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LABORATORY STUDY

Ultrastructural Effects of Acute Organophosphate Poisoning on Rat Kidney

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We investigated the ultrastructural effects of the organophosphate compound methamidophos and treatment with atropine and pralidoxime (2-PAM) on rat kidneys. Male Wistar albino rats were assigned to four groups. Group 1 received 30 mg/kg methamidophos, the LD_{50} for this compound in rats, via oral gavage. Group 2 received only physiologic saline. Group 3 rats received 30 mg/kg methamidophos and were treated with 2-PAM and atropine via intraperitoneal injection when cholinergic symptoms were noted. Group 4 served as a control, and received physiologic saline in equivalent volumes and routes to Group 3. Kidney tissues were prepared for electron microscopic studies. No ultrastructural changes were detected in Group 1 after acute poisoning with methamidophos and in Group 3 treated with antidotes after poisoning. Acute organophosphate poisoning and antidotal treatment in this model are not associated with histopathological changes in the rat kidney but the models with different organophosphate compounds, by administrating the different dosages, may be more illuminative in explaining the effects of these chemicals in kidney.

Keywords atropine, kidney, organophosphate, poisoning, pralidoxime, treatment, ultrastructure

INTRODUCTION

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] Additional effects of organophosphates include delayed polyneuropathy, immunotoxicity, carcinogenesis, and endocrine developmental and reproductive toxicities.[2,3]

Severe organophosphate poisoning has a high mortality rate. Renal impairment and multiple organ failure are relatively rare, but correlated with death. Although anecdotal reports have recently pointed out an association of human acute renal failure and organophosphate intoxication, the pathogenesis of renal injury by

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organophosphate poisoning is unclear.[4–6] The mechanisms, which allegedly induced the entity in these reports, also vary and are inconsistent.

The present study investigates whether administration of a single LD_{50} dose of methamidophos, a highly toxic organophosphate, induces ultrastructural changes in rat kidneys and whether antidotal therapy with atropine and pralidoxime (2-PAM) mitigates or alters these changes.

MATERIALS AND METHODS

Thirty-four male Wistar albino rats were obtained from Cukurova University Medical Sciences Experimental Research Center. The rats weighed between 220 and 230 grams. Animals were housed five to a cage, with a room temperature of 22°C±2°C and a light-dark cycle of 12 hours on and 12 hours off. Food and water were available ad libitum.

In preparation for the study, the animals were anesthetized with 75 mg/kg ketamine and 5 mg/kg xylazine intramuscularly (IM). Methamidophos (O,S-dimethyl phosphoramidothioate, 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. Atropine sulfate and pralidoxime (2-PAM) were dissolved in saline and injected intraperitoneally (IP) with a 23-gauge needle. Each rat was treated and examined separately and independently. Rats were sacrificed by intracardiac blood collecting. Blood samples were centrifuged at 1000 cycles per minute for 10 minutes, and the plasma was kept at −70°C until it was analyzed for pseudocholinesterase (PCE) activity. The PCE was assayed by the enzymatic colormetric method (S-butyrylthiocholineiodide) (Integra Cobas 800 Roche, Germany®).

The kidney tissues were fixed in 5% glutaraldehyde in Millonig's 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 treatment groups. The rats in Group 1 (n=10) were treated with 30 mg/kg methamidophos, the LD_{50} of this compound in rats,[7] whereas Group 2 (n=7) was treated with an equivalent volume of 0.9% saline via oral gavage. Cholinergic signs, such as muscle fasciculations, bronchorrhea, bradycardia, and seizures, were noted to begin within five minutes of treatment with methamidophos, and all treated rats exhibited signs of cholinergic crisis by 8 minutes after treatment. The rats in Groups 1 and 2 were sacrificed 8 minutes following treatment.

Group 3 (n=10) was given 30 mg/kg methamidophos via gavage. Eight minutes after exposure to methamidiphos, Group 3 rats were treated with 40 mg/kg 2-PAM IP and atropine IP. The 2-PAM was administered as a bolus dose, but the atropine was titrated to reverse cholinergic signs such as bronchorrhea or bradycardia. Group 4 (n=7) was given initial gavage with 0.9% saline in equivalent volumes to Group 3, and then received IP injections of 0.9% saline in equal number and volumes to those in Group 3.

After cholinergic signs were identified in Group 1, intracardiac blood samples were taken from the rats and placed into EDTA blood collection tubes. Intracardiac blood samples were drawn from Group 2 after waiting as long as in Group 1. The rats in Groups 3 and 4 were allowed to emerge from anaesthesia and were returned to their previous housing conditions. Four days after the initial interventions, these rats were re-anesthetized and sacrificed by intracardiac blood collection; and kidney biopsies were obtained.

