

Ultrastructural Changes in Rat Liver Treated with Pralidoxime Following Acute Organophosphate Poisoning

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Abstract

We investigated the ultrastructural effects of methamidophos and the positive effects of 2-pralidoxime (2-PAM) on the liver. Male Wistar-albino rats were assigned to 4 groups and all were treated per os: Group 1 (n=10) received 30 mg/kg methamidophos; Group 2 (n=7) (serving as controls for Group 1) received physiologic NaCl; Group 3 (n=10) received 30 mg/kg methamidophos and was treated with 2-PAM and atropine when cholinergic symptoms were noted; and Group 4 (n=7) (serving as controls for Group 3) was treated with physiologic NaCl. Plasma cholinesterase was measured using radioimmunoassay. Liver tissues were prepared for electron microscopic studies. Methamidophos treatment of Group 1 led to serious changes in hepatocytes and organelles. These changes were not detected in Group 3. In Group 1, the chromatin content of some hepatocyte nuclei and cytoplasmic density increased; these cells also became vacuolar in appearance as a result of lysis in the mitochondrial matrices. In some cells, the lipid content constituted the majority of the cytoplasm. Furthermore, these cells were surrounded by glycogen accumulation. In some areas of the perisinusoidal zone, collagen fibers had increased to form bands. None of these changes were noted in Group 3. These findings suggest that acute organophosphate poisoning causes serious histopathological effects in rat liver, but that these changes are reversible with appropriate treatment strategies.

Key Words: Liver, organophosphate, pralidoxime, poisoning, treatment, ultrastructure, hepatotoxicity.

ORGANOPHOSPHATE INSECTICIDES are widely used in both agricultural and landscape pest control, and the potential for human exposure to this class of compounds is significant. The primary toxicity associated with acute exposure to organophosphate insecticides is cholinergic crisis resulting from acetylcholinesterase inhibition (1). However, these compounds also have numerous other specific effects, including delayed polyneuropathy, immunotoxicity and carcinogenesis, as well as endocrine, developmental and reproductive toxicity (2, 3). In the past, research efforts generally focused on neurobiological effects of these chemicals. A review of the literature indicates that peripheral organs such as the liver have been much less inten-

sively studied. Yet it is well documented that numerous drugs and hepatotoxins elicit marked alterations in hepatocyte functioning (4).

The present study was undertaken to investigate whether administration of a single lethal dosage of methamidophos, a highly toxic organophosphate, induces any ultrastructural changes in murine hepatocytes, and to highlight the characteristics of the changes observed after treatment.

Materials And Methods

Thirty-four male Wistar albino rats, obtained from Çukurova University Medical Sciences Experimental Research Center, were used for the study. The rats weighed between 220 and 230 grams. They were housed five to a cage, with a room temperature of $22 \pm 2^\circ\text{C}$ and a light:dark cycle of 12 hours on and 12 hours off. Food and water were available ad libitum. Methamidophos [0,5-dimethyl phosphoramidothioate, median lethal dose (LD_{50}) = 30 mg/kg] with a purity of 99.1% was diluted in tap water to 30 mg/kg/mL and administered via gavage using a 20-gauge feeding needle. At-

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ropine sulfate and pralidoxime (2-PAM) were dissolved in saline and injected intraperitoneally (IP) with a 23-gauge needle. The animals were anesthetized with 75 mg/kg ketamine and 5 mg/kg xylazine intramuscularly (IM). The rats were sacrificed by intracardiac blood collecting. Each rat was treated and examined separately and independently. Blood samples were centrifuged in 1000 cycles for 10 minutes, and their plasmas were kept at -70°C . Then the enzyme, pseudocholinesterase (PCE) underwent the same treatment. Assay was made by radioimmunoassay. The liver tissues were fixed in 5% glutaraldehyde in Millonig phosphate buffer at pH 7.4 for four hours and postfixed in 1% osmium tetroxide in phosphate buffer at pH 7.4 for 2 hours at 4°C . Tissues were dehydrated in graded ethanol and embedded in araldite. Thin sections were cut using Reichert Ultracut S ultramicrotome, stained with uranyl acetate and lead citrate, and then examined with a Zeiss EM 10B electron microscope.

