

Full Length Research Paper

Tissue effects of high dose atropine and 2-PAM on healthy rats: Examining their role in complications during treatment of organophosphate poisoning

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The aim of this study was to determine the effects of high dose atropine and pralidoxime (2-PAM) on different tissues, and to evaluate the drugs' probable roles in complications during organophosphate (OP) treatment, without using pesticides on healthy tissues. This study was designed as a randomized controlled experimental study consisting of three groups of 10 rats (the control group, the atropine group, and the 2-PAM group). The overall drug dosages were set based on those administered in a previous study to counteract the effects of OP toxicity (0.12 mg/kg parathion-methyl). The drugs were administered in divided doses intraperitoneally (i.p) for 96 h. Sample tissues (that is, heart, lungs, liver, spleen, kidneys, intestines, pancreas, ovaries, and both parotid glands) were evaluated using a light microscope by pathologists blinded to the experiment. The only significant tissue findings were nonspecific findings in the parotid glands of the 2-PAM group and in the secretory glands (parotids, pancreas, and ovaries) of the atropine group identified by light microscope. Atropine contributed to nonspecific cellular changes detected in the secretory glands. Macroscopic evaluation demonstrated that the parotid glands were the only tissues affected by 2-PAM. These nonspecific changes could be the initial state of definite pathological changes and require further study over longer periods with an electron microscope.

Key words: Atropine, pralidoxime, tissue.

INTRODUCTION

Organophosphates (OPs) are used as insecticides and cause mortality. A regional two-year study found that the rate of organophosphate usage in suicides was 5.18% (Al et al., 2010). It was determined that insecticides caused 7% of fatal suicides (Canturk et al., 2010). OPs inhibit acetylcholinesterase (AChE) and produce irreversible

effects via accumulation of acetylcholine (Ach) at synapses (Goel et al., 2007). Atropine and 2-pralidoxime (2-PAM) have been used for emergency treatment and in experimental studies to counteract the effects of excess Ach (Gokel et al., 2002; Gulalp et al., 2007). Previous studies found that large doses of atropine and 2-PAM are key in treating toxic and lethal doses of OP intoxication with parathion, mentioned in WHO: Class 1A (Gokel et al., 2002; Gulalp et al., 2007; WHO 2001). However, the negative effects of these agents in high doses on various healthy tissues without OP poisoning have not clearly

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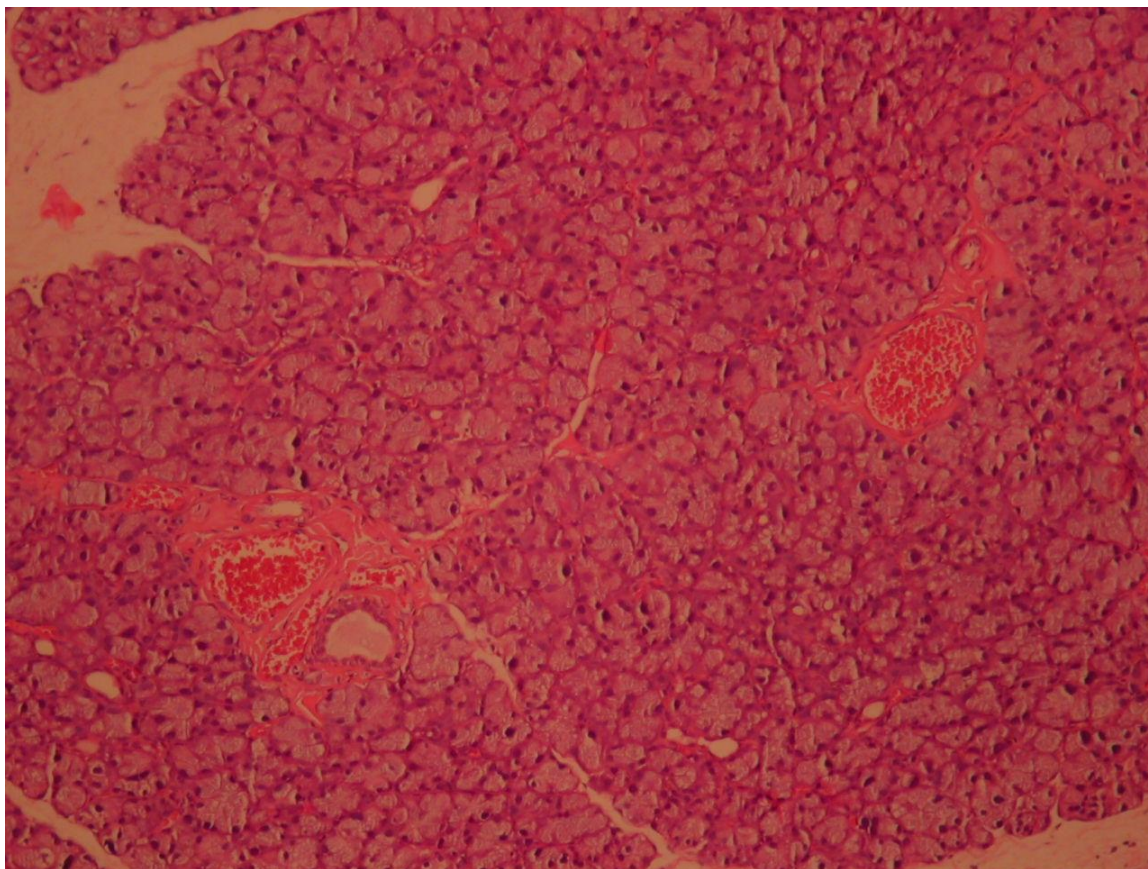


