Histol Histopathol (2003) 18: 703-708

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Ultrastructural evaluation of the effect of endosulfan on mice kidney

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Summary. In this study, the effect of the endosulfan on mice kidney was investigated at ultrastructural level. Moreover, biochemical analyses (G6PD, CAT, SOD, GSH and MDA) were determined in supernatant of kidney tissue. Endosulfan (13mg/kg/day body weight) was administered orally to mices via intragastric-during 10 days. The presence of mitochondrial degeneration in cytoplasm of proximal convoluted tubule cells were a striking feature. Furthermore, there was lipofuscin granules and membranous structures in some of proximal convoluted tubule cells. In some glomeruli, ultrastructural changes such as fusion in pedicels and focal thickening at glomerular basal membrane were seen. There were cytoplasmic bulges in some distal convoluted tubule cells. The biochemical results of the experimental group were significant when compared to the control. The effect of the endosulfan was mainly on the proximal convoluted tubule cells. Morever, the other parts of the nephron were effected. Thus, this degeneration in kidney may be thought that oxidative stress may play a role to the mediator in changing configuration of cell membrane and seem to account for the morphologic alteration of kidney.

Key words: Electron microscopy, Endosulfan, Kidney, Nephrotoxicity

Introduction

Endosulfan, a polycyclic chlorinated hydrocarbon of cyclodien group is an organochlorine insecticide. Endosulfan is widely used for agricultural insecticides. Hence, endosulfan is important for enviromental contamination. In addition, endosulfan is used in plants such as corn, vegetable, cotton and tobacco. Endosulfan may enter into human and animal systems directly or via environmental contamination. The primary effect of endosulfan is neurotoxicity after long term inhalation or oral exposure. Experimental studies have shown that endosulfan may cause inhibition of microsomal enzymes with regard to acute and subacute toxicity in liver, inhibition of enzymes of androgen biosynthesis in testis and inhibition of mixed function oxidases in kidney (Kellogg and Fridovich, 1975; Inbaraj and Harder, 1983; Narayan et al., 1985; Singh and Pandey, 1989, 1990). Furtermore, marked hyperplasia and hypertrophy of tubuler cells in kidney are seen as a result of the effect of chronic exposure some pesticides such as malathion. Besides, an increase in excretion of potassium was significant (Bosco et al., 1997). But, in the literature, the ultrastructural effects of endosulfan on kidney and correlation between the ultrastructural changes and antioxidant systems are uncommon. In this study, therefore, the effects of endosulfan on kidney and its possible results at ultrastructural and biochemical levels were studied in mice.

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Materials and methods

In this study, 12 male albino mice weighing between 26-29 grams were used. Mice were divided into two groups. 6 mice determined as control group and the other 6 mice were used as the experimental group. Throughout the experiment, all mice were fed with standard laboratory diet. The room temparature was normal. Mice comprising the experimental group received dissolved endosulfan in nut oil at doses of 13 mg/kg/day body weight. Endosulfan was administered orally to mices via intragastic-during 10 days. The control group received only nut oil. Mice were sacrificed with servical dislocation at the end of 10th day, and then kidneys were removed for examination. Renal tissue samples were immediately placed in 5% gluteraldehyde buffered at pH 7.4 with Milloning phospate for 4 hours and subsequently fixed in 1% osmium tetraoxide for two hours The samples were dehydrated in graded ethanol and embedded in araldite. Thin sections were stained with lead citrate and uranyl acetate, and examined with a Zeiss EM 10B electron microscope.

Biochemical analyses were determined as U/g tissue (G6PD, CAT and SOD), umol/g tissue (GSH) and

nmol/g tissue (MDA) in supernatant of kidney tissue. G6PD (glucose-6-phosphate dehydrogenase), CAT (catalase) and GSH (gulutatione) were determined by the Beutler method. SOD (superoxide dismutase) activity was determined according to the Fridovich method. MDA (malondialdehit) level was determined by the method of Ohkawa.

For statistical analysis the Mann-Whitney U test was used.

