Iron and Magnesium Levels and Electrocardiogram in Rat fed Selenium Deficient Diet

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ABSTRACT

Rats were fed selenium (Se) deficient or Se-adequate diet (0.1 ppm Se as Na_2SeO_3) for 42 wks from weanling to assess the effects of Se status on iron (Fe) and magnesium (Mg) levels in rat serum and on electrocardiogram. Fe levels in serum were significantly higher in Se-deficient rats than in Se-adequate rats. The serum transferrin concentration was significantly lower in Se-deficient rats than in control rats. Unsaturated iron binding capacity decreased significantly to give a transferrin saturation of greater than 89% in Se-deficient rats. Mg levels in serum were significantly higher in Se-deficient rats than in Se-adequate rats. The concentration of albumin decreased significantly in serum of Se-deficient rats. Se-deficient rats showed a progressive development of a characteristic abnormality in ECG: a depression of S-T segment and bradycardia.

INTRODUCTION

Selenium (Se) is recognized as an essential trace element for animals and humans[1,2]. Through the enzyme gluthathione peroxidase (GSHPx), Se plays a role in curtailing lipid hydroperoxides and hydrogen peroxide which are capable of interacting with other cell constituents to form free radicals and to cause cellular death[3]. Clinically, Se deficiency is responsible for several diseases such as cardiovascular disease, liver necrosis in rats, myopathy in cattle, and Keshan disease, a cardiomyopathy in human found in China.

The purpose of this research was to study the effects of Se-deficiency for 42 wks on the Fe and Mg levels in rat serum. Fe plays an absolutely essential role in the transport of oxygen to the tissues and in the maintenance of oxidative system within the cells[4]. Mg is essential for the maintenance of the functional and structural integrity of the myocardium[5].

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Table 1. Composition of diets (%)

Ingredients	Se (−)	Se (+)
Torula yeast	36	36
Sucrose	46	46
Soybean oil	5	5
Starch	5	5
Mineral mixture[1]	3.5	3.5
Vitamin mixture[2]	1	1
Cellulose power	3	3
D, L-Methionine	0.3	0.3
Choline bitartrate	0.2	0.2
Na_2SeO_3	—	0.00001

1. Based on AIN-76 formula (16) for Se (+) diet. Se (Sodium selenite) was omitted from AIN-76 formula for Se (−) diet.
2. Based on AIN-76 formula.

MATERIALS AND METHODS

Weanling male Wistar rats were divided into 2 groups, and were fed Torula yeast-based diets (Table 1). One group was fed the selenium deficient [Se (−)] diet and other group the selenium adequate [Se (+)] diet, which contained 0.1 ppm Se as sodium selenite. They were fed their respective diets and water *ad libitum*. Each 100 g of both diets contained 3.47 mg Fe and 50.4 mg Mg. From 12wks to 42 wks, blood samples were collected from the orbital veins. Serum was removed by centrifugation and frozen at −20℃ until it was analyzed. The amounts of Fe and Mg were measured by inductively coupled plasma-atomic emission spectrometry (ICP) (Hitachi Model 96-953). Serum transferrin was analyzed by enzyme-linked immunosorbent assay (ELISA)[6]. The iron-binding capacity of serum was determined by using 2, 4, 6-tri-pyridyl-S-triazine (TPTZ)[7]. This provides an indirect measurement of the unsaturated iron-binding capacity (UIBC) of the serum. Total iron-binding capacity (TIBC) and transferrin saturation (%) were assessed by calculation. The concentration of serum albumin was measured by BCG binding method[8]. GSHPx activity was determined by a modified method of Donald et al[9]. Hemoglobin was determined by the cyanmethemoglobin method[10]. Creatine phosphokinase (CPK) was assayed with a test kit (CPK UV Test-Wako, Wako Pure Chemical Ind., Osaka, Japan).

The ECG pattern was observed by the method of Einthoven[11] using an electrocardiograph, Cardiomate (NEC, Japan). The data obtained were accumulated and processed with a computer. All the data were analyzed by Student's t-test.

