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Effect of iron and selenium status on glutathione peroxidase activity and lipid peroxidation in rats

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Abstract : The combined effects of iron and selenium status on glutathione peroxidase (GSHPx) activity, cytochrome P-450 activity, and lipid peroxidation in the liver and intestinal mucosa of rats were investigated. In experiment one, four experimental groups (+Se+Fe, −Se+Fe, +Se++Fe, −Se++Fe) were manipulated for 3 weeks with intramuscular administration of iron-dextran (++Fe) and/or normal diet (+Fe) and deionized water (−Se) and/or selenium-supplemented deionized water (+Se). In experiment two, 2% dietary carbonyl iron (instead of the parenteral administration) was fed for 3 weeks to rats. Body weight of rats was significantly decreased in both parenterally and orally iron-overloaded groups ($p < 0.01$), regardless of Se supplement. Serum iron was significantly increased in parenterally iron-overloaded groups but it was marginally increased in orally iron-overloaded groups. There was no significant difference in hemoglobin content among experimental groups in either experiment one or two. Total iron in the small intestine, intestinal mucosa, and livers was significantly high in both parenterally and orally iron-overloaded rats, regardless of selenium status. In the liver and intestine, GSHPx activity was significantly higher in all selenium-supplemented groups, compared to Se-deficient groups ($p < 0.01$) and lipid peroxidation was significantly enhanced in both parenterally and orally iron-overloaded groups, compared to iron-adequate groups. There was no significant difference in cytochrome P-450 activity in the livers between groups in both experiment one and two.

These results indicated that GSHPx activity in liver and intestinal mucosa was depended on selenium status, regardless of iron status, and iron-overload enhances lipid peroxidation in liver and intestinal mucosa by increasing the tissue iron content.

Key words : cytochrome P-450, glutathione peroxidase, iron-overload, lipid peroxidation, selenium.

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Introduction

Iron is essential for life, but iron-overload is toxic and potentially fatal. It has been recognized that iron-overload is relatively common disorder of iron metabolism such as hereditary hemochromatosis and thalassemia[1,2]. The liver is a major site of iron storage and is particularly susceptible to injury from iron-overload, especially when the iron accumulates in hepatocytes[2,3]. Hepatotoxicity is the most consistent finding in patients with iron overload. Whether derived from increased absorption or parenteral administration, massive deposition of iron in hepatic parenchymal cells eventually produces fibrosis and ultimately cirrhosis and hepatocellular carcinoma[4].

The possible mechanism of iron-induced hepatotoxicity is lipid peroxidation[5]. Lipid peroxidation is a free-radical-mediated process that can result in acute cellular injury, especially if the normal protective mechanisms of the cell are reduced. On the other hand, lipid peroxidation itself and some of its by-products are known to be potential initiators and promoters of cancer[6,7]. Lipid peroxidation is also associated with structural changes and dysfunctions of hepatic organelles[8].

Selenium is an important trace element in human and animal bodies. Selenium diets are known to increase glutathione peroxidase (GSHPx) activity in mammalian tissue[9,10]. Selenium protects the free radical-mediated lipid peroxidation by increasing GSHPx activity[11]. GSHPx is a very important enzyme against lipid peroxidation. It reduced hydrogen peroxide and organic hydroperoxides, thereby blocking the subsequent free radical formation[9,12]. In the intestine, due to the fast turnover rate of mucosal cell, GSHPx is dependent on Se in a diet but not on Se body store[13]. Therefore, the intestinal protection of GSHPx on free radical damage can be seriously compromised by a selenium-deficient diet.

It has been known that treatment with selenium increase liver and plasma levels of GSHPx[11]. Meanwhile, the effect of iron-overload on selenium metabolism is still controversial. In this study, the roles of iron and selenium, as a prooxidant and an antioxidant, respectively, were compared in rats manipulated by parenteral and dietary iron-overload in combination with selenium status. We investigated GSHPx activity, cytochrome P-450 activity, and lipid peroxidation in the liver and small intestine of rats. These results would illustrate an implication of iron and selenium status in the intestinal or liver cancer or free radical-mediated tissue damage.

