 ± 0.10[c]	23.5 ± 0.2	1.6 ± 1.1[c]
Nx-DZ	8	0.47 ± 0.06	1.35 ± 0.2[a]	1.28 ± 0.05[a]	22.3 ± 0.4	4.0 ± 1.4[b]
Nx-PF	8	0.40 ± 0.08	1.17 ± 0.04[c]	1.08 ± 0.07[b]	22.2 ± 0.5	2.1 ± 0.5[a]

Abbreviation and statistical notations see Table 2.

Statistics

Statistical analysis was carried out using one-way ANOVA and Student's t-test. All values were reported as mean ± EM. Correlation between variables was determined by linear regression analysis and regression line comparison was made by t-test. A p values less than 0.05 was considered significantly different. The analysis was carried out on a computer using statistical software (SAS).

Results

Five weeks after 5/6 nephrectomy (Nx), all the 5/6 Nx rats including those in Nx group, Nx-D group, Nx-DZ group and Nx-PF group presented a syndrome of uremia, for example, significantly elevated levels of plasma creatinine and urea nitrogen as well as decreased renal creatinine clearance. But they still had normal plasma calcium and inorganic phosphorus level as well as content of hemoglobin in RBC (Table 1). Increased urinary protein excretion was found in all 5/6 Nx rats. Low plasma selenium and triglyceride levels were only found in Nx-D group. The activities of plasma ceruloplasmin significantly increased in Nx-D group and decreased in Nx-PF group (Table 1).

Five weeks after dietary zinc deficiency, the uremic rats of Nx-D group produced severe growth retardation and anorexia, but the uremic rats of Nx-DZ group with adequate zinc supply in drinking water (120 mg/l zinc sulfate solution) had normal growth rate and appetite. (Table 2). Uremic rats on standard rat chow (Nx group) had a lower plasma zinc level and a higher urine zinc excretion than normal rats, but their body weight, appetite and plasma copper level still were normal (Tables 2 and 3). Food restriction decreased the level of plasma copper (Table 3) and the body weight of 5/6 Nx rats (Nx-PF group, Table 2). Dietary zinc deficiency in 5/6 Nx rats did not reduce RBC hemoglobin, but significantly increased the level of plasma copper (Table 3). The activity of RBC-GPX did not reduce in 5/6 Nx rats, but dietary zinc deficiency decreased RBC GPX activity (Table 4). Zinc supplement in zinc deficiency rats (Nx-DZ group) could recover the levels of plasma zinc and selenium as well as the activity of RBC GPX (Tables 1 and 4).

When the amount of food intake was similar, 5/6 Nx rats in dietary zinc deficiency (Nx-D group) and dietary zinc non-deficiency (Nx-DF group) produced opposite reaction to RBC-SOD activity, Nx-D group reacted to elevate and Nx-PF group reacted to reduce RBC-SOD activity (Table 4). Decreasing RBC-MDA formation after stressing RBC with H_2O_2 was found in Nx-D group, but increasing RBC-MDA formation after stressing RBC with H_2O_2 was found in Nx-PF group. Increased RBC osmotic fragility was found only in Nx-PF group (Table 4). We also observed a positive relationship between plasma copper level and plasma ceruloplasmin (r=0.882, p<0.001, Figure 1) as well as between plasma copper level and RBC-SOD activities (r=0.802, p< 0.01, Figure 1).

Discussion

Although zinc has been shown to have antioxidant and membrane-stabilizing properties (5,8), zinc deficiency in our uremic rats seem no increase RBC oxidative stress, because it dose not increase osmotic fragility of RBC and formation of RBC MDA. when their plasma coppers level is normal. In contrast, if zinc deficiency increase the level of plasma copper, zinc deficiency reduce RBC MDA formation and osmotic

Table 4. Effects of Zinc Deficiency on the Activities of Endogenous Antioxidant Enzymes, Lipid Peroxidation and Osmotic Fragility in Red Cells of Rats with Chronic Uremia

