Effects of Sodium Selenite on the Ultrastructure of the Kidney Cortex in Normal Rats

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Abstract: This study aims to reveal the histological and ultrastructural effects of sodium selenite on the kidney cortex of normal rats. The over supply of selenium in diet are associated with the occurrence of clinical symptoms to humans and animals. Rats used in this study were divided into five groups, one as a control and the other four groups were treated with sodium selenite at doses of 1 and 2 mg/kg body weight for 5 and 10 days. The histopathologic investigation revealed renal cells damage after 5 days of treatment with lower doses of sodium selenite followed by necrosis in the kidney from rats with higher doses at 10 days. Ultrastructural lesions similar to the light microscopic lesions were most common on the proximal convoluted tubules, distal convoluted tubules and collecting tubules. After 5 days of treatment with low doses, initial cellular changes included vacuolation of cells and mild to moderate degenerative lesions were scattered through the kidney cortex with loss of cellular detail and hydropic degeneration and formation of apical buds and cellular rupture, in distal convoluted tubules and collecting tubules observed after 10 days of treatment with both low and high doses. The range of structural damage in proximal convoluted tubules was more restricted than in distal convoluted tubules and collecting tubules cells.

Key words: sodium selenite- rats- kidney- histopathology- ultrastructure.

INTRODUCTION

Selenium (Se) is an essential trace element, and its clinical significant in various pathophysiologic conditions has been increasingly recognized[2,8,16,17].

Selenium is an integral component at catalytic sites of the enzyme glutathione peroxidase in several tissues[19].

The principle role of selenium is associated with the control of lipid peroxidation because this trace element is a component of selenoenzymes contributing to the antioxidant system[4,6,14,21].

Both deficiency and over supply of selenium in a diet are associated with the occurrence of clinical symptoms including Kashan and Kashin Beck disease[22].

Experimental animal studies for selenium toxicity have been reported.

Selenium has protective effects against mercury toxicity in rat kidney[10] and Selenium also used to diminish the toxic effects of the cadmium on the antioxidant enzyme system, which in turn affects the membranes structures such as mitochondria and endoplasmic reticulum[7].

Selenium deficiency reduces glutathione peroxidase activity have been reported in diet of glomular disease and in diet of tubular epithelium in normal rats[11,10].

Acute tubular injury of mice kidney induced by dimethyl selenide intratracheal instillation have been studied before and also noticed swelling and vacuolation of epithelial cells of proximal tubules and in some mice, tubular necrosis was observed[9].

Organic selenium compounds (selenosemicarbazide) are the form more easily assimilated, compared to inorganic sodium selenite, which is confirmed by an elevated content of selenium in the internal organ of mice after organic and inorganic supplementation with selenium have been reported on histopathologic and ultrastructural changes[11,12,18].

The aim of the present study was to investigate possible histological and ultrastructural changes in the kidney cortex of rats treated with sodium selenite.

MATERIALS AND METHODS

Fifty adult male rats weigh 100-150 mg each were used as experimental animals. Rats were assigned at random to five groups of 10 rats each. One group of rats were designated as control group and other four groups were designated as experimental groups of rats were administered orally by stomach tube two doses of sodium selenite (1 mg and 2 mg/kg body weight).

Rats of control groups were administered distilled water.

At 5 and 10 days after treatment, the rats were sacrificed and the kidneys were removed and washed in phosphate buffer. The kidney cortex was cut into 1 mm thick for light and electric microscopic
examination. Then fixed in 2% formaldehyde and 2.5 glutaraldehyde in 0.02 M phosphate buffer and then placed in fresh cold fixative for 24 hrs. in refrigerator. The tissue was rinsed in cold phosphate buffer (pH 7.4) and post fixed in 2% aqueous osmium tetroxide for 2 hrs. at 4°c. The samples were rinsed in cold phosphate buffer, dehydrated in ethanol series and embedded in epoxy resin. Blocks were sectioned and semithin (1 um) sections were stained in 0.5 % toluidine blue in borax and examined under light microscope.

Ultrathin sections were cut and stained with 2% aqueous uranyl acetate for 20 mins. and lead citrate for 20 mins. for examination and photographed using Joel 1200 Ex-II electron microscope.

RESULTS AND DISCUSSIONS

Control Group: Light microscopic examination of the kidney of control rats had normal renal cortical structure, which consist of glomerulus (tuft of blood capillaries surrounded by epithelial capsule, namely Bowman's capsule, proximal and distal convoluted tubules and the collecting tubules (Fig. 1)

Fig. 1: Photomicrograph of a semithin section of the control rat kidney cortex, showing proximal convoluted tubules (P), distal convoluted tubules (D) and collecting tubular cells (C). Toludine blue stain. X1200.