RESULTS AND DISCUSSION

The mean levels of some minerals in sera of rats fed Se (−) or Se (+) diets are shown in Fig. 1. Fe and Mg levels in the Se (−) rat sera were significantly higher than in the Se (+) rat sera. There was

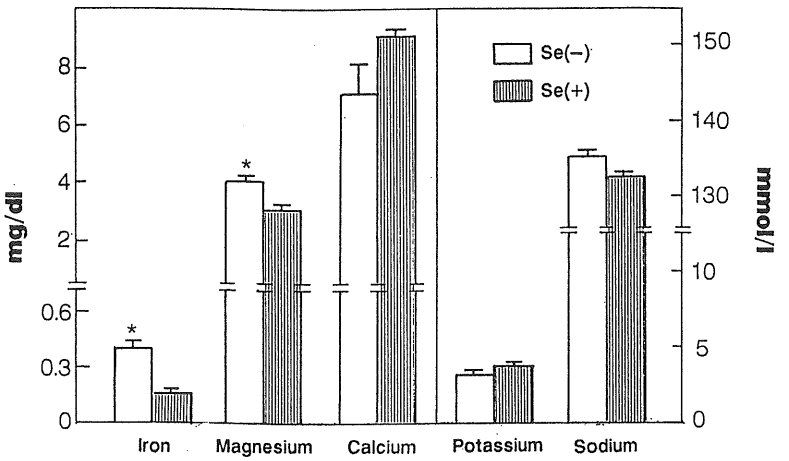

Fig. 1. Mineral levels in sera of Se-deficient and Se-adequate rats. Values represent means ± SEM of 9 and 11 rats respectively. *Significantly different at $p < 0.001$ as compared with Se-adequate rats.

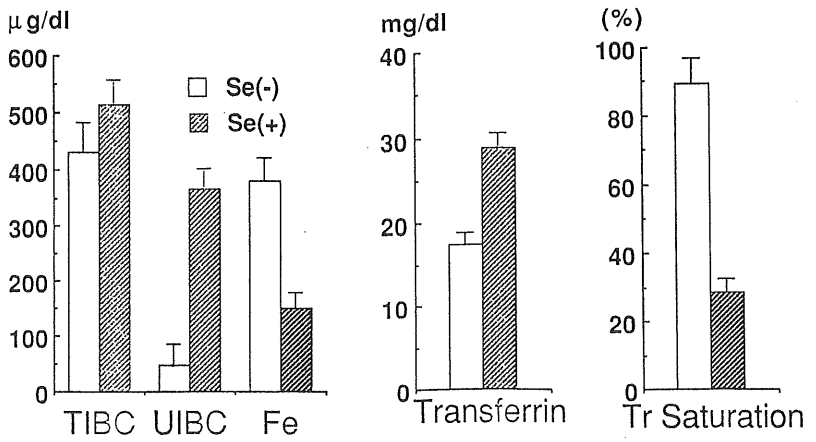

Fig. 2. Serum transferrin level, iron binding capacity and transferrin saturation of Se-deficient and Se-adequate rats. Values represent means ± SEM of 6 rats. *Significantly different at $p < 0.001$ as compared with Se-adequate rats.

no significant difference in the levels of serum calcium, potassium and sodium between the two groups.

Many studies suggested that Fe overload can happen as a result of grossly excessive dietary iron intake[12], an increased rate of red cell destruction[13], a failure of bone function for hemoglobin synthesis[14] and a decreased transferrin concentration, the Fe transport protein[15].

Contrary to the serum Fe levels the serum transferrin concentration was significantly lower in Se (−) rats than in Se (+) rats (Fig 2). Under normal conditions only about 30% of transferrin binds with Fe, and by far a large proportion of iron binding capacity of transferrin is reserved. The reserved being

called unsaturated iron binding capacity (UIBC). In the contary the Se (−) rat serum showed an unsaturated iron binding capacity of nearly zero and a transferrin saturation of greater than 89% (Fig. 2). These results indicated that Se deficiency may disturb the transportation system of Fe. The observed increase in serum Fe levels in Se (−) rats can be interpreted in term of the lack of unsaturated iron-binding capacity; therefore transferrin is no longer available to bind free Fe.

Se deficiency causes a decrease in the activity of GSHPx that may then result in altered cell membrane stability and increased hemolysis. We have repeatedly confirmed that Se deficiency increased hemolysis in these rats. GSHPx activity in erythrocyte, hematocrit and hemoglobin was significantly lower in Se (−) rats than in Se (+) rats (Table 2). These results are consistent with the notion that Se deficiencies affect the erythrocyte membrane to increase hemolysis, which then leads to high Fe levels in the serum. The decreased transferrin concentration and its greatly increased saturation, associated with Se deficiency, hampers the removal of excess free serum Fe.

A majority of serum Mg is associated with albumin and transported to tissues. The concentration of albumin, as assayed by the BCG binding method, decreased significantly in serum of rats fed Se (−) diet (Table 3). This indicates that Se deficiency retards Mg transport into tissue, which results in an increase in serum Mg.

The signs of Keshan disease were cardiac enlargement, arrhythmia, acute or chronic insufficiency of cardiac function, as reflected by ECG abnormality. We have studied heart function in these rats by ECG and assayed one of cardiac enzymes, CPK.