The rats were divided into four groups. The rats in Group 1 ($n=10$) were treated with 30 mg/kg LD_{50} of methamidophos, whereas Group 2 ($n=7$) was treated with physiologic NaCl equal to the proportion administered to Group 1 via gavage. The animals in Group 1 received no treatment for organophosphate poisoning. Group 2 was designed to serve as the controls for Group 1. Cholinergic symptoms were apparent in the rats in Group 1 in 5 minutes. Eight minutes after ingestion of methamidophos, all of the signs of cholinergic crisis were seen, and the rats were sacrificed. Group 3 ($n=10$) was given 30 mg/kg LD_{50} of methamidophos via gavage. Time interval until appearance of cholinergic symptoms was the same as in Group 1, i.e., 5 minutes after administering methamidophos. For this group, however, treatment was provided when all of the signs of cholinergic crisis were recorded (after 8 minutes). As the rats in Group 3 developed the symptoms of cholinergic crisis, they were treated with 40 mg/kg 2-PAM IP and atropine IP. Rats in Group 3 received 2-PAM in one dose, but atropine was administered until the cholinergic signs were reversed. The rats in Group 4 ($n=7$) were treated with physiologic NaCl 0.9% equal to the volume of methamidophos used in Group 3 by means of gavage. In addition, they were given NaCl 0.9% IP in the same number of injections and amount of volume as given in Group 3. The rats in Group 3 received treatment after cholinergic crisis occurred. Group 4 was designed as the control group of Group 3. This design allowed applica-

tion of the same level of treatment stress in Group 4 as in Group 3. After cholinergic signs were identified in Group 1, intracardiac blood samples were taken from the rats and put into tubes with ethylenediaminetetraacetic acid. Intracardiac blood was drawn from Group 2 after waiting the same amount of time as with Group 1. In Group 3, blood samples were taken after cholinergic signs had disappeared; at the end of the same period, intracardiac blood samples from Group 4 were obtained under anesthesia. The rats were followed for four days in Groups 3 and 4, in order to preclude the intermediate syndrome that is associated with certain compounds 24–96 hours after acute, severe cholinergic crisis. There was no cholinergic crisis observed within the study period.

Statistical analyses were performed using the statistical package SPSS v 10.0. Mann-Whitney U test was used for comparison between groups. Bonferroni's correction was done when multiple comparisons were performed. P values below 0.05 were considered significant. Mean values were presented as \pm SD (standard deviation).

Results

The mean level of PCE was significantly lower in Group 1, (32.6 ± 17.0), median: 37.0, range: 45, than in Group 2, (579.4 ± 59.0), median: 595.0, range: 170 ($p=0.000$). It was significantly higher in Group 3, (392.5 ± 39.4), median: 387.5, range: 130, than in Group 1 ($p=0.000$). The mean level of PCE was 616.3 ± 54.3 (median: 601.0, range: 141) in Group 4, and was significantly higher than in Group 3 ($p=0.000$) (Fig. 1). The ultrastructural

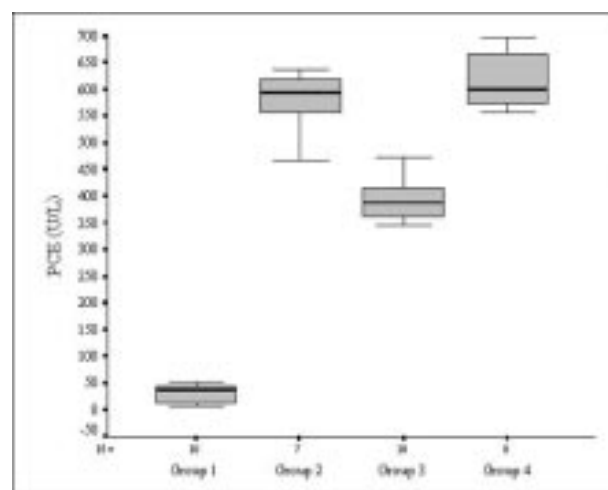


Fig. 1. PCE levels between groups.

changes observed in experiment groups are listed below:

Group 1: Some nuclei of the hepatocytes were irregularly shaped and their chromatin content had increased. Vacuoles were identified in the mitochondria due to lysis of their matrices. In some of the hepatocytes, variously sized lipid droplets were observed throughout the cytoplasm, and glycogen accumulation was also observed in these areas. Increments of collagen fibers, forming bundles, were seen in perisinusoidal spaces (Figs. 2 and 3).