Materials and Methods

Materials : Thiobarbituric acid (TBA), trichloroacetic acid (TCA), tetraethoxypropane, butylated hydroxytoluene (BHT), hydrogen peroxide, potassium cyanide, potassium ferricyanide, bovine albumin, glutathione reductase, glutathione, O-ethyl O-(4-nitrophenyl) phenylphosphonothioate (EPN), and sodium azide were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals : Male Sprague-Dawley rats (in number, 20 rats for experiment one and 20 rats for experiment two), initially weighing 210~230g, were fed a control diet for at least five days to adapt to the experimental environment before starting the experiments. They were housed in pairs in stainless steel cages with wire fronts and bottoms and fed the assigned diets and deionized water *ad libitum* for 3 weeks. The animal facility was maintained to about 23℃ with a 12hr light : dark cycle (07 : 00 to 19 : 00). Animals were weighed before feeding the experimental diet and assigned randomly to treatment groups. Rats were fasted for 16hr before they were sacrificed.

Diet preparation : Animal diets consisted of 34.0% dextrose, 20% casein, 20% corn oil, 15% corn starch, 5% cellulose, 0.3% DL-methionine, 1.7% $CaHPO_4$, 3% mineral mixture, and 1% vitamin mixture (Table 1, 2 and 3). Dextrose (2%) was substituted with 2% carbonyl iron for dietary iron-overload in experiment two. Diet ingredients were purchased from the ICN Biochemical Inc. (Cleveland, OH) and mixed well, and the diet was stored under nitrogen gas in evacuated bags in the refrigerator to prevent the contact with oxygen. Total iron and selenium contents in the normal diet were 60ppm and less than 0.01ppm, respectively.

Experimental design : In experiment one, iron-overload

in rats was induced by intramuscular injections of iron dextran (100mg Fe/ml, Sigma Chemical Co.) up to a total of

Table 1. Composition of semipurified diet

Ingredients[a]	Composition (%)
Dextrose	34 (32)
Casein	20
Corn oil	20
Corn starch	15
Cellulose (Alphacel)	5
DL-Methionine	0.3
Mineral mixture[b]	3
Vitamin mixture[c]	1
$CaHPO_4$	1.7
Carbonyl iron[d]	2 (for dietary Fe load)

[a]Dextrose, corn oil, and corn starch were purchased at local stores. All other ingredients were purchased from the ICN Biochemicals Inc. (Cleveland, OH).

[b]The compositions of vitamin mixture are shown in Table 2.

[c]The compositions of vitamin mixture are shown in Table 3.

[d]The ingredient was used only for dietary iron-overload and the dextrose content was decreased by 2% (34 to 32%).

Table 2. Composition of vitamin mixture

Ingredients	g/kg mixture	mg/kg diet
Vitamin A (500,000 I.U./g)	1.0	5,000 I.U.
Vitamin D (850,000 I.U./g)	0.125	1,063 I.U.
Alpha tocopherol (250 I.U./g)	20.0	50 I.U.
Niacin	4.0	40
Riboflavin	0.6	6
Pyridoxin hydrochloride	0.7	7
Thiamin hydrochloride	0.6	6
Vitamin B_{12}	0.00135	0.0135
Ascorbic acid	45.0	450
Inositol	1.0	10
Choline chloride	150.0	1500
Menadione	5.0	50
p-Aminobenzoic acid	5.0	50
Calcium pantothenate	1.0	10
Biotin	0.02	0.2
Folic acid	0.2	2.0
Dextrose	to make 1,000g	

Table 3. Composition of mineral mixture

Ingredients	Amount(g/kg)	Mineral	Mineral mg/kg diet
$MnSO_4 \cdot H_2O$	6.0	Mn	58.6
$MgCO_3$	50.0	Mg	435
KI	0.02	I	0.46
$ZnSO_4 \cdot 7H_2O$	2.0	Zn	13.8
$CuSO_4 \cdot 5H_2O$	0.8	Cu	6.0
$Na_2MoO_4 \cdot 2H_2O$	0.05	Mo	0.6
$CoCl_3 \cdot 6H_2O$	0.05	Co	0.3
$CrCl_3 \cdot 6H_2O$	0.1	Cr	0.6
Na_2H_2PO	150.0	Na	855
KCl	120.0	K	1872
$FeSO_4 \cdot 7H_2O$	10.0	Fe	60
Dextrose	to make 1,000g		

