Iron Overload Secondary to Selenium Deficiency in Rat

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ABSTRACT

The present study was aimed to examine the effects of dietary deficiency of selenium (Se) and/or dietary excess of iron (Fe) on the iron levels in serum and various tissues of rats. The animals were fed either of following diets : Se-deficient/Fe-adequate (0.0035% Fe as ferric citrate), Se-adequate (0.1ppm Se as sodium selenite) /Fe adequate, Se-deficient/Fe-excess (0.25% carbonyl Fe) and, Se-adequate/Fe-excess. Rats were killed after 30 weeks of feeding period and the levels of Fe and Se in serum, liver, kidney, spleen, heart and lung were determined by an inductively coupled argon emission spectrophotometer. Hematological parameters were obtained by usual procedures. Within Fe-adequate groups the Fe levels were significantly higher in serum and most of analyzed tissues of Se-deficient rats than those of Se-adequate rats. The conclusion derived is that excess dietary Fe did not augment the conditions produced by Se deficiency. These results confirm the previously held notion that Se deficiency increases Fe levels in serum and other tissues because of Fe liberated by promoted hemolysis.

INTRODUCTION

Selenium deficiency causes a compensated hemolysis in animals, which is characterized by the increases in sensitivity to hemolysis (1,2,3), in the methemoglobin formation in vitro (3,4) and in the population of abnormal erythrocytes containing Heinz bodies (3,5,6,7), and the decrease in the proportion of aged erythrocytes (8). Such abnormal erythrocytes would be readily sequestered on passage through the spleen and elsewhere. The increased hemolysis in circulating blood and the ready sequestration of erythrocytes may well result in increased iron in serum and among various tissues. Previous studies in our laboratory have shown by using chemical and histological methods that selenium deficiency for a prolonged period leads to an iron excess condition in animals (9,10). The purpose of this study was to confirm these changes not to occur by excess dietary iron in conjunction with selenium deficiency.

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MATERIALS AND METHODS

Weanling male Wistar rats were divided into 4 groups and fed either of following Torula yeast-based diets (Table 1) : selenium-deficient/iron-adequate (0.0035% iron as ferric citrate), selenium-adequate (0.1 ppm selenium as sodium selenite) /iron-adequate, selenium-deficient/iron-excess (0.25% carbonyl iron), and selenium-adequate/iron-excess. On analysis, each kg of selenium-adequate and selenium-deficient diets contained 0.12±0.01 and 0.02±0.01 mg (means±SEM, n= 4) of selenium, respectively. They were fed their respective diets and water *ad libitum*. After 30 wks of the feeding period, blood was collected from vena cava of animals anethetized with sodium pentobarbital, and then the animals were killed by decapitation. Serum was centrifugally separated from the blood clot. Various tissues were excised, cleaned of adhering materials, blotted, weighed and stored at -70℃ until analysis, The levels of iron were determined by an inductive coupled argon emission spectrophotometer (ICAP-96-953, Nippon Jarrell-Ash Co, Ltd, Uji, Japan) after digestion of the samples in a nitric acid-perchloric acid mixture. Glutathione peroxidase activity was assayed by a modification of the method of Plaglia and Valentine (11) using *tert*-butyl hydroperoxide as substrate as previously described (1). Hemoglobin was determined by cyanmethemoglobin method of International Commitee for Standardization in Haematology using a test kit (Hemoglobin Test-Wako, Wako Pure Chemicsl lnd, Osaka, Japan). Hematocrit was read from the percentage of red cells after centrifugation of the whole blood in capillary tube at 6000 rpm for 10 min in a hematiocrit centrifuge (MC-202, Hitachi koki, Tokyo, Japan).