By using the electron microscope control rats kidney cortex has normal cortical ultrastructural appearances. The cortex constitutes the major portion of the kidney. The proximal convoluted tubules (PCT), distal convoluted tubules (DCT) and collecting tubules (CT) which are common in control rat kidney cortex. The proximal convoluted tubules have luminal brush border (Fig.2), these cells had large central nuclei. Golgi apparatus lies in a supranuclear portions. These brush border is composed of long closely packed microvilli, and these cells contained abundant elongated mitochondria and some lysosomes (Fig.2).

Distal convoluted tubules are shorter than the proximal tubules and the cells have no brush border and the distal convoluted cells have central or apical nuclei. In the basal cytoplasm there is a complex interdigitation of lateral cell processes, radially oriented mitochondria (Fig.3).

The collecting tubules vary in size contain mitochondria and the cytoplasm containing apical vesicles and the plasma membrane of the basal surface is infolded but not deeply. Free ribosomes are prominent and the nucleus is centrally located (Fig.4).

Fig. 2: Photoelectron micrograph of proximal convoluted tubular cell of control rat kidney cortex, showing the apical microvilli (arrow), mitochondria (M), lysosmes (L) and nucleus (N). X15000.

Fig. 3: Photoelectron micrograph of distal convoluted tubular cell of control rat kidney cortex, showing the basal enfolding membrane (arrow), nucleus (N) and elongated mitochondria (M). X12000.

Fig. 4: Photoelectron micrograph of collecting tubular cells of control rat kidney cortex, showing the nucleus (N), mitochondria (M), basal membrane (arrow) and tubular lumen (L). X10000.
Fig. 5: Photomicrograph of a semithin section kidney cortex after 5 days of treatment with sodium selenite (1 mg/kg body weight), showing cloudy, swelling tubular cells and vacuolar degeneration (head arrows). X1200.

Fig. 6: Photomicrograph of a semithin section kidney cortex 10 days after treatment with sodium selenite (2 mg/kg body weight), showing the necrosis of the tubular cells (head arrows) with darkly stained pyknotic nuclei (arrow). X900.

Experimental Groups: Light and electron microscopic examination revealed subcellular changes after 5 and 10 days of treatment with two doses of sodium selenite (1 and 2 mg/kg body weight).

After Five Days: Sodium selenite supplementation in a lower dose (1 mg/kg, body weight), for five days, caused a slight histopathological changes the cells exhibited cloudy swelling and showed smudgy appearance in the renal tubular cells (proximal and distal convoluted tubules, and collecting tubules) and showed also, vacuolar degeneration that is obvious in narrowing of cells lumina and slight alterations of their nuclear appearance (Fig.5).

A higher dose (2 mg/kg body weight) of sodium selenite, revealed progressive histopathological changes.

The epithelial cells lining the convoluted tubules (proximal and distal convoluted tubules and collecting tubules) were partially detached and showed necrotic changes with damage of brush borders and with darkly stained pyknotic nuclei (Fig.6).

Electron microscopic examination revealed subcellular changes. The most significant ultrastructural changes occurred in the proximal and distal convoluted tubules and also in collecting tubules of the kidney cortex after 5 days of treating rats with sodium selenite (1 and 2 mg/kg body weight). Most proximal tubules show a variety of ultrastructural changes. Some cells show swollen microvilli, whereas others have morphologically unchanged microvilli and many vesicles were seen in apical region of these cells.

Swollen mitochondria were condensed with diffusely darkened matrices and undistinguishable cristae. The rough endoplasmic reticulum was dilated in some areas of the cytoplasm and also dilated Golgi apparatus was noticed (Figs.7 & 8).

The range of structural change in distal convoluted tubules and collecting tubules cells were less affected than in proximal collecting tubules cells after lower doses of sodium selenite. Some distal convoluted tubular cells had cytoplasmic vacuoles and the mitochondria had abnormalities in appearance and were condensed. And the nuclear chromatin was condensed (Fig.9).

In the collecting tubules cells, the apical portion is slight swollen forming apical cytoplasmic buds, protrude into the tubular lumen. The mitochondria are condensed and the chromatin clumped in nucleus (Fig.10).
Fig. 9: Photoelectron micrograph of distal convoluted tubules after 5 days of treatment with sodium selenite (1 mg/kg body weight), showing condensed mitochondria (M), nucleus (N) and cytoplasmic vacuoles (V). X12000.