75mg Fe per 100g body weight. The dose was divided in three equal injections delivered on days 5, 10, and 15 of the experiment. The remaining 10 rats were sham-injected with physiological saline. In experiment two, iron-overload in rats was induced by a diet containing 2% carbonyl iron for 3 weeks. For each experiment one or two, animals were randomized into 4 groups of 5 animals each : Se- and Fe-supplemented group (+Se+Fe), Se-deficient and Fe-supplemented group (- Se+Fe), Se-supplemented and Fe-overload (+Se++Fe), and Se-deficient and Fe-overload (- Se++Fe). Selenium was supplemented with adding 0.5ppm Se (as Na_2SeO_3) to deionized water. Se-deficient rats were manipulated with deionized water.

Homogenate preparation : Rats were anesthetized with ether and the abdomen was opened. Blood was collected from the abdominal aorta. Livers were dissected and placed in isotonic KCl. The intestinal tract was incised from the stomach at the pyrolic junction and gently pulled away from the mesentery and placed in isotonic KCl. The intestine was incised at the same position and the sections were flushed with isotonic KCl to wash out to the intestinal contents. Excess liquid was removed with ashless paper tissue and the epithelial mucosa gently scraped with the edge of a metal spatula. The mucosal scrapes were weighed and homogenized in 3 vol of KCl tris buffer (150/25mM, pH 7.4) with soft tissue homogenizer. Livers were also homogenized using a polytron homogenizer. All steps were performed in a cool room. Aliquots of homogenate were frozen in liquid nitrogen and stored at - 70℃ for further analysis.

Chemical analysis

Thiobarbituric acid reactive substance (TBARS) : TBARS was determined as an indication for lipid peroxidation using the method described by Buege and Aust[14]. The reaction mixture contained 1.0ml of 10% homogenate and 2.0ml of 15% TCA-0.375% TBA-0.25N HCl reagent solution. The mixture was heated for 15 min in a boiling water bath (95℃) to develop a pink color, cooled with tap water, centrifuged at 2,000×g for 15 min, and measured spectrophotometrically at 532nm with a UV 2100U spectrophotometer (Shimadzu Co., Kyoto, Japan). Prior to the heating step, 0.2% BHT will be added to prevent spurious lipid peroxidation. The standard curve was made by determining the absorbance of known amount (1, 2, 4, and 8nM/ml) of tetraethoxypropane, a precursor of malonedialdehyde (MDA). TBARS was calculated by the standard curve.

Total iron concentration : Iron was determined in wet-ashed samples by using the ferrozine colorimetric method[15]. Homogenized samples (0.5ml) were digested on a hot plate until being white ash with 3 ml of concentrated nitric acid and 0.2ml of 30% hydrogen peroxide. The white ash was dissolved into 0.2ml of 1.0N hydrochloric acid and diluted with 0.8ml deionized water. One ml of 0.5% ascorbic acid was added and mixed thoroughly. After 20 min, one ml of 10% ammonium acetate buffer and 1 ml of 1 mM ferrozine color reagent was added. After standing at room temperature for 45 min, the absorbance of the samples was measured at 562nm.

Hemoglobin (Hb) : Hb was determined by the cyanomethemoglobin method[16]. Blood (0.02ml) was mixed with 5ml Drabkin solution containing 8.0mM potassium cyanide, 0.6mM potassium ferricyanide and 12mM sodium bicarbonate and the mixture was allowed to stand for 20 min. Absorbance was read at 540nm by spectrophotometer and the Hb concentration was calculated with standard curve (5, 10, 15 and 20g Hb/dl).

Glutathione peroxidase activity : GSHPx activity was measured in the supernatant obtained from centrifugation of homogenate using hydrogen peroxide as a substrate[17]. GSHPx activity in human blood was also measured and its value compared with the established standard, to validate the assay. One hundred microliter of sample was added to the reaction mixture containing 2.58ml of 50mM phosphate buffer (pH 7.4), 0.1ml of 8.4mm NADPH, 0.01ml of glutathione reductase (100EU/ml), 0.01ml of 1.125M sodium azide, and 0.1 ml of 0.15M reduced glutathione and let it equilibrate at 20℃ for 5 min. To initiate the reaction, 0.1ml of 2.2mM hydrogen peroxide was added and absorbance was read at 340nm. The coversion of NADPH to NADP is followed spectrophotometrically by continuous recording of the change in absorbance of the system at 340nm between 2 and 4 min after initiation of the reaction, employing a cuvette with a 1 cm light path. An unit of activity was ex-

pressed as micromole of NADPH oxidized per mg of protein.