Results

Electron microscopic findings

Control Group

In micrographs, glomerular basal membrane and podocytes in renal corpuscles appeared intact (Fig. 1). Proximal and distal convoluted tubular cells showed a normal structure (Figs. 2, 3). Occasional, small apical vacuoles were observed beneath the brush border.

Experimental Group

There was prominent mitochondrial degeneration in proximal convoluted tubular cells. Furthermore, there were widespread free ribosomes in the cytoplasm (Fig. 4). There were prominent lipofuscin granules in the cytoplasm of some proximal convoluted tubular cells (Fig. 5). In addition, the presence of prominent membranous structures was observed in some proximal convoluted tubular cells (Fig. 6). There were histological changes in some glomeruli. Ultrastructural alteration was seen in the cytoplasm of some podocytes. In addition, there was fusion in pedicels in some glomeruli. A slight increase in the thickness of the glomerular basal membrane was focally seen. Urinary space of the Bowman's capsule was extremely narrowed (Fig. 7). There was an increase in the cytoplasmic density of some of the distal convoluted tubule cells. Extenstion in the length of some cells, and cytoplasmic bulges toward the lumen from the apical cytoplasm were observed (Fig. 8).

Biochemical analyses

The values of activities of G6PD, CAT, SOD enzymes, and the values of GSH, MDA are shown in (Table 1). G6PD was asseyed by the increase of absorbance at 340 nm of NADPH. CAT activity was determined by measuring the decrease in hydrogen peroxide concentration at 230 nm. GSH was determined in a metaphospforic acid filtrate at 412 nm. SOD activity was measured at 505 nm. MDA level was based on the intensity of the colour after treatment of the sample with thiobarbituric acid rectant. The amount of the lipid peroxidase was measured at 542 nm. Values of biochemical analysis at experimental group were statistical significant when compared to the control group ($p<0,05$). There was increment in values of activities of G6PD, SOD enzymes. However, there was prominent decrease in value of enzyme activity CAT. Furtermore, an increment in GSH and MDA was seen.

Discussion

In biological systems, the most common causes of oxidative stress are metabolites of foreign chemical substances. Some of the nonactive chemical substances are transformed to active toxic metabolites by specific organs (Rao and Murty, 1980). Metabolism of endosulfan is especially formed in liver by mixed function oxidases and then reactive metabolites occur (Hassall, 1993). These metabolites may be toxic for cells. In experimental studies, effects of endosulfan on respiratory enzymes, porfirin enzymes and small intestinal enzymes have been shown. Morphologically, hypertrophy and focal degenerative changes in proximal convoluted tubular cells in kidney have been reported (Hanck et al., 1995).

In our study, prominent degeneration was seen in proximal convoluted tubule cells. This degeneration may become evident of toxic phenomena in these cells, as a

Table 1. The values of biochemical studies of control and experimental group as statistical.

	Control (X±SD)	Experimental Group (X±SD)
G6PD (U/g tissue)	0.69±0.41	2.14±0.93
CAT (U/g tissue)	1415±464.44	614.16±217.94
SOD (U/g tissue)	459.66±221.21	1019.33±331.35
GSH (umol/g tissue)	0.41±0.29	0.89±0.53
MDA(nmol/g tissue)	7.33±1.75	17.16±6.01