Statistical analyses were performed using the statistical package SPSS v 10.0. The 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 as significant. Mean values are presented as ±SD (standard deviation).

RESULTS

The mean activity of PCE was significantly lower in the methamidophos-treated rats (Group 1; 32.6±17.0

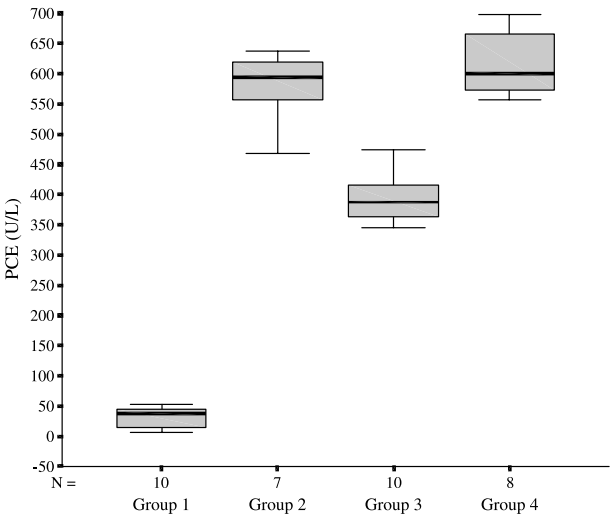


Figure 1. PCE levels in the four groups of rats.

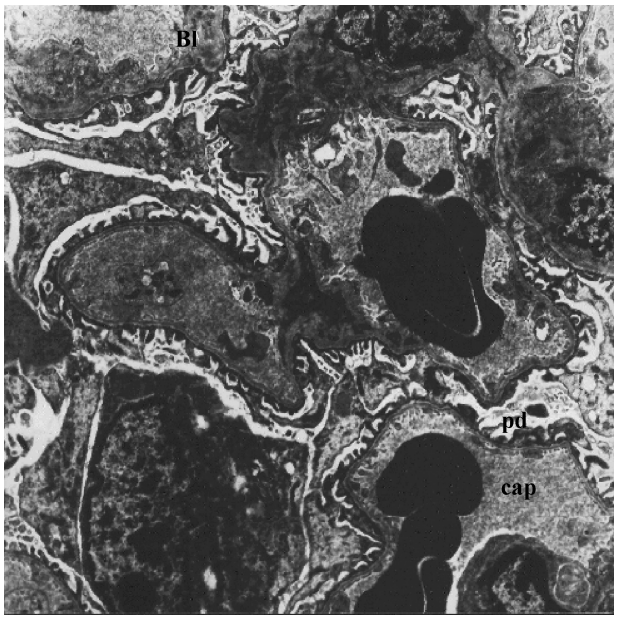


Figure 2. Normal glomerulus was seen in Group 1. Capillary (cap), Basal laminae (Bl), pedicels of podocytes (pd). X 7087.

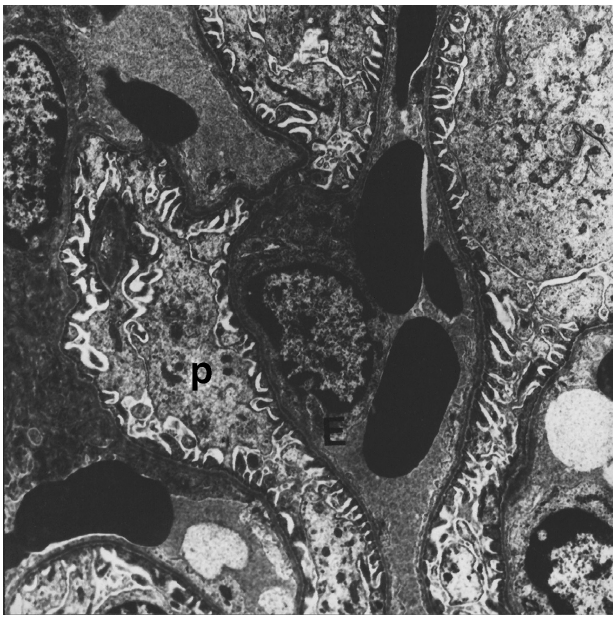


Figure 4. Podocytes (p) and endothelial cells (E) are seen in the electron microscopic photography of the kidney in Group 3. X 8837.