Cytochrome P-450 activity : Cytochrome P-450 activity was assayed in liver homogenates, using EPN as a substrate [18]. Cytochrome P-450 system metabolizes the substrate into *p*-nitrophenol. The absorbance was measured at 410nm and the enzyme activity was calculated as μg *p*-nitrophenol produced/hr/mg protein by the standard curve.

Protein content : Protein was estimated by the Lowry method using bovine albumin as a standard[19].

Statistical analysis : Data was analyzed using SAS program for two-way ANOVA[20]. Whenever F is found to be statistically significant ($p < 0.01$), least significant values were calculated to identify statistically significant differences among treatment means.

Results

Parenteral iron-overload : Body weights of iron-overloaded rats (++Fe) with iron dextran were significantly lower than those of iron-adequate (+Fe) rats, regardless of selenium supplementation (Table 4). It is probable that the injection of iron dextran is stressful or toxic to the rats. The body weight of Se-supplemented (+Se) rats was slightly higher than Se-deficient (-Se) rats in experimental groups with the same iron status (Table 4). There was no significant difference in Hb concentration between all treated groups. However, a marginally high Hb concentration was shown in the iron-overload rats. Serum iron was significantly higher in iron-overload (++Fe) rats, regardless of Se-supplementation ($p < 0.01$). In addition, total iron in the intestinal mucosa, small intestine, and liver was significantly higher in iron-overloaded (++Fe) rats ($p < 0.01$), probably indicating the distribution of injected iron to parenchymal cells. Although most of tissues accumulate iron, the liver seemed to be most sensitive to iron-overload. Iron-overload increased the liver iron content by approximately 20-fold compared to normal rats (+Se+Fe). In the liver and intestinal mucosa, GSHPx activity was dependent on the Se-supplement but not dependent on the iron-overload (Table 5). However, there was an actual decrease in GSHPx activity in iron-overloaded rats compared to iron-adequate rats (Table 5). Cytochrome P-450 activity was measured in only the liver because of low intestinal cytochrome P-450 activity. Cytochrome P-450 activity in the liver had no significant difference between experimental groups. Lipid peroxidation in the liver and intestinal mucosa of iron-overloaded rats was elevated (Table 5). However, selenium-supplementation significantly inhibited the lipid peroxidation as measured by TBARS formation in the liver of iron-overloaded rats ($p < 0.01$).

Dietary iron-overload : A diet with 2% carbonyl iron caused a decrease in body weight of rats, independently on

Table 4. Body weight, hemoglobin, serum iron, and total tissue iron in parenterally iron-overloaded rats

Parameters	Experimental groups			
	+Se+Fe	-Se+Fe	+Se++Fe	-Se++Fe
Body weight (g)	280±15[a]	262±18[a]	222±21[b]	213±20[b]
Hemoglobin (g/dl)	17.2±2.4[a]	17.4±1.7[a]	18.1±2.3[a]	18.6±2.9[a]
Serum iron (μg/ml)	3.5±0.5[a]	3.6±0.9[a]	7.5±1.1[b]	7.8±1.0[b]
Tissue iron (μg/mg protein)				
Intestinal mucosa	1.8±0.5[a]	1.7±0.6[a]	3.9±0.6[b]	4.5±0.4[b]
Small intestine	2.3±0.8[a]	2.2±0.9[a]	15.9±2.3[b]	16.8±2.0[b]
Liver	1.4±0.4[a]	1.2±0.2[a]	29.1±4.3[b]	29.7±3.8[b]

[ab]Means in the same row with the same superscript letter are not significantly different ($p < 0.01$).