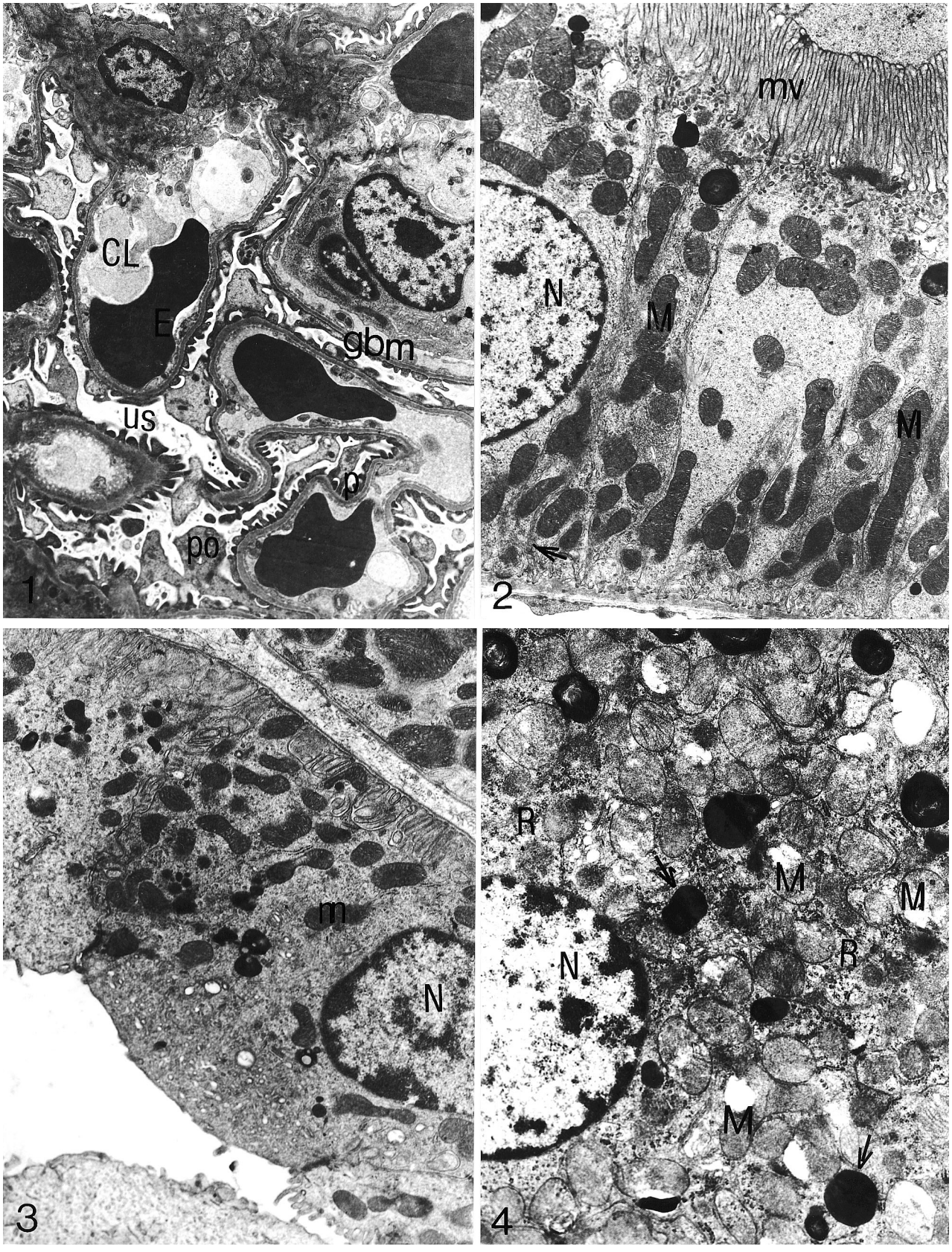
Values are expressed as mean (X)±standard deviation (SD) from six animals in each group. Increase in G6PD, SOD, GSH, MDA, and decrease in CAT compared with control group was significant ($P<0,05$).

Fig. 1. Normal glomerulus. Podocytes (po), pedicels (p), glomerular basal membrane (gbm), capillar lumen (CL), erythrocytes (E), urinary space (us). x 6,300

Fig. 2. Normal proximal convoluted tubule cell. Microvilli (mv), nucleus (N), mitochondria (M), basal infoldings of plasma membrane(arrow). x 8400

Fig. 3. Normal distal convoluted tubule cell. Nucleus (N), mitochondria (m). x 12,400

