Histological Study of Iron Deposits in Tissues of Selenium-deficient Rats

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ABSTRACT

Previous studies performed in our laboratory have demonstrated that selenium (Se) deficiency for a prolonged period leads to an iron excess condition in rats; iron contents of serum and tissues including heart, liver, kidney, spleen and femur are significantly increased and serum total iron binding capacity is almost saturated in Se-deficient rats. This report is of histological studies on the location of excess iron deposit in these tissues. Male Wistar rats were fed Torula yeast-based Se-deficient or Se-adequate (0.1 ppm Se as Na_2SeO_3) diet for 82 weeks. Excised tissues were embedded in either paraffin or epoxy resin. Iron deposit was stained in dark blue with Prussian blue on optical microscopic examination of liver (parenchymal cells) and kidney from Se-deficient rats. Iron deposits in kidney were located in the secondary lysosomes existing in proximal tubule by X-ray microanalysis (Kevex system 7000) in conjunction with electron microscopy (Hitachi H-700). Basing on these results, we propose that Se deficiency-induced damages to various tissues are largely, if not completely, interpreted as the consequences of iron excess; excess iron in tissues leads to peroxidation of cell membranes and oxidation of intracellular proteins, which ultimately results in tissue damages.

INTRODUCTION

It has been known for a long time that the body has no mechanism for excreting excess iron, which is distributed preferentially within the parenchymal cells of the liver, pancreas, heart, and other organs, leading to cellular damage, organ dysfunction, and hepatic malignancy unless treated (1, 2). Intravascular hemolysis results in parenchymal iron deposition in the liver and, if severe, in the kidney (3, 4). In our previous studies have demonstrated that selenium (Se) deficiency for a prolonged period leads to an iron excess condition in rats; iron contents of serum and tissues including heart, liver, kidney and femur are significantly increased and serum total iron binding capacity is almost saturated in Se-deficient rats.

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However, in the previous studies we did not utilize histochemical methods, while the histologic examination together with biochemical evaluation is expected to provide the most accurate means of iron assessment. In the present study we utilized the Prussian blue method to define the iron deposits in tissues, to evaluate relative size of the iron deposits, and to identify the sites of iron deposits in the subcellular level with X-ray microanalysis in conjunction with electron microscopy.

MATERIALS AND METHODS

Animals and Diets

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 the other group the selenium adequate [Se(+)] diet (5), which contained 0.1 ppm Se as sodium selenite. They were fed their respective diets and water (deionized water) *ad libitum*. At the 82 weeks of feeding period, rats were killed, organs were excised and histological evaluations were done. Portions of the liver and kidney were fixed in 10% neutralized formalin, dehydrated with a graded series of ethyl alcohol, and embedded in paraffin. Thin sections (5-10 μm) were prepared and mounted on a glass slide for Prussian blue staining with a 2% acid ferrocyanide solution (6). The specimens were examined under a light microscope (Nikon, Japan).

Blocks of liver and kidney were finely minced in 2.5% glutaraldehyde in 0.05 M phosphate buffer at pH 7.2, kept for 2 hours at room temperature, and then rinsed three times in 0.05 M phosphate buffer at pH 7.2. After rinsing, all specimens were postfixed for 1 hr in 1% OsO_4 in 0.05 M phosphate buffer, pH 7.2; routinely dehydrated with a graded series of ethyl alcohol, and embedded in Epon low-viscosity medium (60℃, 48 hrs), as modified by the method of Bacon et al (7). Ultrathin sections were cut on a Reichert-Jung microtome OmU_4 (Austria) with a glass knife and examined in a Hitachi H-700 electron microscope at an accelerating voltage of 100 kV. Representative areas for iron deposits found in

Table 1. Composition of Basal Diet (%)

Ingredients	Se (+) diet
Torula yeast	36
Sucrose	46
Soybean oil	5
Mineral mix*	3.5
Vitamin mix**	1
Cellulose powder	3
DL-Methionine	0.3
Choline chloride	0.2

*Based on AIN-76 formula (5) without Na_2SeO_3. To the basal diet, 0.1 ppm of Se as sodium selenite was added for Se-adequate diet.

**Based on AIN-76 formula (5).

ultrathin sections were investigated with X-ray microanalysis (Kevex system 7000) in conjunction with an electron microscopy (Hitachi H-700).

RESULTS AND DISCUSSION

At autopsy, the livers of Se-deficient rats and of control rats appeared to be of similar size. But the size of kidney appeared enlarged in rats fed Se-deficient diet than in those fed control diet (Fig. 1).

In rat fed Se-deficient diet, the Prussian blue staining showed iron deposition predominantly in parenchymal cells in the peripheral parts of the hepatic lobules; a few particles were present in the space of pericentral vein and abundant particles were present in the lateral space of hepatic lobules of Se-deficient rats (Fig. 2). Fat deposits were also observed in a Se-deficient rat liver (Fig. 2, indicated by arrows).

