Effects of vitamin C on pathology and caspase-3 activity of kidneys with subacute endosulfan toxicity

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Abstract

Endosulfan is an insecticide that is composed of two stereoisomers: α- and β- endosulfan in an approximate ratio of 70:30. Owing to its widespread use, poisoning of both humans and animals is possible. We examined the toxic effects of endosulfan on New Zealand white rabbit kidneys. Rabbit kidneys were examined histopathologically and caspase-3 activity was detected using immunohistochemistry. Animals were divided into four groups: Group 1 was given a sublethal dose of endosulfan in corn oil by oral gavage daily for 6 weeks, Group 2 was given endosulfan + vitamin C during the same period, Group 3 was given corn oil daily and vitamin C on alternate days, Group 4 was given only corn oil daily throughout the experiment. By the end of experimental period, the concentration of α-endosulfan was greater than the β-endosulfan concentration in the kidneys of both of endosulfan treated groups (Groups 1 and 2). Decreased accumulation of α- and β-endosulfan was observed in Group 2, possibly because of the antioxidant effect of the vitamin C. Histopathological examination revealed hemorrhages, tubule cell necrosis, glomerular infiltration, glomerulosclerosis and proteinaceous material in the tubules, and Bowman spaces in the kidneys of Group 1. Caspase-3 reaction was stronger in Group 1 than in the other groups. Apoptotic activity was most frequent in proximal tubule cells. Endosulfan is toxic to rabbit kidneys. Vitamin C treatment reduced the accumulation of endosulfan in kidneys and reduced its toxicity.

Key words: endosulfan, immunohistochemistry, kidney, pathology, rabbits, toxicity

Endosulfan is an organochlorine cyclodiene insecticide and it is a persistent organic pollutant (Hayes and Laws 1991, Naqvi and Vaishnavi 1993). The chemical name of endosulfan is 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide. Technical endosulfan is a brown crystalline substance that consists of α and β isomers in a ratio of approximately 70:30. Endosulfan still is in use in many countries including Turkey. It has low volatility and a slow rate of biotransformation. It is highly lipid soluble and resistant to degradation (Gonzales-Farias et al. 2002, Oktay et al. 2002, Yavuz et al. 2007, Mor and Ozmen 2003). Because of its widespread use in agriculture, humans and animals commonly are exposed to endosulfan by different routes (Hack et al. 1995, Uboh et al. 2011). Because of its toxic effects, the World Health Organization (WHO) has classified endosulfan as a moderately hazardous Class II pesticide (WHO 2002). The half-life of endosulfan in water varies from 3–7 days to approximately 5 months, depending on the dissolved oxygen, turbidity, pH and other contaminants in the water. Endosulfan and its breakdown products remain in the environment with an estimated half-life of 9 months to 6 years (Hayes and Laws 1991, WHO 2002).
Vitamin C (ascorbic acid) is a natural antioxidant that can prevent tissue damage by scavenging reactive oxygen species (ROS) (Muruguesan et al. 2005). Vitamin C can reduce lipid oxidation caused by toxic substances (Altuntas et al. 2002, Serbecic and Beutelspacher 2002). The role of vitamin C in oxidative stress is related to inhibition of cell death (Muruguesan et al. 2005, Howard 1991).

Caspases are inactive proenzymes in cytosol that are activated when apoptosis is initiated; they play an essential role during various stages of apoptosis. The activation of these enzymes is controlled by their production as inactive zymogens that become catalytic following signaling events that promote their aggregation into dimers or macromolecular complexes (Llopis et al. 2003).

We have reported many degenerative effects of endosulfan in different organs (Mor and Ozmen 2010a,b, Ozmen et al. 2010, Ozmen and Mor 2012, Ozmen 2013). Others have examined the histopathology and ultrastructure of rat kidneys exposed to endosulfan and reported blood biochemistry related to kidney damage caused by endosulfan (Uboh et al. 2011). The literature contains no report, however, concerning the mechanisms of toxicity and apoptosis in kidneys that have been exposed to endosulfan. Therefore, we investigated the pathological effects of subacute endosulfan toxicity and the prophylactic effects of vitamin C.

The caspases are crucial mediators of apoptosis and especially because caspase-3 is a frequently activated apoptosis protease that catalyzes the specific cleavage of many key cellular proteins. Antioxidant effects of vitamin C have been demonstrated earlier (Altuntas et al. 2002, Serbecic and Beutelspacher 2002). We studied especially caspase-3 expression in the kidneys of New Zealand white rabbits that suffered endosulfan toxicity.