Group 2: The nuclei of the hepatocytes had normal chromatin dispersion and cytoplasmic organelles were normal in structure (Fig. 4).

Group 3: Nuclei of the hepatocytes were regularly outlined and their chromatin content were normal, although cytoplasmic lytic areas were observed in some cells. Overall, cytoplasmic organelles, bile canaliculi and perisinusoidal areas appeared to be normal (Figs. 5 and 6).

Group 4: The cytoplasm and nuclei of the hepatocytes had normal structures. The mitochondria, endoplasmic reticulum cisternae and Golgi apparatus were all within normal limits. Glycogen particles and rare lipid droplets that were the normal structural components of the liver, were also observed (Fig. 7).

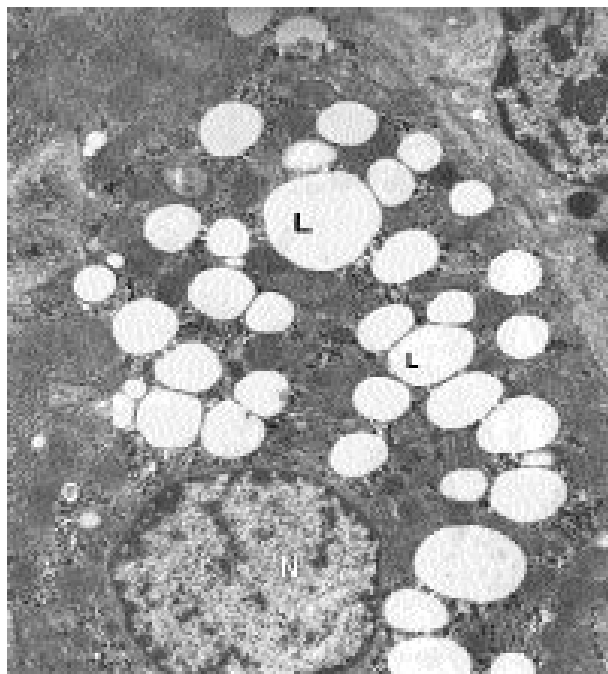


Fig. 2. Increased lipid droplets (L) and glycogen particles (G) are observed throughout the cytoplasm of the hepatocytes of the rat in Group 1. Nucleus (N). ($\times 8837$)

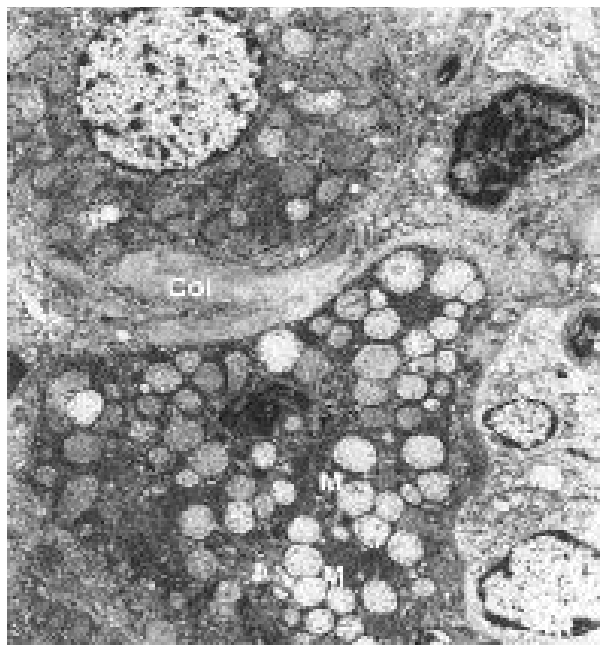


Fig. 3. Lytic mitochondrial matrix (M) appeared in the cytoplasm of hepatocytes of the rats in Group 1. Collagen (Col). ($\times 5512$)

