Iron Overload Secondary to Selenium Deficiency Changes the Electrocardiographic Pattern in Rats

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ABSTRACT

Selenium (Se) deficiency causes an abnormal hematological profile in animals, and an increased accumulation of iron in various organs. The aim of this study was to investigate whether excess iron has a direct effect on cardiac muscle, leading to increased abnormalities in heart functions, as observed in Keshan disease. Male Wistar rats fed either of following Torula yeast-based Se-deficient [Se(−)] or Se-adequate [Se(+)] (containing 0.1 ppm Se as sodium selenite) diet for 8 weeks. The animals were injected intramuscularly with iron-dextran (totaling 500 mg of iron). Iron concentrations in the liver and spleen as well as the percentage of transferrin saturation were significantly higher in Se(−) rats without iron overload than in Se(+) rats without iron overload. However, iron concentrations in the sera, hearts and kidneys did not significantly differ between the groups. Se(−) and Se(+) rats with iron overload ahowed a 2 to 40-fold increase in iron concentrations of all tissues examined, and 2-fold increase in the percentage of transferrin saturation, regardless of Se status. Se(−) rats with and without iron overload and Se(+) with iron overload all showed a greater T-wave height, a depression of S-T segment and a shallow S-wave. There was a 2-fold increase in T-wave height in Se(−) rats with iron overload compared with Se(−) rats without iron overload. The conclusion derived is that electrocardiogram is abnormal in Se deficiency, probably due to increased myocardial iron deposits.

INTRODUCTION

Selenium (Se) is an essential trace element for mammals. In humans, a lower Se status has been associated with Keshan disease. Supportive evidence for the pathologic importance of Se deficiency in heart disease has been reported from Finland (1), which clearly demonstrated that the relative risks of coronary heart disease and myocardial infarctions are high in the people with lower serum Se level (≤ 34 μg/L) compared with those with the higher level (≥ 45 μg/L). Previous studies in this and other

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laboratories have shown that Se deficiency results in a hematological abnormality, which may lead to an increased distribution of iron in various tissues (2).

Recently, even normal levels of stored iron are suggested to promote ischemic heart dissase and iron depletion protects against it (3, 4). Salonen *et al* (5) have also suggested an association between stored iron and increased risk of myocardial infarction in eastern Finnish men. The purpose of this study was to determine whether Se-induced excess iron has a direct effect on cardiac muscle, leading to increased abnormalities in heart functions.

MATERIALS AND METHODS

Animals and diets. Weanling male Wistar rats were divided into 4 groups of six animals each and fed Torula yeast-based diets which were either selenium-deficient [Se(−)] or Se-adequate [Se(+)] to their respective (Table 1) for 8 weeks. They were given free access to water and diets. The animals were loaded with 100 mg of iron dextran (Imferon, Sigma Chemicals Co. St. Louis, MO), weekly, to a total of 500 mg, injected intramuscularly, beginning at week 2 of the study. Control animals received an equialent volume of sterile isotonic sodium chloride. There were initially six animals per group but one rat in the Se(−) group with iron overload, died midway through the study. Animals were weighed in the morning at weekly intervals.

Electrocardiographic recording and analysis. Electrocardiograms (ECG) were taken by using the bipolar standard leads that were originally described by Einthoven (6). By utilizing the limbs (extremities) to hold the electrodes, all leads are sufficiently distant from the heart so that none are disproportionately influenced.

From week 3 to 8 on the experimental diet feeding, rats were anesthetized (lightly) with ether,

Table 1 Composititon of Basal Diet (g / kg diet)

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

[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.

placed in a supine position. The ECGs were recorded on a Cardiomate (NEC, Japan) for humans at a voltage setting of 1 mV/cm and at a paper speed of 25 mm/sec. The analogue data from the Cardiomate were converted to digital data by an A/D converter interfaced with a personal computer (PC 9801-VX, NEC, Japan).

After 8 weeks of the feeding period, the rats were anaesthetized with sodium pentobarbital (50 mg/kg body wt, intraperitoneally). The abdominal cavity was opened and blood samples for analysis were collected from the vena cava. The animals were then sacrificed by decapitation. The livers, hearts, kidneys and spleens were immediately excised, cleansed of adhering materials, blotted dry and then stored at −70℃ until analysis.

Biochemical Analyses. Hematological parameters, iron status, glutathione peroxidase ($GSHP_x$) activity and iron concentration were measured and have been reported previously (2).

Statistics. The data were expressed as means ± SEM. The Student's t test was used to compare the group mean.