Table 2. Whole blood hemoglobin, hematocrit and erythrocyte glutathione peroxidase activity levels of rats fed Se-deficient or Se-adequate diet for 42 weeks

	Se (−)	Se (+)
GSHPx (U/g Hb)	8.1±3.1* (6)	231±11 (6)
Hematocrit (%)	40.6±1.1* (9)	47.8±0.9(12)
Hemoglobin (g/dl)	14.2±0.9**(11)	17.2±1.0(12)

Values are means ± SEM for the numbers of rats indicated in parentheses. Asterisks indicate significant difference from Se-adequate rats: $^{*}p < 0.001$, $^{**}p < 0.05$.

Table 3. Albumin level and creatine phosphokinase activity in the serum of rats fed Se-deficient or Se-adequate diet for 42 weeks.

	Se (−)	Se (+)
Albumin (g/dl)	3.75±0.17**(11)	4.38±0.02(12)
CPK activity (mU/ml)	209±11* (5)	47±14 (5)

Values are means ± SEM for the numbers of rats shown in parentheses. Asterisks indicate significant difference from Se-adequate group: $^{*}p < 0.05$, $^{**}p < 0.001$.

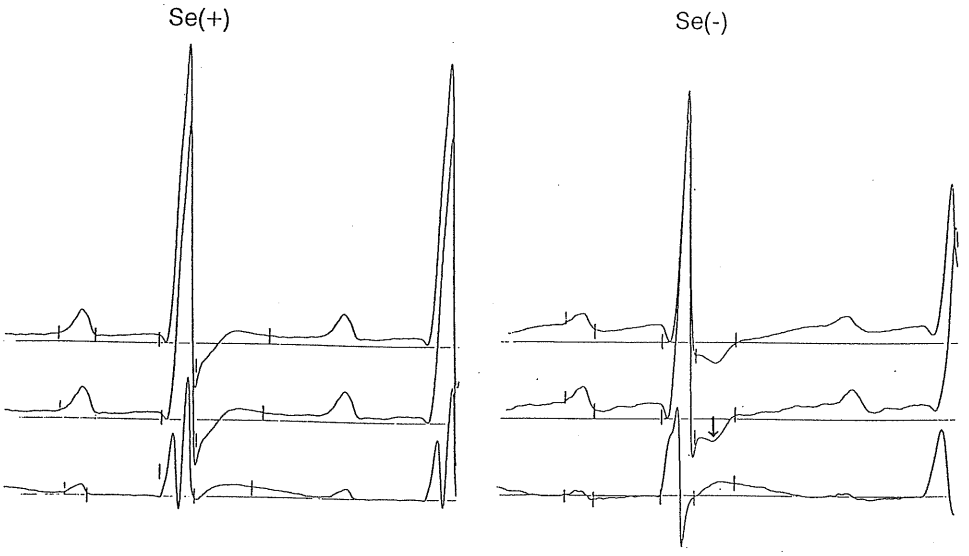

Fig. 3. Abnormal ECG patterns in Se-deficient rats; S-T segment depression.

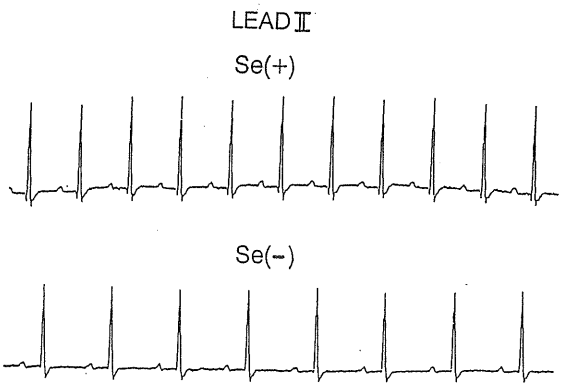

Fig. 4. Abnormal ECG patterns in Se-deficient rats; Bradycardia.

In Figs. 3 and 4, we compare ECG patterns of Se adequate and Se deficient rats by the method of Einthoven. S-T depression was observed in Se deficient rats (Fig. 3). This suggests a possible disorder to occur in the contraction of the left ventricle at the early stage of repolarization. Bradycardia, a prolonged period of contraction of cardiac muscle, was observed in some rats fed a Se deficient diet (Fig. 4).

The serum CPK activities were significantly greater in Se (−) rats when compared with Se (+) rats (Table 3). These results suggest that Se deficiency is closely associated with the injury of myocardium. Therefore, it is possible to interpret that Fe and Mg excess in serum are associated with observed abnor-

mal ECG and elevated CPK with Se deficient rats. Further extensive study is need for more precise interpretation of the findings described in this paper.

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