U/L) than in controls (Group 2; 579.4±59.0 U/L) (p=0.000). It was significantly higher in rats treated with 2-PAM and atropine (Group 3; 392.5 ± 39.4 U/L) than in those not receiving antidotal therapy (Group 1) (p=0.000). The mean activity of PCE in antidote-treated rats (Group 3) was still significantly lower than among controls (Group 4; 616.3±54.3 U/L) (p=0.000). See Figure 1.

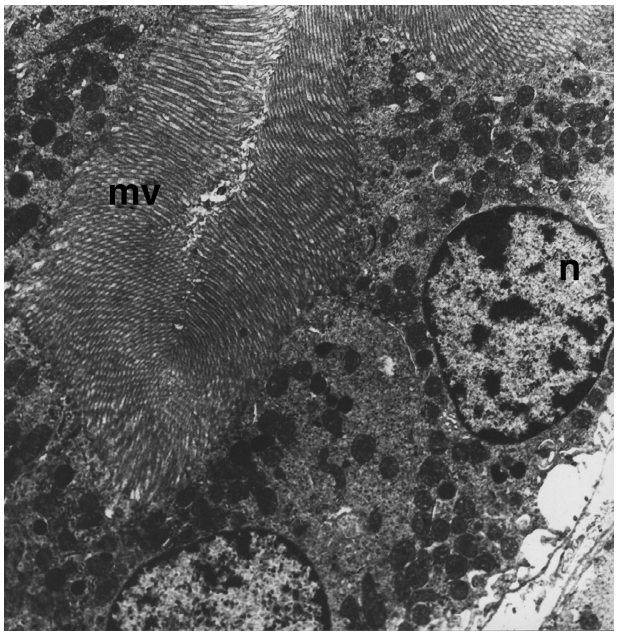


Figure 3. Proximal convoluted tubule is seen. Nucleus (n), microvilli (mv). X 8837.

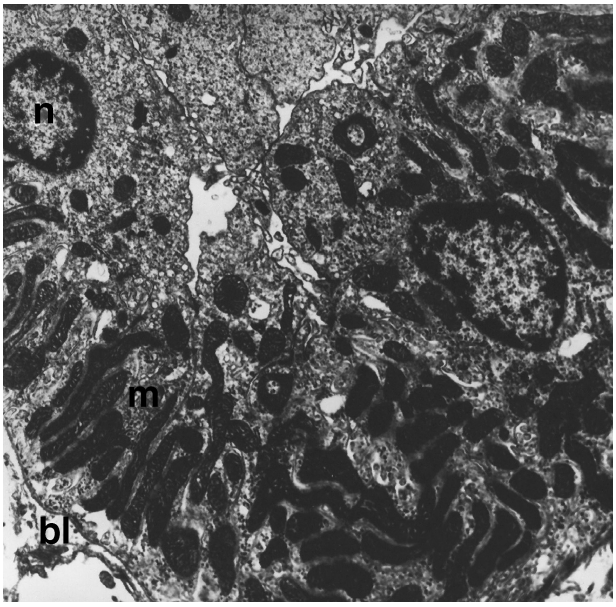


Figure 5. Distal tubule is seen. Nucleus (n), mitochondria (m), basal laminae (bl). X 8837.

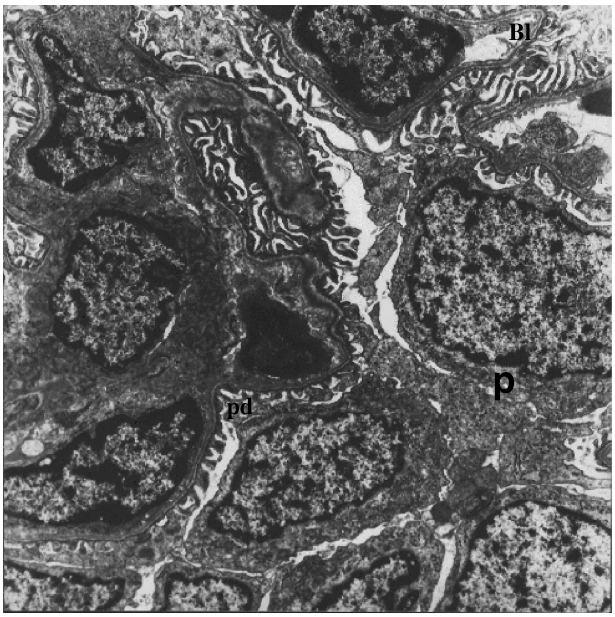


Figure 6. Glomerulus with normal appearance in Group 4. Podocytes (p), Basal laminae (Bl), pedicels of podocytes (pd). X 7087.