	Control (8)	Nx (8)	Nx-D (8)	Nx-DZ (8)	Nx-PF (8)
GSH μmol/g Hb	0.44 ± 0.06	0.42 ± 0.05	0.46 ± 0.10	0.43 ± 0.07	0.43 ± 0.08
GSSG/GSH %	8.8 ± 0.8	9.0 ± 0.7	8.9 ± 1.1	9.3 ± 1.3	9.2 ± 1.0
GPX unit/mg Hb	0.35 ± 0.05	0.29 ± 0.08	0.18 ± 0.05[a]	0.25 ± 0.03	0.23 ± 0.04
SOD unit/g Hb	0.78 ± 0.05	0.72 ± 0.08	1.95 ± 0.27[a]	0.68 ± 0.10	0.44 ± 0.11[a]
CAT unit/mg Hb	95.4 ± 3.5	98.5 ± 4.4	92.4 ± 4.6	96.3 ± 12.1	93.8 ± 11.1
Cp unit/L	182.7 ± 2.9	176.4 ± 2.4	216.2 ± 2.2[a]	180.1 ± 3.1	155.7 ± 1.9[a]
MDA nmol/g Hb	42.6 ± 1.2	43.8 ± 1.8	37.2 ± 1.1[a]	44.1 ± 1.5	48.6 ± 1.5[a]
OS %	68.5 ± 1.8	70.8 ± 2.0	67.5 ± 2.4	71.2 ± 1.9	78.4 ± 1.2[a]

Values are expressed as mean ±SEM. Values in paretheses represent number of animal. GSH:Total glutathione, GSSG:Oxidative glutathione, GPX:Glutathione peroxidase, SOD:Superoxide dismutase, CAT:Catalase. Cp:Ceruloplasmin, MDA:Malondialdehyde, OS: Osmotic fragility, [a]$p<0.05$ when compared with control group.

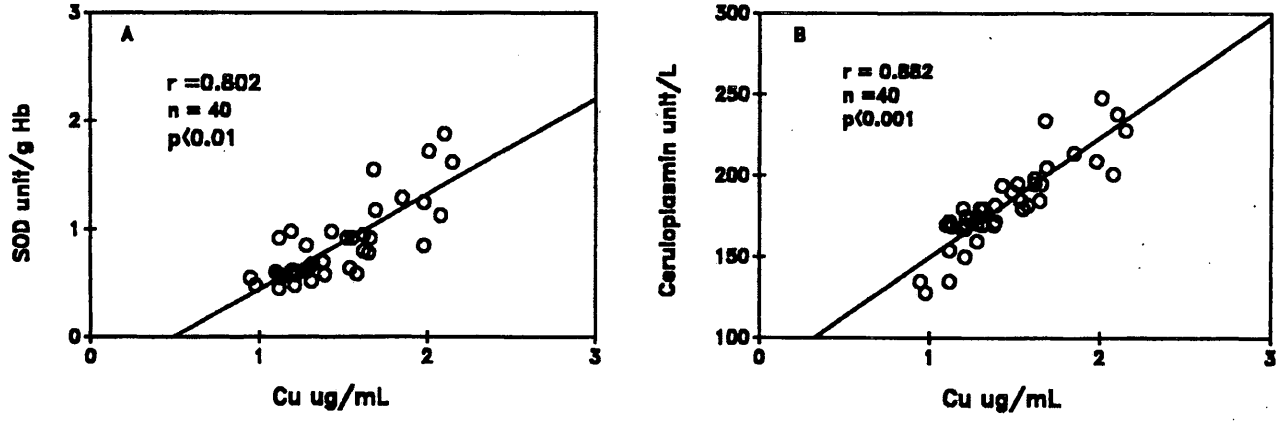

Fig. 1. Relationship between plasma copper and erythrocyte superoxide dismutase (A) as well as between plasma copper and plasma ceruloplasmin (B) in normal and uremic rats with different treatments