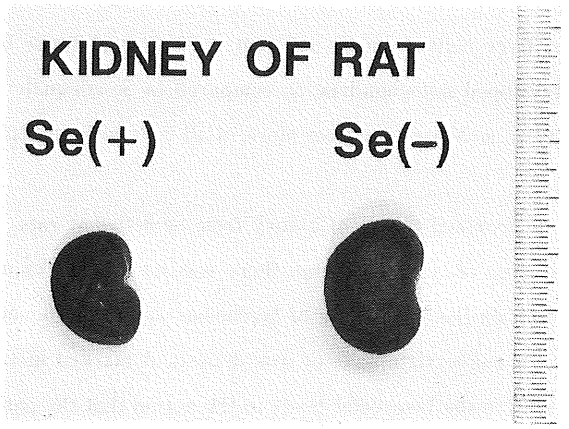

Fig. 1. Enlargement of kidney size in Se-deficient rats after 82 weeks of feeding period.

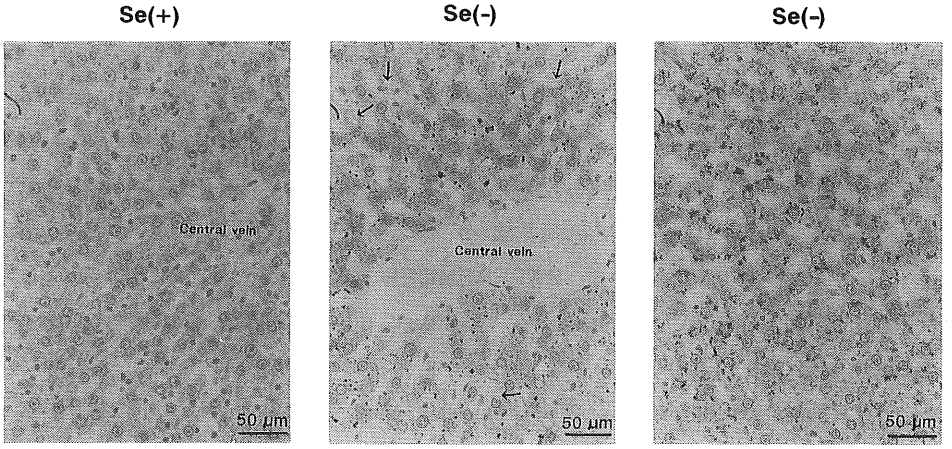

Fig. 2. Prussian blue staining of liver cells from Se-deficient and Se-adequate rats after 82 weeks of feeding periods. Left, locus containing a central vein from Se-adequate rat; middle, locus containing a central vein from Se-deficient rat; right, lateral locus from Se-deficient rat.

There was no evidence of iron deposits in a Se-adequate rat liver (Fig. 2).

The Prussian blue staining of kidney from Se-deficient rats show abundant positive iron granules in the proximal tubule. There was no evidence of iron deposits in the kidney of Se-adequate rats.

A marked increase of iron deposits in parenchymal cells of liver and proximal tubule of kidney could perhaps be explained by the intravascular hemolysis. When red blood cells were destroyed within the circulation, the free hemoglobin binds to plasma haptoglobin and it taken up by hepatic parenchymal cells, not by reticuloendothelial cells. If the plasma haptoglobin becomes saturated with hemoglobin, free hemoglobin is filtered by the kidney and is both reabsorbed and stored by the epithelial cells of the proximal convoluted tubules (3). It is thus possible that Se deficiency causes intravascular hemolysis rather than extravascular hemolysis, leading to an accumulation of iron in parenchymal cell of the liver and in proximal tubule of kidney.

Electron micrographs of liver cells from Se-deficient rats showed large lipid droplets, irregularly-shaped nucleus and less developed mitochondria, as compared in Se-adequate rats. A mitochondrial disorder might lead to inability to mobilize iron from store in all cell, with resultant buildup of iron deposit (8, 9).

On X-ray microanalysis of proximal tubule of kidney from Se-deficient rats, the X-ray emission of iron peaks were observed at the area of lysosomes. But, there was no iron peaks in other areas, for example in mitochondria. The analysis disclosed staining of lysosomes more significantly than of other parts indicating a greater capacity for endocytic uptake of iron deposit. A marked increase of kidney size as well as an increase in iron deposits in the lysosomes leads to the notion that the pathologic changes because of iron loading of lysosomes may lead to their disruption, with consequent release of proteolytic enzymes and hemosiderin into cytosol, and thus to cellular damage (10). It is therefore possible that Se-deficiency causes pathological changes in kidney via excess iron.

Basing on these results, we propose that cellular damages produced by Se-deficiency in various tissues are largely, if not completely interpreted as the consequences of iron excess; excess iron in tissues leads to peroxidation of cell membranes and oxidation of intracellular protein, which ultimately results in cellular damage.

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