Fig. 10: Photoelectron micrograph of collecting tubular cells after 5 days of treatment with sodium selenite (1 mg/kg body weight), showing apical cytoplasmic buds (head arrow), protrude into the tubular lumen (L), mitochondria (M) and nucleus condensed with chromatin (N). X12000.

A higher dose (2 mg/kg body weight) of sodium selenite, caused more restricted in structural change. In renal tubular epithelium, the proximal convoluted tubular cells showed more damage than those of low doses. The apex of the proximal convoluted tubular cells are swollen and extend into the lumen and also observed vacuolization of the cytoplasm. The condensed mitochondria are irregular in shape and structure. The nucleus chromatin was also clumped at nuclear membrane (Fig. 11). Distal convoluted tubule cells showed more damage in the cytoplasm, the basal membrane is folded and the dense irregular elongated mitochondria are noticed. On the other hand, the nuclei of the lining cells of these tubules had obviously migrated to the apices of these cells displaying an irregular appearance (Fig. 12).

Collecting tubular cells had severe damage in some cells by forming apical buds or by shrinkage and decreased in length. The nuclei marked clumping of their chromatin, the basal cell membrane are

Fig. 11: Photoelectron micrograph of proximal convoluted tubules after 5 days of treatment with sodium selenite (2 mg/kg body weight), showing damaged mitochondria (M), nucleus with clumped chromatin (N), cytoplasmic vacuoles (V) and swollen apical microvilli (head arrow). X12000.

Fig. 12: Photoelectron micrograph of distal convoluted tubules after 5 days of treatment with sodium selenite (2 mg/kg body weight), showing basal folded membrane (arrow), irregular nuclei (N), dense mitochondria (M) and apical cytoplasmic buds (head arrow). X12000.

Fig. 13: Photoelectron micrograph of collecting tubular cells and a part of distal convoluted tubular cells after 5 days of treatment with sodium selenite (2 mg/kg body weight), showing the apical cytoplasmic buds (head arrow), nucleus (N), basal folded membrane (arrow) and damage mitochondria (M). X
Fig. 14: Photomicrograph of a semithin section of the kidney cortex cells after 10 days of treatment with sodium selenite (1 mg/kg body weight), showing moderate degeneration with loss of cellular detail architecture (head arrow) and pyknosis nuclei (N). X1200.

Fig. 15: Photomicrograph of a semithin section of the kidney cortex cells after 10 days of treatment with sodium selenite (2 mg/kg body weight), showing more severe degeneration on the tubular cells (head arrow) than the above treated one (1 mg/kg body weight). X1200.

folded in some areas and the dense mitochondria are damaged (Fig. 13).

After 10 Days: Tubular cells affected by mild to moderate degenerative lesions were scattered through the cortex with loss of cellular detail and hydropic degeneration after 10 days of treatment with lower and higher doses of sodium selenite. The pathological symptoms of these renal tubules were the loss of usual cellular architecture, deterioration of cell membranes, pyknosis of nuclei and necrosis of some cells in tubular Lumina (Figs.14 & 15).

Electron microscopic examination of the section from this subgroup revealed progressive degenerative changes in renal tubular epithelium after 10 days for lower and higher doses of treated groups.

The proximal convoluted tubules cells showed coagulative necrosis, such cells were exceeding by electron opaque and most of their organelles have been obviously demolished except for some heavily condensed lysosomes. The cytoplasm of these cells contained many degenerated mitochondria. Some of these mitochondria are either hypertrophied and had condensed matrices and also it was noticed in the cytoplasm electro-dense inclusion body. The nuclei of some affected cells, showed shrinkage in size contain many vacuoles and with chromatin clumped at the nuclear membrane (Figs 16 & 17).

The distal convoluted tubules were swollen and have electron lucent cytoplasm with few organelles as degenerated mitochondria. The lumina of these tubules were highly reduced (Fig.18).

The collecting tubules, showed marked degeneration, these cells have electron-dense cytoplasm with few organelles. The nuclei have clumped chromatin (Fig.19). After a higher dose (2mg/kg body weight) of treatment, the proximal convoluted tubules more affected than those in low doses. The microvilli were broken and swollen mitochondria are often disrupted. In severally damaged tubular cells, vacuolization of the cytoplasm is observed and the nuclei marked clumping of their chromatin (Figs. 20 & 21).

The distal convoluted tubular cells and collecting tubular cells showed more damage than those in low
Fig. 18: Photoelectron micrograph of distal convoluted tubules after 10 days of treatment with low doses of sodium selenite, showing elongated mitochondria (M), nucleus (N) and lumen (L). X12000.