Table 5. Glutathione peroxidase and cytochrome P-450 activity and lipid peroxidation in the liver and intestine of parenterally iron-overloaded rats

Parameters	Experimental groups			
	+Se+Fe	−Se+Fe	+Se++Fe	−Se++Fe
Liver				
GSHPx (μM NADP/min/mg protein)	0.61 ± 0.07^{a}	0.21 ± 0.05^{b}	0.56 ± 0.08^{a}	0.19 ± 0.07^{b}
P-450(μg *p*-nitrophenol/hr/mg protein)	1.49 ± 0.16^{a}	1.43 ± 0.13^{a}	1.38 ± 0.12^{a}	1.38 ± 0.20^{a}
Lipid peroxidation (nM MDA/mg protein)	0.26 ± 0.02^{a}	0.28 ± 0.04^{a}	0.57 ± 0.10^{b}	0.82 ± 0.12^{c}
Intestinal mucosa				
GSHPx (μM NADP/min/mg protein)	0.08 ± 0.01^{a}	0.05 ± 0.01^{b}	0.08 ± 0.02^{a}	0.04 ± 0.01^{b}
Lipid peroxidation (nM MDA/mg protein)	0.57 ± 0.08^{a}	0.88 ± 0.11^{b}	1.27 ± 0.16^{c}	1.42 ± 0.18^{c}

^{abc}Means in the same row with the same superscript letter are not significantly different ($p < 0.01$).

Table 6. Body weight, hemoglobin, serum iron, and total tissue iron in orally iron-overloaded rats

Parameters	Experimental groups			
	+Se+Fe	−Se+Fe	+Se++Fe	−Se++Fe
Body weight (g)	282 ± 18^{a}	269 ± 22^{a}	227 ± 16^{b}	221 ± 20^{b}
Hemoglobin (g/dl)	17.2 ± 2.0^{a}	17.7 ± 1.7^{a}	18.1 ± 1.3^{a}	18.3 ± 1.9^{a}
Serum iron (μg/ml)	3.8 ± 0.5^{a}	3.9 ± 0.3^{a}	4.6 ± 0.4^{a}	4.4 ± 0.3^{a}
Tissue iron (μg/mg protein)				
Intestinal mucosa	1.6 ± 0.2^{a}	1.5 ± 0.4^{a}	2.6 ± 0.4^{b}	2.3 ± 0.3^{b}
Small intestine	2.4 ± 0.3^{a}	2.5 ± 0.2^{a}	3.2 ± 0.3^{b}	3.4 ± 0.3^{b}
Liver	1.4 ± 0.4^{a}	2.0 ± 0.4^{b}	2.8 ± 0.3^{c}	3.1 ± 0.5^{c}

^{abc}Means in the same row with the same superscript letter are not significantly different ($p < 0.01$).

selenium status (Table 6). A similar result was shown in rats treated with iron dextran (Table 4). There was no significant difference in Hb concentration among experimental groups. However, iron-overloaded (++Fe) rats showed a slightly higher Hb concentration, compared to iron-adequate (+Fe) rats. Serum iron was significantly higher in iron-overloaded (++Fe) rats, regardless of selenium status. The increase in serum iron may be due to increased iron absorption but not due to decreased erythropoiesis and/or increased hemolysis, because Hb concentration was higher in iron-overloaded (++Fe) rats. The high serum iron might be associated with high total iron in tissues. Total iron content in liver was significantly higher in iron-overloaded rats, compared to Fe-adequate rats ($p < 0.01$). In the intestinal mucosa, the absorbed iron may be retained for a while to regulate body iron store, thereby a significant increase in iron in the intestinal mucosa was shown in the iron-overloaded rats. In the small intestine, however, the increased iron content may be reflected by increased serum iron. Selenium-deficiency (−Se) caused significantly decreased GSHPx activity in the liver and intestinal mucosa of rats (Table 7). Iron-overload did not affect GSHPx activity (Table 7). Lipid peroxidation

Table 7. Glutathione peroxidase and cytochrome P-450 activity and lipid peroxidation in the liver and intestine of orally iron-overloaded rats