Table 1. Composition of Basal Diet (g/kg diet)

Ingredient	Se(−)	Se(+)	Se(−)Fe	Se(+)Fe
Torula yeast	360	360	360	360
DL-Methionine	3	3	3	3
Sucrose	460	460	460	460
Soybean oil	50	50	50	50
Cellulose	30	30	27.5	27.5
α-Corn starch	50	50	50	50
Mineral mix[1]	35	35	35	35
Vitamin mix[2]	10	10	10	10
Choline bitartrate	2	2	2	2
Carbonyl iron	—	—	2.5	2.5

[1]AIN-76 mineral mix for selenium-adequate diet (0.1 ppm Se) and AIN-76 mineral mix modified without sodium selenite for selenium-deficient diet. Both diets contained 34-36 mg iron/kg diet as ferric citrate.
[2]AIN-76 vitamin mix.

RESULTS AND DISCUSSION

The mean iron levels in serum, liver, spleen, heart, lung, kidney and intestine in two selenium-deficient groups were significantly higher than in other two groups (Table 2). There is no significant difference between iron adequate groups and iron excess groups. Therefore, it can be presumed that excess iron diet has no effect on the absorption of iron in selenium-deficient rats. These results leads to the conclusion that the excess iron deposits are not attributable to an increased absorption of dietary iron, as occurs in hemochromatosis, iron-deficient anemia and other conditions, but are responsible for the iron liberated by compensated hemolysis.

Previous studies in this and other labolatories have shown that selenium deficiency is associated with hematological abnormality, which is characterized by the increases in the sensitivity to hemolysis (1,2,3), in the methemoglbin formation in vitro (3,4) amd in the population of abnormal erythrocytes containing Heinz bodies (3,5,6,7), and by the decrease in the proportion of aged erythrocytes in mice (8). Therefore, the selenium deficiency is associated with promoted hemolysis and sequestration of defective erythrocytes from the circulating blood, because the defective erythrcytes are to be readily hemolyzed during circulation or sequestered on passages through the reticuloendothelial system. However, this hemolysis state is not associated with severe anemia, so the condition is described as compensated hemolysis (1). The increased hemolysis in circulating blood and the ready sequestration of erythrocytes may well result in increased in iron in serum and amoung various tissues. Increase in heme oxygenase activity is observed in selenium deficiency (12). This suggests that in selenium deficiency, the level of substrate heme is increased sufficiently enough to induce the enzyme liberating free iron from heme.

The Prussian Blue staining showed iron deposition in bone marrow in rat fed selenium-deficient diet

Table 2. Iron levels in serum and other tissues of rats fed selenium-deficient and iron-excess diet

	Se(−)	Se(−)Fe	Se(+)	Se(+)Fe
Serum	91.5 ± 5.3^{a}	77.1 ± 3.9^{a}	36.6 ± 3.3^{b}	43.4 ± 6.2^{b}
Liver	13.8 ± 2.0^{a}	17.0 ± 1.7^{a}	2.13 ± 0.35^{b}	2.56 ± 0.38^{b}
Spleen	70.6 ± 6.5^{a}	60.8 ± 4.9^{a}	22.6 ± 1.8^{b}	20.1 ± 2.1^{b}
Heart	1.67 ± 0.09^{a}	1.49 ± 0.07^{a}	1.18 ± 0.04^{b}	1.03 ± 0.07^{b}
Lung	1.66 ± 0.05^{a}	1.56 ± 0.04^{a}	1.16 ± 0.19^{ab}	1.02 ± 0.07^{b}
Kidney	5.4 ± 0.66^{a}	4.8 ± 0.77^{a}	1.32 ± 0.13^{b}	1.51 ± 0.23^{b}
Intestine	0.85 ± 0.03^{ab}	0.98 ± 0.05^{a}	0.57 ± 0.05^{c}	0.69 ± 0.11^{bc}

Values are means±SEM ; n=6 for the Se (−) and Se (−) Fe groups and n=5 for the Se (+) and Se (+) Fe groups.Values are represented as μmol/L for serum and μmol/g wet wt for other tissues. Values with different superscripts within a same row are significantly different ($P<0.05$).