**Material and methods**

Our study was approved by the Institutional Animal Use and Care Committee of Akdeniz University and was performed in accordance with the National Institutes of Health Guidelines for the Care and Handling of Animals. We used 24 male 6–8-month-old New Zealand white rabbits. Rabbits were fed standard rabbit chow and tap water ad libitum and they were housed in cages at controlled temperature (22°C) and a 12 h light/dark cycle throughout the study. The physical condition of each rabbit was assessed daily for obvious signs of illness.

The rabbits were allocated randomly to four groups of six. Rabbits in Group 1 (endosulfan) were exposed to sublethal concentrations of endosulfan (1 mg/kg/day) in corn oil for 6 weeks. Group 2 (endosulfan + vitamin C) was given 1 mg/kg/day endosulfan and 20 mg/kg vitamin C every other day. Group 3 (oil and vitamin C) was given corn oil daily by oral gavage + 20 mg/kg vitamin C every other day for 6 weeks. Group 4 (control group) was given only corn oil by oral gavage.

Rabbits were sacrificed 1 week after the last treatment. Intracardiac blood samples were obtained prior to euthanasia, then a necropsy was performed on all animals. Kidney samples were fixed in 10% buffered formalin, processed routinely to paraffin, sectioned, then stained with hematoxylin and eosin (H & E) for light microscopy. Histopathological changes were graded in a blinded manner. Hyperemia, edema, inflammatory reaction and degeneration were evaluated according to the severity of lesions using a 0–3 scoring system where 0, normal; 1, slight hyperemia, degeneration of tubules; 2, degeneration of numerous tubules, slight inflammatory reactions; 3, severe degeneration, vacuolization and severe inflammatory reactions.

For evaluation of apoptosis, the slides were immunostained for caspase-3 reaction using immunohistochemical methods (Mor and Ozmen 2010a). Selected tissue sections were stained to demonstrate caspase reaction by immunohistochemistry (Neomarker, Fremont, CA) (caspase 3 (CPP32) Ab-4, 1:100 dilution) using a routine streptavidin-biotin peroxidase technique according to manufacturer’s instructions.

Endosulfan residues in the kidney samples were detected using the methods of Luke et al. (1975) and Shyre et al. (1998). All of the solvents used were pesticide-free grade. The tissues were homogenized in 100 ml acetone using an Edmund Bühlner 7400 shaker (Tübingen, Germany). Endosulfan and its metabolites in the kidney samples were extracted using florisil solid-phase extraction and analyzed by gas chromatography with electron capture detection (GC/ECD). The samples were dissolved in 40 ml 1:1 diethyl-ether:petroleum ether. Extraction was carried out using a 30 x 1 cm glass column containing 10 g florisil and 2 g sodium sulfate. Florisil was activated at 200°C for 12 h, then deactivated by adding water until the water content of the florisil was approximately 2%. Anhydrous sodium sulfate was placed on the top 2 cm of the column to remove moisture. The pesticides were eluted from the column with acetone. The eluate was evaporated under a flow of nitrogen gas at 30°C, and the residue was resuspended in 2 ml hexane for gas chromatography. Glassware used in the experiment was cleaned.
Results

All animals survived the experiment. Clinical signs including loss of appetite and lethargy were observed in Groups 1 and 2; no clinical symptoms were evident in the other groups. The most prominent gross findings at necropsy were pale and swollen kidneys in Group 1. Macroscopic examination of kidneys of the Group 2 showed slight swelling, but less than was seen in Group 1. The kidneys of Groups 3 and 4 appeared normal.

Normal kidney histology was observed in Groups 3 and 4 (Fig.1A). Small hemorrhages and single cell necrosis were detected in some kidneys from Group 2, but no inflammatory reaction was observed in this group (Fig.1B). Histopathological examination of the kidneys of rabbits in Group 1 showed hemorrhages, tubule cell necrosis, glomerular infiltration, glomerulosclerosis, and proteinaceous material in the tubules and Bowman spaces (Fig. 1C–D). Glomerular infiltration evaluated that related endosulfan toxicity, because there were no inflammatory reaction in the other groups except Group 1. Similarly, tubule cell necrosis and inflammatory reaction were observed only in Group 1.

Numerous strong caspase-3 positive tubule epithelial cells were observed in Group 1 (Fig. 1E); the strongest positive reaction was seen in the proximal tubule cells. Small numbers of caspase-3 positive tubule cells were observed in Group 2 (Fig.1F). Caspase-3 positive reactions in a few scattered cells also were seen in Groups 3 and 4.

The concentration of endosulfan in the kidneys of Groups 1 and 2 are shown in Table 1. We observed a marked decrease in kidney α- and β-endosulfan as indicated by GC-ECD concentrations as a result of vitamin C treatment. The difference in the endosulfan concentrations between Groups 1 and 2 were statistically significant ($p < 0.001$).