Parameters	Experimental groups			
	+Se+Fe	−Se+Fe	+Se++Fe	−Se++Fe
Liver				
GSHPx (μM NADP/min/mg protein)	0.74 ± 0.08^{a}	0.47 ± 0.06^{b}	0.68 ± 0.07^{a}	0.39 ± 0.06^{b}
P-450(μg *p*-nitrophenol/hr/mg protein)	1.40 ± 0.18^{a}	1.33 ± 0.15^{a}	1.58 ± 0.22^{a}	1.48 ± 0.17^{a}
Lipid peroxidation (nM MDA/mg protein)	0.08 ± 0.01^{a}	0.13 ± 0.03^{b}	0.10 ± 0.02^{b}	0.21 ± 0.04^{c}
Intestinal mucosa				
GSHPx (μM NADP/min/mg protein)	0.08 ± 0.01^{a}	0.05 ± 0.01^{b}	0.07 ± 0.01^{a}	0.05 ± 0.01^{b}
Lipid peroxidation (nM MDA/mg protein)	0.60 ± 0.11^{a}	1.12 ± 0.18^{b}	0.97 ± 0.17^{bc}	1.22 ± 0.24^{b}

^{abc}Means in the same row with the same superscript letter are not significantly different ($p < 0.01$).

occurred highly in iron-overloaded rats. In the liver and intestinal mucosa, the lipid peroxidation was strongly associated with the tissue iron store. In experimental groups with the same iron status, selenium-deficient (−Se) rats showed a high MDA value, but not significantly different from selenium-adequate (+Se) rats (Table 7). In the liver, there was no significant difference in cytochrome P-450 activity between experimental groups (Table 7).

Discussion

Iron overload in human may be produced by an increased absorption of dietary iron and/or by parenteral administration of iron. In our study, iron-overload was induced by either parenteral or dietary manipulation. The increased serum iron concentration may be results of consumption of large amounts of bioavailable dietary iron. However, intestinal mucosa may control or regulate the absorption of dietary iron, dependently on body iron store. Parenteral iron loading is produced by repeated blood transfusion or by injection of therapeutic iron preparations. In our study, iron dextran was injected intramuscularly to rats. The excretion of injected iron may be limited by urinary, biliary and exfoliation of intestinal mucosal cells. Iron overload in hereditary hemochromatosis which is the most common of the iron-loading disorder first occurs in parenchymal cells of the liver[1]. Thalassemia, the iron loading anemia, in which there is an increased absorption of iron has similar pattern of iron overload to hereditary hemochromatosis. In these patients, elevated iron absorption is probably the result of ineffective erythropoiesis and/or hemolysis of erythrocytes[2].

The excess iron stores by parenteral iron overload are found primarily in the reticuloendothelial system, but eventually iron is accumulated in parenchymal cells of the liver[5]. Iron within hepatocytes can be formed in several biochemical forms: ferritin, hemosiderin, heme, and iron in the putative 'intracellular transit pool' or 'low molecular weight chelate pool'. Although the form of intracellular iron responsible for initiating hepatic lipid peroxidation in iron-overload is not known, ferritin and hemosiderin may be involved. Both ferritin and hemosiderin can initiate lipid peroxidation *in vitro* at neutral pH when a reductant such as ascorbate or superoxide anion is present; however, at acidic pH, ferritin and hemosiderin can stimulate lipid peroxidation without a reductant[21]. Another possibility is that severe iron overload results in an increase in the putative 'intracellular transit pool' of iron from which certain components could be catalytically active in stimulating lipid peroxidation[22].

Iron can catalyze lipid peroxidation cascades. Fe (II) reacts with preformed lipid hydroperoxides to form alkoxyl radicals (1) and Fe (III) reacts with lipid hydroperoxides to form peroxyl radicals (2).

$LOOH + Fe^{2+} \rightarrow Fe^{3+} + OH^- + LO^{\cdot}$ ····· (1)

$LOOH + Fe^{3+} \rightarrow Fe^{2+} + H^+ + LOO^{\cdot}$ ····· (2)

Both alkoxyl and peroxyl radicals can stimulate the chain reaction of lipid peroxidation by extracting further hydrogen atoms. Hydroxyl radical produced either by the Fenton reaction (3) may also be responsible for initiating the peroxidative reaction[23].

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\cdot}OH$ ····· (3)

Regardless of the initiating species, the peroxidative decomposition of the polyunsaturated fatty acids of organelle membrane phospholipids would result in specific abnormalities of organelle function which are dependent on intact membrane[8].