Fig. 4. Proximal convoluted tubule cell. Experimental group. Prominent mitochondrial degeneration (M), lysosomes (arrow), free ribosomes (R), nucleus (N). x 16,100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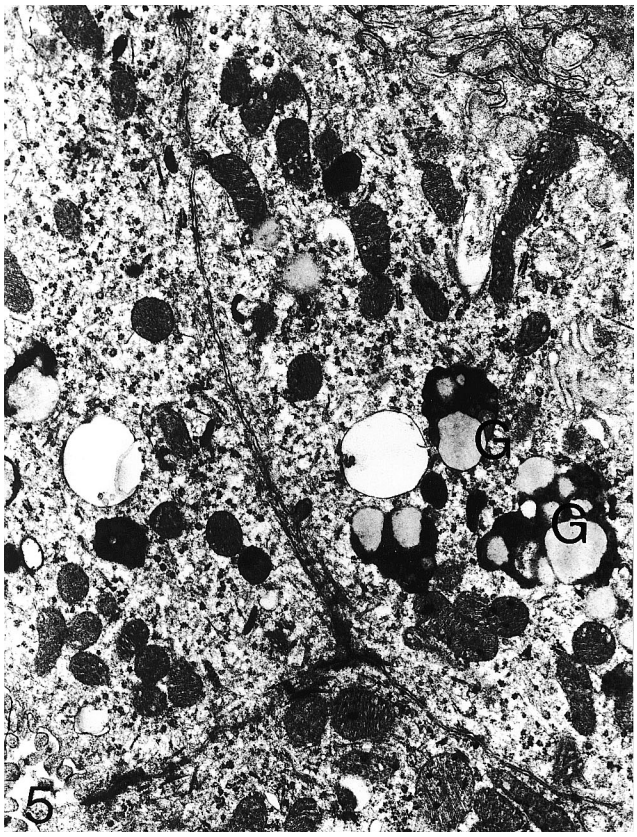
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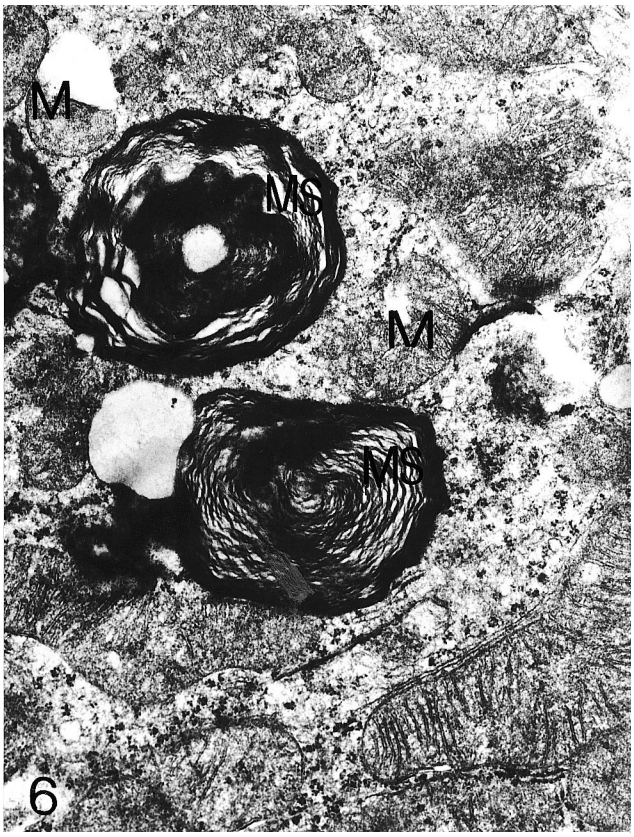