Discussion

Exposure to nephrotoxic substances may cause impairment of renal functions (Uboh et al. 2011). We studied the clinical, pathological, immunohistochemical and toxicological characteristics of rabbit kidneys in which endosulfan toxicity was induced experimentally. We also examined the effects of vitamin C on endosulfan toxicity because of the antioxidant property of ascorbic acid. Undiluted endosulfan is absorbed slowly and incompletely in the digestive tract of warm blooded animals, but absorption is enhanced in the pres-
in humans and animals (Yavuz et al. 2007, Mor and Ozmen 2003). We showed that endosulfan is toxic to rabbits and that even subacute toxicity can cause kidney lesions. We observed the most severe kidney lesions in Group 1 and we observed amelioration.

Pesticides are important chemicals economically. Endosulfan is widely used and can cause poisoning of alcohols, oils and emulsifiers (Maier-Bode 1968). Therefore, we administered endosulfan emulsified in corn oil.

Fig. 1. A) Normal appearance of rabbit kidney in Group 3. H & E staining. Bar = 100 μm. B) Histopathology of a kidney from Group 2 shows slight degeneration of the tubule cells (arrows). H & E staining. Bar = 100 μm. C) Severe vacuolization (arrows) and degeneration in tubule cells in a kidney of Group 1. H & E staining. Bar = 100 μm. D) Inflammatory reaction at the interstitial tissue (thick arrows), cystic tubules (thin arrows) and proteinaceous material reaction in the tubule lumen (arrowhead) in a kidney of Group 1. H & E staining. Bar = 200 μm. E) Strong caspase-3 immunoreaction, which indicates apoptosis in tubule cells in a kidney of Group 1. Avidin-biotin-peroxidase (ABP) method, with DAB and Harris’ hematoxylin counterstain, Bar = 100 μm. F) Slight caspase-3 immunoreaction in tubule cells in a kidney of Group 2. ABP method with DAB and Harris’ hematoxylin counterstain, Bar = 100 μm.
of the toxic effects in the vitamin C treated group (Group 2). We attribute the glomerular infiltration and glomerulosclerosis to the toxic effects of endosulfan on kidney cells.

Vitamin C is an essential nutrient (Carr and Frei 1999). It prevents genetic damage caused by toxic substances. Vitamin C is an antimutagen that acts mainly by interfering with free radical generation and formation of toxic metabolites (Abdelai et al. 1995). We found that vitamin C not only protected kidney tissue from damage, but also decreased the accumulation of endosulfan in the kidneys. Although severe histopathological lesions and inflammation were observed in the endosulfan treated kidneys (Groups 1 and 2), there were only slight lesions and no inflammatory reaction in the vitamin C treated group (Group 2). Hemorrhages and necroses also were less severe in Group 2 than in Group 1. The severe lesions in the kidneys of Group 1 appear to reflect marked accumulation of endosulfan in this organ.

Apoptosis is a highly regulated and conserved process of cell suicide (Green and Kroemer 1998). Caspases play a central role in the initiation and execution of apoptosis (Gross et al. 1999). Insecticides also can induce apoptosis. When cells are exposed to oxidative stress, they often die by apoptosis or necrosis (Ishihara et al. 2005). Strong and abundant caspase positive reaction was more prominent in the endosulfan only group (Group 1) than in the other groups. Only a few caspase-3 positive cells were seen in the endosulfan + vitamin C group (Group 2). We found that endosulfan exposure caused severe kidney damage and that vitamin C had an ameliorating effect on endosulfan toxicity in rabbit kidneys.

We found decreased α- and β-endosulfan levels in rabbits exposed to endosulfan and treated with vitamin C (Group 2) compared to Group 1. Our results indicated a differential ability to accumulate the two isomers of endosulfan, which may explain in part the difference in the toxic potential of the α and β isomers.

We demonstrated that subacute endosulfan toxicity can cause hemorrhages, tubule cell necrosis, glomerular infiltration, glomerulosclerosis, and accumulation of proteinaceous materials in tubules and Bowman’s spaces. Endosulfan exposure caused increased apoptosis in kidney cells. We also demonstrated the preventive effects of vitamin C on accumulation of endosulfan in kidneys. We suggest that endosulfan is nephrotoxic and that vitamin C has an protective effect on subacute endosulfan toxicity in kidneys.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

Table 1. Statistical analysis of α- and β-endosulfan mean residue levels in rabbit kidney samples (μg/g wet weight)

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<th>Endosulfan</th>
<th>Endosulfan + vitamin C</th>
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<tr>
<td>α</td>
<td>0.18 ± 0.011</td>
<td>0.03 ± 0.004</td>
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<tr>
<td>β</td>
<td>0.12 ± 0.006</td>
<td>0.01 ± 0.001</td>
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References