In the early 1970s, Se was discovered as an integral part of GSHPx[12] . Of which the activity in animal tissue is directly related to the availability of dietary Se[24]. Pascoe *et al*[13] found significant lower GSHPx activity in the intestinal cells of rats after 3 days of a Se-deficient diet (0.01ppm Se). Se-dependent GSHPx plays an important role in prevention of the membrane lipid peroxidation[9]. GSHPx reduces H_2O_2 and other hydroperoxides possibly present in the soluble compartment of the cell, whereas phospholipid hydroperoxide GSHPx reduces phospholipid hydroperoxides in the membrane, resulting in prevention of lipid peroxidation[9].

In this study, the roles of Fe and Se, as a prooxidant and an antioxidant, respectively, were compared in rats manipulated by Fe-overload in combination with Se status. Generally, it is known that treatment with Se increases liver and plasma levels of GSHPx[11]. Meanwhile, dietary iron had no significant effects on selenium metabolism, especially if adequate amounts of selenium are being consumed[25]. In our study, GSHPx activity was dependent on selenium status, but not on iron status. Chareonpong-Kawamoto and Yasumoto[26] found that serum iron concentration was decreased by 40-58% in Se-deficient rats, compared to Se-adequate rats. Iron concentration in tissues ranged from 1.1 to 2.5 times higher in Se-deficient rats than in Se-adequate rats. They concluded that Se-deficiency might cause a secondary overload of iron. However, our result did not accord with their data. Moriarty *et al*[27] reported that during iron-deficiency Se-dependent GSHPx1 mRNA levels and Se-dependent GSHPx activity were decreased in the liver of rats. Se concentration in the liver was also decreased by 42%.

There is evidence of decreased Se-dependent GSHPx activity in animals under Fe overload[28]. Reffet *et al*[29] induced dietary Fe overload in lambs and observed decreased Se-dependent GSHPx activity in the liver. Lee *et al*[28] evaluated GSHPx activity during intake of excess dietary iron and Se deficiency : a decrease in liver Se-dependent GSHPx activity was observed. Liver Se-dependent GSHPx activity was decreased markedly during Se-deficiency.

Lipid peroxidation in liver was elevated in the Fe-overload animals compared to the controls[28]. However, total GSHPx in liver remained constant or decreased in animals receiving high iron. GSHPx did not respond to increased oxidative stress associated with elevated dietary iron. Also, iron-overload can lower the hepatic Se-dependent GSHPx activity[28,29]. A supplement of both selenium and vitamin E increased significantly heart selenium and GSHPx activity in iron-overload mice. This treatment also decreased iron concentration in heart[10]. Pascoe *et al*[13] demonstrated that dietary deprivation of Fe could decrease the intestinal cytochrome P-450 content and activity. By contrast, liver cytochrome P-450 has been shown to be highly resistant to Fe-deficiency[30]. In the intestine, cytochrome P-450 content is much less than that of the liver (0.1 vs 0.9nmol/mg microsomal protein).

In both parenteral and dietary iron-loaded rats, GSHPx activity in the liver and intestinal mucosa was depended on selenium status. The iron-overload increased lipid peroxidation, probably resulting from increased tissue iron content. GSHPx seemed to be partly participated in the prevention of lipid peroxidation.

References

1. Edwards LQ, Griffen LM, Kushner JP. Comparison of stainable liver iron between symptomatic and asymptomatic hemochromatosis homozygotes and their homozy-gous relatives. *AM J Med Sci*, 301:44-46, 1991.
2. Gordeuk VR, Bacon BR, Brittenham GM. Iron overload : causes and consequences. *Annu Rev Nutr*, 7: 485-508, 1987.