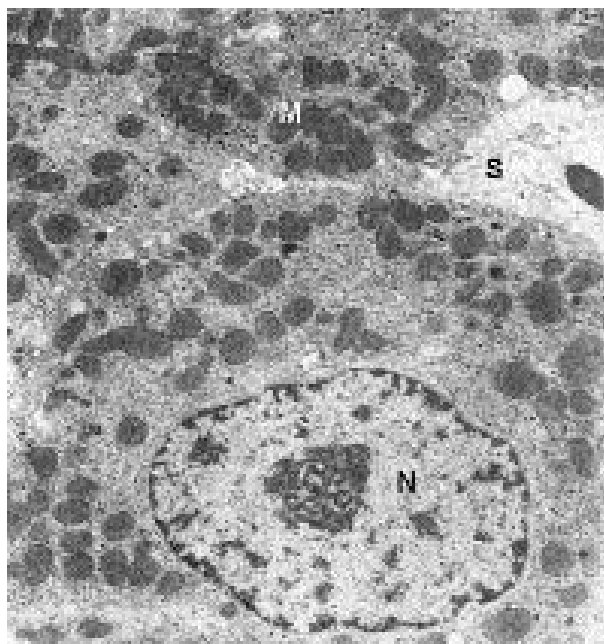


Fig. 4. Nucleus (N) and cytoplasmic organelles are normally seen in the hepatocytes of the rats in Group 2. Mitochondria (M), sinusoidal space (S). ($\times 8837$)

Discussion

The liver is involved in about 8% of reported adverse drug reactions. This is a reflection of its central role in the metabolism and excretion of many drugs. A number of drugs may

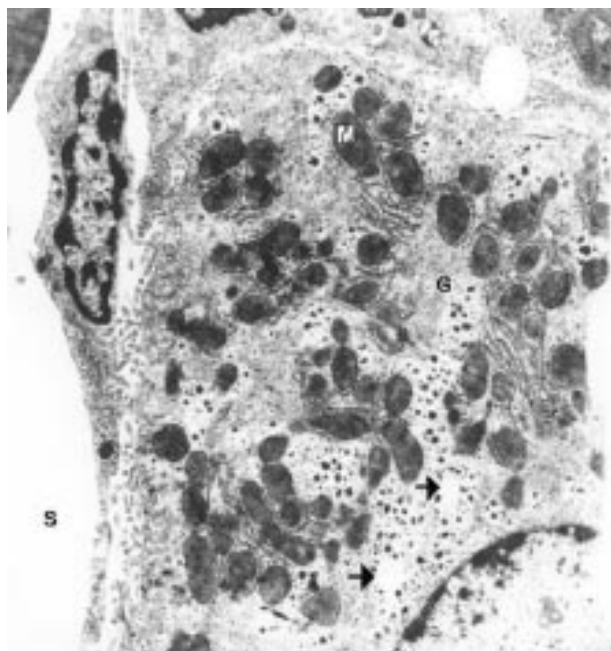


Fig. 5. Mitochondria (M), in variously shaped and small lytic areas (arrows) are seen in the hepatocytes of the rats in Group 3. Glycogen (G), sinusoidal space (S). ($\times 10850$)