(results not shown). Glutathione peroxidase activity in plasma and erythrocytes and serum selenium levels were significantly lower in selenium-deficient rats with or without excess iron than in other two groups (Table 3). These results suggested that selenium deficiency may destabilize the cell membrane to hemolysis.

Hematocrit and hemoglobin levels were also significantly lower in selenium-deficient rats with or without exsess iron than in other two groups, but no significant difference in serum albumin levels (Table 4). The selenium-deficient rats had insign of anemia because hematological data tended to be in the normal range. The observed insignificant difference in albumin level is inconsistent with the previous observation (9,13). This may be explained by the limited period of duration of selenium deficiency in these rats. The observed iron deposits in bone marrow and hematological data in selenium-deficient rats indicate that these rats were under the condition of compensated hemolysis, a hemolytic state that is not associated with severe anemia.

There appears to be a close interrelationship between the mechanisms underlying the pathophysiological effects of selenium deficiency and those of iron overload. Selenium deficiency decreases the antioxidative defense mechanism that is dependent on the functions of glutathione peroxidase and of selenium of yet uncharacterized froms (14), while excess iron activates peroxidation of membrane lipids leading to cell death (15). Therefore, the pathophysiological consequence of selenium deficiency could necessarily be the results of multiple mechanisms, including the decrease of antioxidative defense protection and the

Table 3. Selenium level in serum and glutathione peroxidase in rats fed selenium-deficient and iron-excess diet

	Se(−)	Se(−)Fe	Se(+)	Se(+)Fe
Se (nmol/L)	0.95 ± 0.01^{a}	0.90 ± 0.01^{a}	6.9 ± 0.08^{b}	6.8 ± 0.07^{b}
Glutathione peroxidase activity :				
Plasma (U/g protein)	1.21 ± 0.23^{a}	1.13 ± 0.16^{a}	20 ± 2.9^{b}	21 ± 2.5^{b}
Erythrocyte (U/g Hb)	7.8 ± 0.65^{a}	7.2 ± 1.13^{a}	172 ± 6.3^{b}	169 ± 3.5^{b}

Values are means±SEM, n=6 for the se (−) and for Se (−) Fe groups and n=5 for the Se (+) and Se (+) Fe groups. Values not sharing the same superscript within a row are significantly different ($P<0.05$)

Table 4. Hematological data in rats fed selenium-deficient and iron-excess diet

	Se(−)	Se(−)Fe	Se(+)	Se(+)Fe
Hematocrit(%)	43 ± 0.3^{a}	44 ± 0.7^{ac}	48 ± 0.8^{b}	46 ± 0.6^{bc}
Hemoglobin(g/L)	132 ± 5^{a}	144 ± 4^{ac}	157 ± 4^{b}	159 ± 6^{bc}
Albumin(g/L)	35±2.1	36±1.6	38±1.9	39±1.4

Values are means±SEM ; n=6 for the Se (−) and for Se (−) Fe groups and n=5 for the Se (+) and Se (+) Fe groups. Values not sharing the same supperscript within a row are significantly different ($p<0.05$).

induction of deleterious oxidative damage by excess iron.

Deleterious actions of excessive iron include the widespread iron-induced damages to liver, heart, pancreas, and other organs : overwhelming liver damage, cardiac disease, diabetes mellitus, hormonal changes, skin changes and increased risk of infection (16). Selenium deficiency also causes retarded growth, cardiomyopathies, increased infection in animals (17). Besides, lowered selenium status in humans has been associated with Keshan disease and Kaschin-Beck disease, a cardiomyopathy and a disorder characterized by skeletal deformities and widespread hemosiderosis, respectively (18). Further research is needed to explain whether an excess iron produced by selenium deficiency is involved in the development of cardiovascular diseases.

The conclusion derived is that excess dietary iron did not augment the conditions produced by selenium deficiency. These results confirm the previously held notion that selenium deficiency increases iron levels in serum and other tissues because of iron liberated by promoted hemolysis.

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