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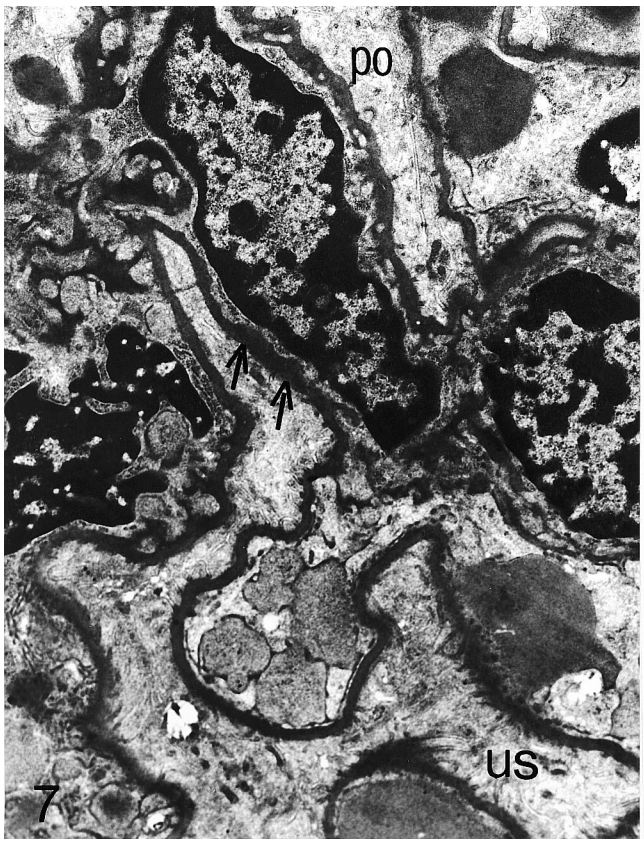
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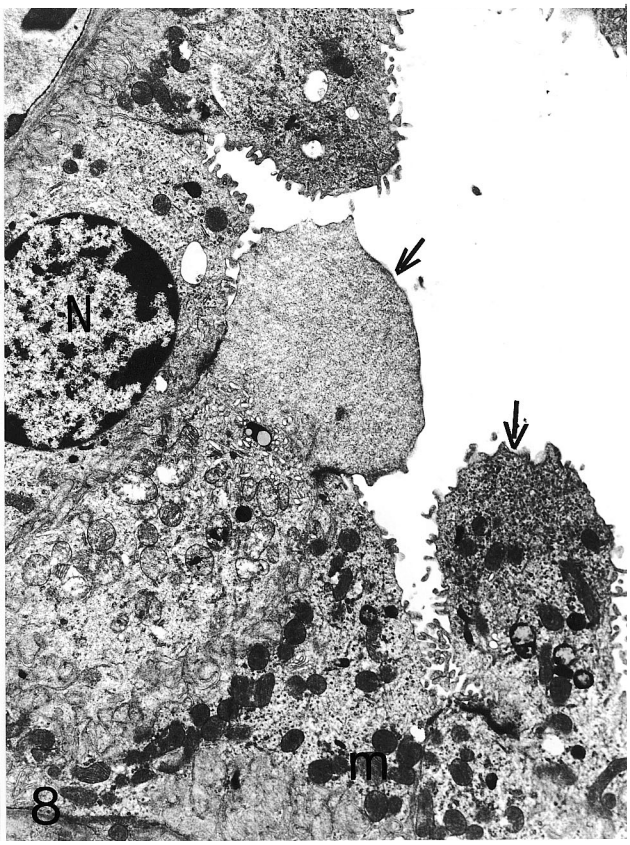
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Fig. 5. Proximal convoluted tubule cell. Experimental group. Prominent lipofuscin granules (G). x 12,400.