fragility, since they increase the activities of RBC Zn. Cu-SOD and plasma ceruloplasmin. RBC-SOD is an antioxidant enzyme and plays an important role in antioxidant defense mechanism of RBC. Because it could fully scavenge superoxide radical and then prevent superoxide radical to catalyze the degradation of H_2O_2 to produce highly reactive hydroxyl radical (29,32). This reaction, known as the Haber-Weiss reaction, occurs in the presence of a trace metal, usually Fe^{3+} acting as an oxidation-reduction catalyst. Ceruloplasmin is a ferroxidase (37) and is also an important antioxidant enzyme (43). Since it catalyzes the oxidation of ferrous to the less reactive ferric state to inhibit iron dependent-lipid peroxidation (52). Copper is an essential cofactor for Zn,Cu-SOD and ceruloplasmin activities (58), is the only metal ion that can confer the activities of Zn,Cu-SOD (27) and ceruloplasmin (31). A positive correlation between plasma copper level and RBC Zn,Cu-SOD activity (r=0.802, $p<0.01$) as well as between plasma copper level and plasma ceruloplasmin activity (r=0.882, $p<0.001$) have been found in normal and uremic rats. Thus, the present result and other author's report (2) suggest that plasma copper affect the activities of RBC-SOD and plasma ceruloplasmin.

Increased urinary zinc and copper excretion have been found in patients with chronic renal failure (13, 35). Food restriction in chronic uremia may thus enhance the deficiency of zinc and copper in body. Food

restriction in our 5/6 Nx rats have been found to reduce plasma copper level. We thus suggest that in chronic uremia, copper deficiency may partially result from the food restriction. But uremic patients may be high plasma copper level (53), low plasma copper level (23) or normal plasma copper level (60). This diverse result may partially be linked to the amount of food intake and the content of food zinc and copper, because between food zinc and copper has biological competition on the luminal side of the intestinal epithelia for absorption of both metals. (28). Dietary zinc deficiency may thus enhance the absorption of intestinal copper. The present study demonstrates the possibility that dietary zinc deficiency increases the absorption of intestinal copper because it elevates the concentration of plasma copper in our uremic rats.

Lipid peroxidation of RBC membrane destroys the integrity of RBC membrane and the activity of RBC membrane Na^+-K^+ ATPase (9), and leads to increased RBC osmotic fragility and hemolysis, because of increased RBC membrane permeability and the retention of sodium ion inside the cell. It is well documented that normal RBC contain very little lipid peroxide because they possess enzymatic antioxidant defense mechanism to cope effectively with oxygen free radical to block the lipid peroxidation, for example, SOD, catalase and GPX enzymes. SOD catalyzes the dismutation of superoxide anion to H_2O_2 (34), which is the first step of enzymatic cascade leading to the complete inactivation of reactive oxygen system formed, thus to prevent the formation of highly reactive hydroxyl radical. The second step depends on the catabolism of H_2O_2 by the catalysis of catalase and/or GPX enzymes into water and oxygen (29). Although a low RBC GPX activity was found in Nx-D group, they dose not find to increase RBC osmotic fragility or the formation of RBC MDA, because they had high RBC Zn,Cu-SOD activity. Thus, the present result and other author's report (54) suggest that the activity of RBC Zn,Cu-SOD may play a major role in antioxidant defense mechanism of RBC.

The reduction of RBC GPX activity in Nx-D group may result from the decrease of plasma selenium level, since selenium is an important cofactor for GPX activity. Zinc deficiency has been suggested to reduce the absorption of intestinal nutrient and electrolytes (57,59), because it active guanylate, adenylate cyclases, stimulating chloride sécretion, producing diarrhea and diminshing absorption of nutrients, thus exacerbating an already compromised mineral status. In present study, we found dietary zinc deficiency result in the decrease of plasma selenium and triglycerides levels. Zinc supplementation recovered the levels of plasma selenium and triglyceride in Nx-D rats.

In chronic uremia, a defective glutathione redox cycle in erythrocyte may play an important role in oxidative stress of red cells. (10,11,44), Our previous study and other author's reports (18,19) suggest that a moderate to severe uremia could affect the glutathione redox in red cells. In the present study, in order to reduce the factors of oxidative stress in red cells, we reduced the damage of residual renal tissues in the surgery of 5/6 nephrectomy. A normal glutathione level and, GSSG/GSH ratio were found in red cells of these uremic rats. Dietary zinc deficiency or food restriction did not alter glutathione level and GSSG/GSH ratio in red cells. Thus, we suggest that zinc deficiency may not affect the redox state of glutathione in red cells.

Although the Nx-PF rats have been found to increase the oxidative stress of RBC, the Nx-PF rats doses not find to decrease the number of RBC. The cause may result from our 5/6 Nx rats have still normal erythropoietin secretion in their remnant kidney tissues to stimulate erythrogenesis of bone marrow.

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