RESULTS AND DISCUSSION

Se deficiency was verified dy a significantly lower $GSHP_x$ activity in erythrocytes, livers and hearts in the Se(−) rats with and without iron overload compared to the other two groups (Table 2). Blood hemoglobin, hematocrit and serum albumin levels were not affected in all groups (Table 2). These results suggest that iron overload has no effect on $GSHP_x$ and hematological parameters.

Table 3 shows iron concentrations in serum and various tissues. Se(−) rats without iron overload had a significantly higher iron concentration in the liver and spleen than Se(+) rats without iron overload. However, iron concentrations in the sera, hearts and kidneys did not significantly differ between the Se(−) rats and Se(+) rats without iron overload. Iron overload in Se(−) and Se(+) rats caused a significant increase in iron concentration in sera, livers, spleens, hearts and kidneys.

Table 2. Glutathione peroxidase activity and hematological data in Se-deficient rats and Se-deficient with iron overload for 8 wk

	Se(−)	Se(−)Fe	Se(+)	Se(+)Fe
Glutathione peroxidase activity :				
Erythocyte (U / g Hb)	13± 2 [a]	12±1[a]	159±11[b]	151±12[b]
Liver (U / g protein)	16±2[a]	17±1[a]	115±5[b]	105±6[b]
Heart (U / g protein)	29±3[a]	29±4[a]	171±12[b]	187±10[b]
Hematocrit (%)	46±0.7	45±0.8	46±0.5	46±0.5
Hemoglobin (g / L blood)	142±4	141±3	145±3	142±2
Albumin (g / L serum)	35±2	34±2	38±2	37±1

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

Table 3. Iron levels in serum and other tissues of Se-deficient rats and Se-deficient rats with iron overload for 8 wk

	Se(−)	Se(−)Fe	Se(+)	Se(+)Fe
		μmol / L		
Serum	32 ± 4^{a}	56 ± 5^{b}	26 ± 1^{a}	43 ± 3^{b}
		μmol / g fresh wt		
Liver	2.11 ± 0.11^{b}	17.4 ± 1.7^{c}	1.38 ± 0.07^{a}	15.4 ± 1.4^{c}
Kidney	0.76 ± 0.03^{a}	5.68 ± 0.27^{b}	0.76 ± 0.04^{a}	5.22 ± 0.31^{b}
Heart	1.17 ± 0.06^{a}	2.42 ± 0.13^{b}	1.13 ± 0.07^{a}	2.35 ± 0.13^{b}
Spleen	30 ± 2^{b}	64 ± 6^{c}	12 ± 1^{a}	61 ± 5^{c}

Values are means ± SEM; n = 5 for the Se(−)Fe and n = 6 for the Se(−), Se(+) and Se(+)Fe groups. Values with different superscripts within the same raw are significantly different ($p<0.05$).