Fig. 6. Proximal convoluted tubule cell. Experimental group. Degenerated mitochondria (M), prominent membranous structure (MS). x 20,000

Fig. 7. Glomerulus. Experimental group. Focally thickened glomerular basal membrane (arrow), degenerated podocyte (po), narrowed urinary space (us). x 10,100

Fig. 8. Distal convoluted tubule cell. Experimental group. Cytoplasmic bulges (arrow), mitochondria (m), nucleus (N). x 6,300

result of administration of endosulfan. Our micrographs revealed that most mitochondrial membranes were ruptured. It may be thought that metabolites of endosulfan may effect configuration and active transport of the cell membrane. It may be said that lipofilic metabolites of endosulfan may impair the membrane of the mitochondria, and it may possibly play a role in mitochondrial dysfunction. Verma et al. (1978) reported that inhibition of Mg, Na, K and ATPase in a high concentration of aldrin and dieldrin in homogenate of kidney tissue was observed as a result of possible alteration of membrane configuration caused by pesticides. Kiran and Varma (1990) reported that erythrocyte membrane-associated Na-K ATPase and Mg ATPase activites in rat were significantly decreased as a result of oral administration of 12,5 mg/kg/day body weight of endosulfan for 4 days. Furtermore, membranes of endoplasmic reticulum and mitochondria, which are sensitive to free radicals, are rich with unsaturated fatty acid (McCord and Fridovich, 1978). It is belived that the effect on unsaturated fatty acids of oxygen free radicals can cause lipid peroxidative injury in membranes (Fridovich, 1978). In our micrographs, the presence of widespread free ribosomes in the cytoplasm of proximal convoluted tubular cells is seen. Therefore, it might be thought that widespread free ribosomes in the cytoplasm may be related with toxic effect of endosulfan. Furtermore, the presence of membranous structure and lipofuscin granules in the cytoplasm of some proximal convoluted tubul cells may reflect the probabl injury caused by oxidative effect. Poovala et al. (1999) suggested that organophosphate-induced oxidative stress may play a role in injuring of tubular cells in kidney. Singh and Pandey (1989) reported that the effect of this insecticide on kidney may be releated with lipid peroxidative damage of the microsomal membrane. In our study, it is shown that the glomerular structure was also affected by administration of endosulfan. The presence of prominent degeneration in the cytoplasm of podocytes and fusion of pedicels were striking. Fusion of pedicels might indicate the toxic effect of endosulfan. Degeneration in glomerules may be related with the interaction of the biological membrane with endosulfan during the passage through the filtration barrier. Bertani et al. (1982) reported that fusion of pedicels and proteinuri in kidney were a result of administration of adriamiycin in rat. They suggested that this degeneration may develop as a result of loss of electrical load in pedicels. Cytoplasmic bulges are seen in some distal convoluted tubular cells. This degeneration in distal convoluted tubular cells may suggest that the degeneration could be related with the alteration of cytoskeleton structure as a result of toxic effect in these cells. Walker et al. (1983) suggested that the cell surface changes in hepatocytes in administration of acetaminophen may develop as a result of dysfunction of the cytoskeleton. Gabai et al. (1990) reported that lipid peroxidation and the rates of free oxidation were increased during incubation of the cells with menadione. In addition, cellular ATP was decreased. They suggested that oxidative stress and a decrease in the level of cellular ATP may result in bleb formation and damage to the cytoskeleton.

Normally, reactive oxygen radicals are scarcely produced in the cells, and oxydative damage in cells is prevented by antioxidant systems (Cheeseman and Stater, 1993). According to our biochemical findings, the effect of endosulfan metabolites to membrane enzymes may cause to increase of superoxide radicals in the cell. Endosulfan is possibly showed negative effect decreasing CAT activity in the cell. Endosulfan may cause the occurrence of superoxide radicals in the cell, and these radicals are broken by SOD. Karnaukhova et al. (1990) reported that an increase in activity of SOD may reflect the toxic behavior of the pestıcide. Destruction of hydrogen peroxide is provided by CAT (Jones and Mosters, 1976). Thus, the cytotoxic effect of hydrogen peroxide is important in the cell. Therefore, in our finding, a decrease in activity of CAT and increase of MDA activity may reflect the presence of decreasing free radical in the cell. On the other hand , an increase in GSH and G6PD is possibly showed defence of the cell toward forming free radical in the cell. But, the inability to antioxidant defence system may contribute in occuring of the cell injury. Thus, reactive oxygen species may play an important role in this kidney injury.

Finally, the effect of the endosulfan was mainly on the proximal convoluted tubular cells. Morever, glomerular structure and distal convoluted tubular cells were also effected. Thus, observing changes in kidney may be thought that oxidative stress may play a role to the mediator in changing configuration of cell membrane and seem to account for the morphologic alteration of kidney.

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Accepted February 19, 2003