The ultrastructural findings in the kidney tissue observed in experiment groups are listed here:

Group 1: Podocytes and slit pores connecting to the foot processes and basal membrane were in normal structure (Figure 2). Normal appearance of proximal and distal tubular structures was also observed (Figure 3).

Group 2: The cytoplasmic organelles and nuclei of the tubular epithelial were observed in normal structure. Glomerular capillaries, basal membrane, and podocytes in the renal corpuscles appeared intact.

Group 3: The ultrastructure of glomerular capillary wall, basal membrane, and podocytes were observed in normal structure (Figure 4). Proximal and distal tubular cells had normal structure (Figure 5).

Group 4: Glomerular and tubular components were observed in normal histologic appearance (Figure 6).

DISCUSSION

Renal injury by chemicals can manifest itself in different ways, depending on target site, extent, and duration of damage. Toxic nephropathies are not restricted to a single type of renal injury. Some chemicals target specific anatomical regions of the kidney and may affect only one cell type. The kidney is a target for toxic chemicals because it has a high blood flow, is metabolically active, can generate reactive intermediates, concentrate chemicals, and is sensitive to immune reactions. Although the kidney has an effective repair capacity, there are circumstances where damage is irreversible and a series of degenerative changes takes place that may stabilize or else progress to acute or chronic renal failure.[8]

The cell types most frequently involved in nephrotoxic processes are smooth muscle cells of the renal vasculature, endothelial cells of renal arteries, arterioles, capillaries and veins, podocytes and mesangium cells of the glomerulus, the epithelial cells of both segments of the proximal convoluted tubule and the proximal tubular straight portion.[9–11] The more distal portions of the nephron are rarely target compounds.[8]

Kidneys receive about 20%–25% of the resting cardiac output. Consequently, any drug or chemical in the systemic circulation will be delivered to these organs in relatively high amounts. The process involved in forming concentrated urine also serves to concentrate potential toxicants in the tubular fluid. As water and electrolytes are reabsorbed from the glomerular filtrate, chemicals in the tubular fluid may be concentrated, thereby driving passive diffusion of toxicants into tubular cells. Thus, a nontoxic concentration of a chemical in the plasma may reach toxic concentrations in the kidney.[12]

In this study we used methamidopos, an anticholinesterase organophosphorus ester, which is rapidly absorbed, distributed, metabolized, and excreted in mammals. Elimination is mainly via the urine in the form of acid metabolites; some is also eliminated via the lungs as carbon dioxide.[13] Rats in Group 1 received an LD_{50} dose of methamidophos but maintained normal kidney ultrastructure. This finding is different from previous investigations with organophosphates. Donchev et al. and Hettwer used pinochelin ether of methyl fluoride-phosphoric acid (PEMA) for demonstrating ultrastructural effects in the kidney.[14,15] These effects manifested as vacuolization of podocytes and destruction of mitochondria of the tubule epithelium, which was accompanied by an elevation in the activity of lysosomes. The normal kidney ultrastructure in our study could possibly result from detoxification of methamidophos by aliesterase, which has moderate activity in the kidney.[16]

In other studies demonstrating the effects of organophosphates on the kidney, the authors administered the organophosphate compounds in different dosages and at multiple times.[17–20] Vandana et al. demonstrated that organophosphates cause renal tubular cytotoxicity in association with hydrogen peroxide accumulation and lipid peroxidation; oxidant stress was believed to play a role in the pathogenesis of organophosphate-induced acute tubular necrosis and renal dysfunction.[17] Renal damage may be independent of acetylcholinesterase inhibition[21] and a free radical mechanism might directly contribute to renal cell injury.[20] These studies were achieved in cell culture and during the experiments, the

exposure time and the dosage of organophosphates produced free radicals that may have induced the renal cell injury.[21,22] In our in vivo study, the timing of animal sacrifice after only a few minutes (in Group 1), and the administration of antidotal therapy (in Group 3) may have allowed maintenance of the normal renal ultrastructure.

In organophosphate poisoning, renal impairment is relatively rare but correlated with death. In our study we tried to determine the ultrastructural reasons for the renal impairment in organophosphate poisoning, but no experimental group showed an abnormal kidney ultrastructure. As a result, the pathogenesis of renal injury in acute organophosphate poisoning is still unclear. Additional studies, perhaps using other doses or more prolonged exposure times, may better elucidate the mechanism of organophosphate-induced renal injury.

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