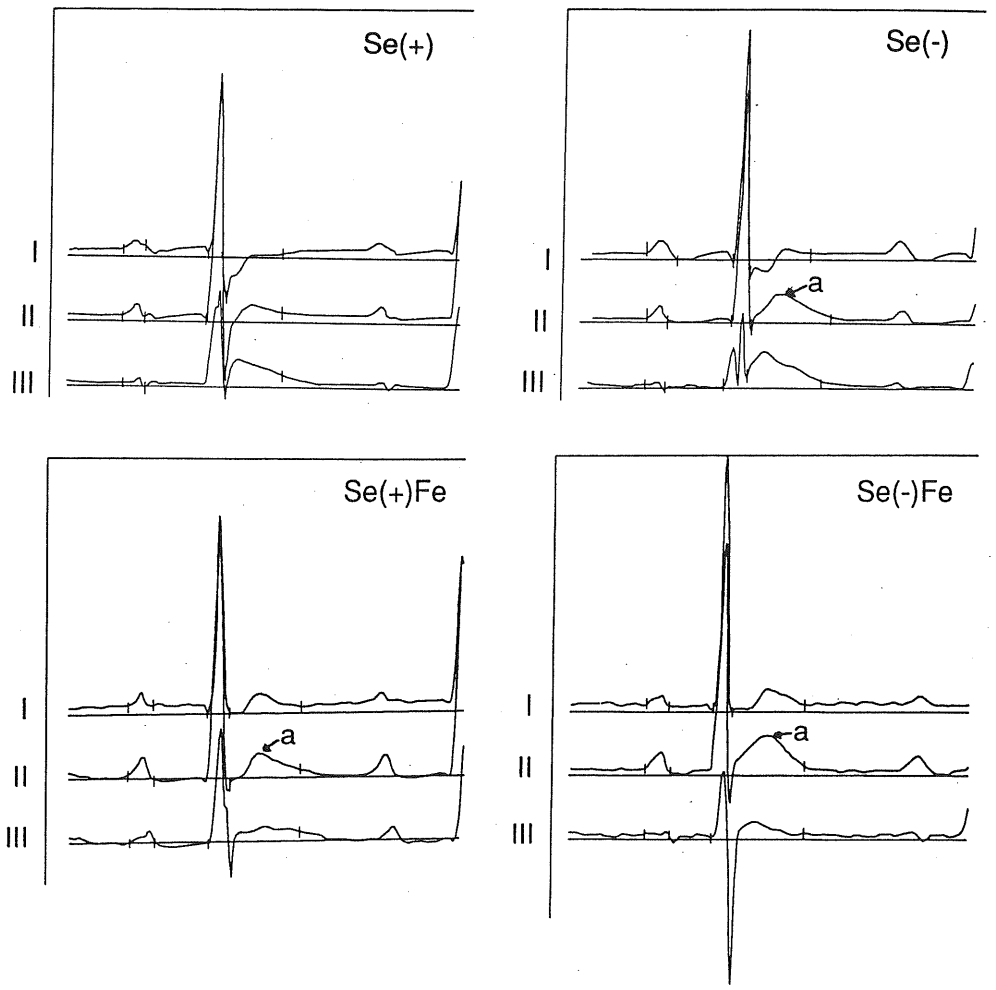

Fig. 1. Abnormal ECG patterns in Se-deficient rats and Se-deficient rats with iron overload. a, enhanced T-wave.

Se(−) rats with and without iron overload and Se(+) rats with iron overload showed ECG abnormalities, predominantly enhanced T-waves (Fig. 1). S-T segment depressions and shallowing of S-wave were also observed. The enhanced T-waves observed in this study were similar to those reported previously in Se(−) infant rats (5-6 weeks old) born from Se(−) female rats spau out(7). In light of our study and the aforementioned study, it is reasonable to predicate that Se deficiency may cause a disorder in the contraction of the ventricle.

However, iron deposition in cardiac muscle is associated with the occurrence of supraventricular arrhymias (8), flattened T-waves and low voltage of the QRS complex (9). We did not observe these abnormalities. However, we did observe a 2 fold increase in T-waves in Se(−) rats with iron overload compared with Se(−) rats without iron overload. These observations prompted us to suspect that those iron deposits in the heat influence the heart function in Se deficiency.

This abnormality may be caused by toxicity of iron. The mechanism by which iron produces tissue damage has not been well established, but it is generally accepted that iron toxicity begins when the iron load exceeds the tissues-or blood-binding capacity and joins a free pool defined as nontransferrin iron (10). The amount of iron that can be stored in a tissue depends on the capacity of the tissue to generate storage proteins; we evaluated transferrin saturation values, which reflects the proportion of iron-bivding sites on serum transferrin molecules that are bound to iron. The transferrin saturation values were significantly higher in Se(−) rats with and without iron overload and Se(+) rats with iron overload than in Se(+) rats without iron overload (Table 4). Thus, the increased transferrin saturation may result in an increase of non-protein-bound iron, which may induce oxygen radicals or peroxides which are directly responsible for cell damage.

Physiological activity of Se has been accounted for largely as a cellular defense mechanism. Se constitutes the catalytic site of $GSHP_x$ (11),which detoxicates hydrogen peroxide and organic hydroperoxides. In contrast, Se(+) rats were not protected against the effect of excess iron as were Se(+) rats with iron overload.

In conclusion, Se(−) rats with and without iron overload and Se(+) rats with iron overload

Table 4. Total iron-binding capacity, transferrin saturation, transferrin and ferritin in Se-deficient rats and Se-deficient rats with iron overload for 8 wk

	Se(−)	Se(−)Fe	Se(+)	Se(+)Fe
TIBC (μmol / L serum)	90±2	89±2	95±3	88±1
TS (%)	45±2^b	78±3^c	32±1^a	71±5^c
Transferrin (g / L serum)	2.9±0.2	2.7±0.3	3.1±0.1	3.0±0.2
Ferritin (mg / L serum)	0.40±0.04^a	0.79±0.07^b	0.40±0.06^a	0.68±0.06^b

Values are means ± SEM ; n = 5 for the Se(−)Fe and n = 6 for the Se(−), Se(+) and Se(+)Fe groups.
Values not sharing the same superscripts within the same row are significantly different ($p < 0.05$).
Abbreviation : TIBC, total iron-binding capacity ; TS, transferrin saturation.

showed ECG abnormalities, predominantly enhanced T-waves. This observation suggests that abnormalities in the heart functions in the Se(−) groups are enhanced by iron, not only at concentrations indicating massive iron overload, but also at normal concentrations. Iron in tissues may lead to peroxidation of cell membranes and oxidation of intracellular protein, ultimately resulting in cell damage.

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