3. Bonkovsky HL. Iron and the liver. *Am J Med Sci*, 301: 32-43, 1991.
4. Hann H, Kim CY, London WT, *et al*. Increased serum ferritin in chronic liver disease: a risk factor for primary hepatocellular carcinoma. *Intl J Cancer*, 43:376-379, 1989.
5. Fletcher LM, Roberts FD, Irving MG, *et al*. Effects of iron loading on free radical scavenging enzymes and lipid peroxidation in rats liver. *Gastroenterology*, 97: 1011-1018, 1989.
6. Vaca EE, Wilhelm J, Harms R. Interaction of lipid peroxidation products with DNA. A review. *Mutat Res*, 195:137-149, 1988.
7. Weinstein IB. Current concepts and controversies in chemical carcinogens. *J Supramol Struc Cell Biochem*, 17:107-128, 1981.
8. Britton RS, Bacon Br, Recknagel RO. Lipid peroxidation and associated hepatic organelle dysfunction in iron overload. *Chem Phys Lipids*, 45:207-239, 1987.
9. Ursini F, Bindoli A. The role of selenium peroxides in the protection against oxidative damage of membranes. *Chem Phys Lipids*, 44:255-276, 1987.
10. Bartfay WJ, Hou D, Brittenham GM, *et al*. The synergistic effects of vitamin E and selenium in iron-overloaded mouse hears. *Can J Cardiol*, 14:937-941, 1998.
11. Simoni J, Simoni G, Garcia EL, *et al*. Protective effect of selenium on hemoglobin mediated lipid peroxidation *in vivo*. *Artif Cell Blood Substit Immobil Biotechnol*, 23:469-486, 1995.
12. Flohe L, Gunzler WA, Schock HH. Glutathione peroxidase: a selenoenzyme. *FEBS Lett*, 32:132-134, 1973.
13. Pascoe GA, Sakai-Wong J, Soliven E, *et al*. Regulation of intestinal cytochrome P-450 and heme by dietary nutrients. *Biochem Parmacol*, 32:3027-3035, 1983.
14. Buege TA, Aust SD. Microsomal lipid peroxidation. *Meths Enzymol*, 105:302-310, 1978.
15. Stookey LL. Ferrozine-a new spectrophotometric reagent for iron. *Anal Chem*, 42:779-781, 1970.
16. Crosby WM, Munn SL, Furth FW. Standardized methods for clinical hemoglobinometry. *US Armed Forces Med J*, 5:693-703, 1954.
17. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythro-glutathione peroxidase. *J Lab Clin Med*, 70:158-169, 1967.
18. Kinoshita FK, Frawley JP, DuBois KP. Quantitative measurement of reduction of hepatic microsomal enzymes by various dietary levels of DDT and toxaphene in rats. *Toxicol Appl Pharmacol*, 9:505-513, 1966.
19. Lowry OH, Rosebrough NJ, Farr AL, *et al*. Protein measurement with the folin phenol reagent. *J Biol Chem*, 93:265-275, 1951.
20. SAS Institute, Inc. *SAS User Guide : Statistics*, 5th ed. Cary, NC, 1985.
21. O'Connell MJ, Ward RJ, Baum H, *et al*. The role of iron in ferritin- and hemosiderin-mediated lipid peroxidation in liposomes. *Biochem J*, 229:135-139, 1985.
22. Britton RS, Ferrali M, Magiera CJ, *et al*. Increased prooxidant action of hepatic cytosolic low-molecular-weight iron in experimental iron overload. *Hepatology*, 11:1038-1043, 1990.
23. Aikens J, Dix TA. Perhydroxyl radical (HOO ·) initiated lipid peroxidation. *J Biol Chem*, 266:15091-15098, 1991.
24. Knight SA, Sunde RA. The effect of progressive selenium deficiency on antiglutathione peroxidase antibody reactive protein in rat liver. *J Nutr*, 117:732-738, 1987.
25. Abdel Rahim AG, Arthur JR, Mills CF. Effects of dietary copper, cadmium, iron, molybdenum and manganese on selenium utilization by the rat. *J Nutr*, 116: 403-411, 1986.
26. Chareonpong-Kawamoto N, Tasumoto K. Selenium deficiency as a cause of overload of iron and unbalanced distribution of other minerals. *Biosci Biotechnol Biochem*, 59:302-306, 1995.
27. Moriarty PM, Picciano MF, Beard JL, *et al*. Classical selenium-dependent glutathione peroxidase expression in decreased secondary to iron deficiency in rats. *J Nutr*, 125:292-301, 1995.
28. Lee YH, Layman DK, Bell RR, *et al*. Response of glutathione peroxidase and catalase to excess dietary

iron in rats. *J Nutr*, 111:2195-2202, 1981.

29. Reffet JK, Spear JW, Prabowo A. Lipid peroxidation and glutathione peroxidase, catalase and superoxide dismutase activities in lambs fed high dietary iron. *Nut Rpt Intl*, 34:977-983, 1986.
30. Dallman PR, Goodman JR. Iron deficiency and hepatic cytochrome P-450 activity. *J Cell Biol*, 48:79-90. 1971.