Fig.19: Photoelectron micrograph of collecting tubular cells after 10 days of treatment with low doses of sodium selenite, showing the degradation of cellular organelles mitochondria (M) and nucleus (N). X18000.

doses of treatment. The distal convoluted tubular cells showed marked feature of degeneration, the apical region of the cytoplasm are vacuolated and the swollen dense irregular shape mitochondria are noticed and also the nucleus of some cells showed clear signs of pyknosis (Fig.22).

The lining cells of collecting tubules were marked damaged. Their nuclei, showed pyknosis i.e. they were small in size and darkly stained. And, also the apical surface appeared smooth and protruded into the tubular lumen and also the mitochondria are condensed and irregular in shape (Fig.23).

Fig. 20, 21: Photoelectron micrographs of proximal convoluted tubules after 10 days of treatment with high doses of sodium selenite (2 mg/kg body weight), showing cells degeneration, broken microvilli (arrow) and nucleus (N). X9000, X12000 respectively.

Discussion: Selenium is an essential micronutrient in all known forms of life. In humans, selenium is a trace element nutrient which functions as cofactor for reduction of antioxidant enzymes such as glutathione peroxidases and thioredoxin reductase. Although in large doses it caused toxicity. Glutathione peroxidase is an active form occurs in cytosol and in the matrix of mitochondria, whereas it is an inactive form in nuclei, liposomes and peroxysomes[6]. Its activity increases in the course of adding selenium to the diet.

Selenium both in excess and deficiency can exert a pathogenic effect. An excess of this element, especially in the form of inorganic compounds and some of its metabolites, lead to changes similar to those observed in the case of deficiency, i.e. to the degeneration of free radicals, intensification of lipid peroxidation and even to inhibition of synthesis of proteins[6].

Results of this study show that sodium selenite had adverse effect on kidney cortex histology and ultrastructure. Changes observed in the light microscope occurred after treatment for 5 and 10 days with low and high doses of sodium selenite.

During this study histological lesions were first seen at 5 days. The types, distribution and progression of light microscopic lesion observed in submicroscopic ultrastructure studies showed great changes occurred in proximal and distal convoluted tubular cells and collecting tubular cells, dilation of the endoplasmic reticulum, changes in the appearance of mitochondria as well as liposomes were observed. These are cellular
Fig. 22: Photoelectron micrograph of distal convoluted tubules after 10 days of treatment with high doses of sodium selenite, showing swollen mitochondria (M), degeneration of the apical region of the cell (arrow) and nucleus (N). X12000.

Fig. 23: Photoelectron micrograph of collecting tubular cells after 10 days of treatment with high doses of sodium selenite, showing damaged cells with condensed mitochondria (M) and apical dilatation of the cell cytoplasm (arrow). X12000.

organelles which due to their protein lipid membranes or the presence of them of glutathione peroxidase, may be a site for selenium activity.

Our results obtained with low and high doses of sodium selenite treatment agree with results that have been classically reported for sodium selenite\cite{9}, after mercuric chloride administration histopathological lesions in kidney and tubular necrosis were produced. And also when sodium selenite was administered pathological changes were noticed.

But when both compounds sodium selenite and mercuric chloride were administrated protective effect on histopathology of kidney was found. Whereas it seems that both histopathologic and ultrastructural changes were more intensified in the case of supplementation of higher doses of selenium, that intensification of the changes observed being greater in the application of sodium selenite with high doses after 10 days of treatment and our studies indicate a dose-dependant effect of sodium selenite on histopathologic and ultrastructural changes.

These results similar to that have been reported after administration of sodium selenite for 10 days\cite{18}. It has been reported that the effect of selenium deficient on diet on two experimental models of glomerular diseases showed significant increased reduction of glutathione peroxidase\cite{11}, thus indicating an important role of glutathione peroxidase in the models of glomerular injury.

Similar results have been studied on the histology of the rat kidney cortex after treatment with selenium deficient diet for 1 to 12 weeks and found that selenium deficiency induces protein urea and glucosuria with renal calcification, which may be primarily induced by injury of proximal tubule via oxidative stress\cite{10}.

Also, the inhalation of selenium derivatives such as dimethyl selenide has been associated with the tubular injury of the kidney as swelling and vacillation of the proximal tubules cells\cite{31}.

Similar histological examination of the renal tubular cells showed abnormalities in selenium deficient, showed focal areas of tubular dilation, atrophy and interstitial fibrosis\cite{13}.

Sloughing or internalization of the brush border was noticed in this study and also has been reported by other authors after treatment with different toxic substances\cite{13, 10, 11}.

In summary, the results of this study demonstrate that the oral administration of sodium selenite in rats is kidney toxic as indicate by histopathological and ultrastructural changes in the kidney cortex cells.

REFERENCES