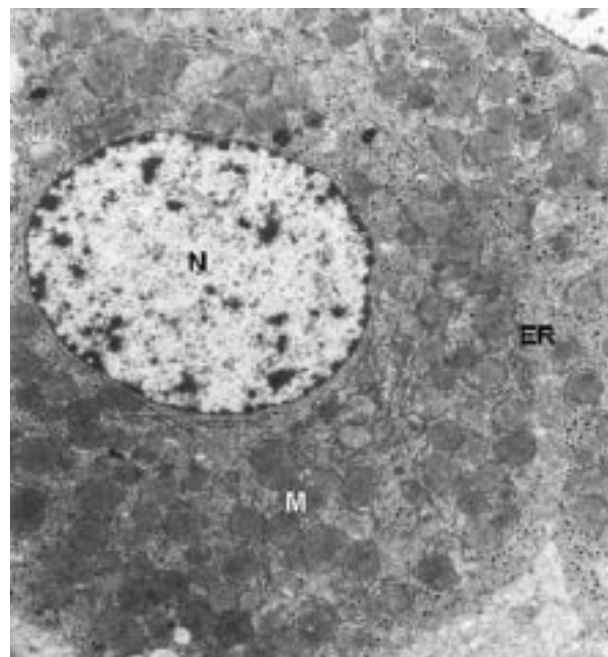


Fig. 7. Mitochondria (M), endoplasmic reticulum cisternae (ER), and nucleus (N) are seen with normal structures in the hepatocytes of the rats in Group 4. ($\times 8837$)

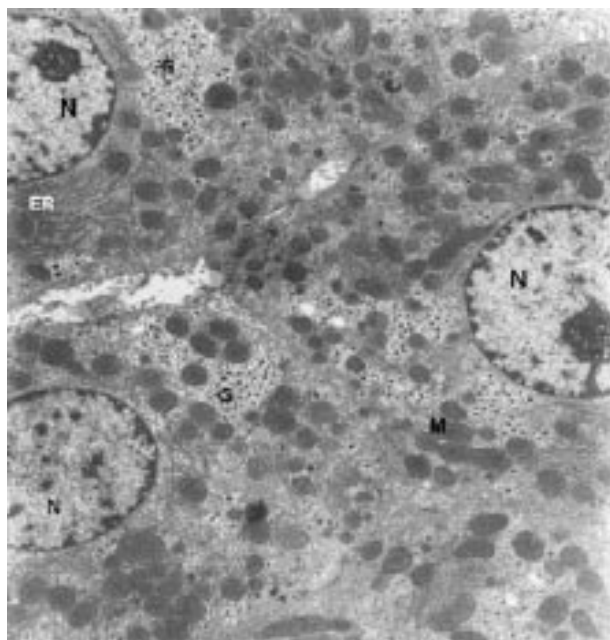


Fig. 6. Nucleus (N), mitochondria (M), endoplasmic reticulum cisternae (ER) and glycogen particles (G) are seen in the hepatocytes of the rats in Group 3. ($\times 7087$)

cause induction of the enzymes involved in the metabolism of these drugs (5).

It is well documented that anticholinesterase agents, such as organophosphates, affect lipid metabolism (6–8). Ryhanen et al. showed that

low-density lipoprotein and cholesterol contents decreased, whereas high-density lipoprotein concentration increased and very-low-density lipoprotein remained unaffected, in dichlorvos-treated rats. The same study also showed that triglycerides, as well as esterified fatty acids, increased significantly (8). PCE is found mainly in the plasma, and to a lesser extent in the adipose tissue, small intestine, smooth muscle cells and other cells. Clitherow et al. have suggested that PCE may be involved in hepatic and fatty acid metabolism (9). PCE is found to be increased in a significant number of patients with hyperlipoproteinemia (10). It has been suggested that elevation of PCE activity could be attributed to a mechanism leading to an increased secretion rate of lipoproteins (11). Concurrent with the inhibition of PCE activity, levels of serum triglyceride, low-density lipoprotein and glycerol decreased significantly (7). Organophosphates may phosphorylate hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase, which is the major regulatory enzyme in cholesterol biosynthesis. It was found that phosphorylation results in inhibition of this enzyme (12). In our study, following the inhibition of PCE in Group 1, lipid accumulation was remarkable. This finding in Group 1 may be linked directly to the toxic effects of organophosphate and/or distribution of lipid

metabolism by the phosphorylation of enzymes in lipid metabolism after poisoning. However, in Group 3, the animals received antidotes after poisoning, and this finding was not observed. This may be because of the temporal relationship between the administering of antidotes and the regeneration of PCE.

It is known that organophosphate insecticides disturb carbohydrate metabolism (13). In organophosphate poisoning, nonketotic hyperglycemia and glycosuria have been reported (14). Signs of organophosphate poisoning may be understood by ascribing them to muscarinic, nicotinic and central nervous system effects. Overstimulation of the nicotinic receptors in the sympathetic ganglia may overwhelm parasympathetic stimulation and produce tachycardia, hypertension, and stimulation of the adrenal medulla (15, 16). The increase in serum glucose is believed to be secondary to the release of catecholamines from adrenal medulla, which is activated by hyperactivity of sympathetic ganglia (17). The accumulation of glycogen in our observations may be attributed to inhibition of glucagon secretion by hyperglycemia and also to the stimulation of liver gluconeogenesis by increased epinephrine secretion. Our findings might also be the result of disruption of carbohydrate metabolism by organophosphates, which was resolved by treatment.

Currently, the precise nature of anticholinesterase-toxicant-induced alterations in liver metabolism has not been clearly ascertained for related organophosphorus toxicants. According to Sitkiewicz, several organophosphates impair respiration and oxidative phosphorylation in liver mitochondrial preparations (18). Rao et al. showed that the total adenosine triphosphatase (ATPase) activity decreased in all tissues, suggesting inhibition of active transport and oxidative phosphorylation under methyl parathion impact (19). Organophosphate intoxication in animals results in mitochondrial swelling, increased proliferation of rough endoplasmic reticulum (RER) and disintegration of smooth endoplasmic reticulum, increased glycogen content and narrowing of biliary pathways. Mitochondrial changes in organophosphate-intoxicated animals are indicative of the increased energy requirements necessary for the cells to overcome the toxic effects of the organophosphorus compound (20). It has also been documented that the increased number of mitochondria in any pathological condition reflects the process of uncoupling of oxidative phosphorylation (21). In con-

trast, another author has reported that organophosphate intoxication eventuates in actual elevation in hepatocyte metabolic functioning (22). A recent publication showed that organophosphate compounds have cytotoxic effects (23). Mitochondria are primary targets in organophosphate-induced cytotoxicity (24). Our study showed that methamidophos causes damage to mitochondria and nuclei to a lesser extent. The ultrastructural alterations in mitochondria hint at a decrease in mitochondrial energy production, which is probably an important cause of the nuclear changes observed in methamidophos-treated rat liver cells. It is postulated that nuclear changes represent an early proliferative response, whereas cytoplasmic changes reflect the disruption of normal function and hepatotoxic injury (6).

With most forms of injury, in addition to loss of specialized cells, the surrounding matrix of connective tissue is damaged. The fibroblasts synthesize and secrete fibronectin and proteoglycans, and these form the scaffolding for rebuilding the matrix (25). In our study, the collagen fiber increment in Group 1 may be due to injury of hepatocytes because of acute intoxication by organophosphate. After treatment (in Group 3), these ultrastructural changes disappeared, since the induction due to injury stopped with the administration of antidotes. The collagen fiber increment in Group 1 might also have occurred because of lipid accumulation. The fat-storing cells exhibited intense positive stainings for type I and type III collagens and prolyl hydroxylase (26). It has been showed that prolyl hydroxylase plays an important role in collagen fiber development (27). Hepatocyte injury resulting from acute exposure to organophosphate compounds, alterations in prolyl hydroxylase enzyme activity following lipid accumulation, and its return to normal limits after treatment, may be causes of collagen fiber increment in Group 1 and its absence in Group 3.

A drawback of the present study was the time interval between the occurrence and the disappearance of the ultrastructural changes in Group 3, since the disappearance of the ultrastructural changes might be related to time factors. Indeed, it is obvious that the metabolism rate in rats is very fast (relative to humans) and the healing of these changes might be due to this factor. But it must also be noted that the effect of time factor in this study has been alleviated by designing another group.

In regard to ultrastructural changes, hepatocytes exposed to highly toxic organophosphate

were seriously affected, but after an appropriately tailored treatment these changes disappeared. Deaths following this kind of poisoning may be linked to the morphological alterations in the liver and the detoxification factory of the body. Further studies are needed to clarify these causal relationships.

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