Figure 1. Nonspecific reactive minimal nuclear changes in the parotid glands of atropine group rats (Group II) received a total of 1 mg/kg of atropine in the first 24 h followed 0.25 mg/kg on the second day, 0.125 mg/kg on the third day, and 0.0625 mg/kg on the fourth day.

been demonstrated. This experimental study was designed to demonstrate the side effects of high doses of standard medications on tissues.

MATERIALS AND METHODS

Thirty four-month-old female Wistar rats (Central Vivarium Animal Laboratory), weighing approximately 250 g, were randomly divided into three equal groups (10 rats each). The rats were kept in Nalgene metabolic cages, maintained at 19 to 23°C and 45 to 60% humidity, and fed 16 mm standard rat food. The cages were kept well-lit for 12 h and in darkness for 12 h each day.

All experiments were conducted following the guidelines for international care and use of laboratory animals after acquiring approval from the institutional review board of Cukurova University. In this study, no organophosphates were used and none of the study groups received organophosphates. The control group, Group I, received no medication. Each rat in Group II received a total of 1 mg/kg of atropine during the first 24 h. Doses of 0.02 mg/kg were administered every 3 to 5 min for the first 90 min and then every 70 min (approximately 50 injections in 24 h). After the first day, atropine was administered as follows: 0.25 mg/kg on the second day, 0.125 mg/kg on the third day, and 0.0625 mg/kg on the fourth day. Group III received 40 mg/kg/d of 2-PAM i.p. for 96 h. A total of 40 mg of 2-PAM was administered to each rat. These doses

were determined based on a previous experimental organophosphate poisoning study in which rats administered toxic doses of oral 0.12 mg/kg parathion-methyl were treated (Gulalp et al., 2007). All substances were administered i.p. using 28-G insulin syringes. All groups received anesthesia in the form of 2.5 mg/kg i.m. xylazine and 60 mg/kg i.m. ketamine at the end of the 96 h period. In all groups, the heart, liver, spleen, stomach, intestines, lungs, kidneys, ovaries, pancreas, and bilateral parotid glands were removed from each rat. All tissues were fixed in 10% neutral buffered formalin for light microscopic examination. Hematoxylin-eosin was applied to samples; the prepared samples were then examined at 100x (Figure 2) and 200x (Figure 3) magnification. The Fisher's Exact Test was used to statistically compare the findings of light microscope evaluation and normal tissues.

RESULTS

Pathological changes in the secretory glands of the atropine group: Cellular effects (Figure 1) such as non-specific reactive nuclear changes are demonstrated in Table 1. Pathological changes in the parotid glands of the PAM group: cellular changes, such as non-specific reactive nuclear changes (Figures 2 and 3), are shown in Table 1. These changes can be related to the increase in

Table 1. The pathologic evaluation of removed organs by group.

Organ	Control		Atropine		PAM	
	N	CC	N	CC	N	CC
Heart	10	0	10	0	10	0
Intestines	10	0	10	0	10	0
Kidneys	10	0	10	0	10	0
Liver	10	0	10	0	10	0
Lungs	10	0	10	0	10	0
Ovaries	10	0	4	6*	10	0
Pancreas	10	0	5	5**	10	0
Parotids	10	0	3	7***	4	6*
Spleen	10	0	10	0	10	0

N: Normal pathology, CC: Nonspecific reactive cellular changes, *p=0.011, **p =0.032 and ***p=0.003 compared with the control group.

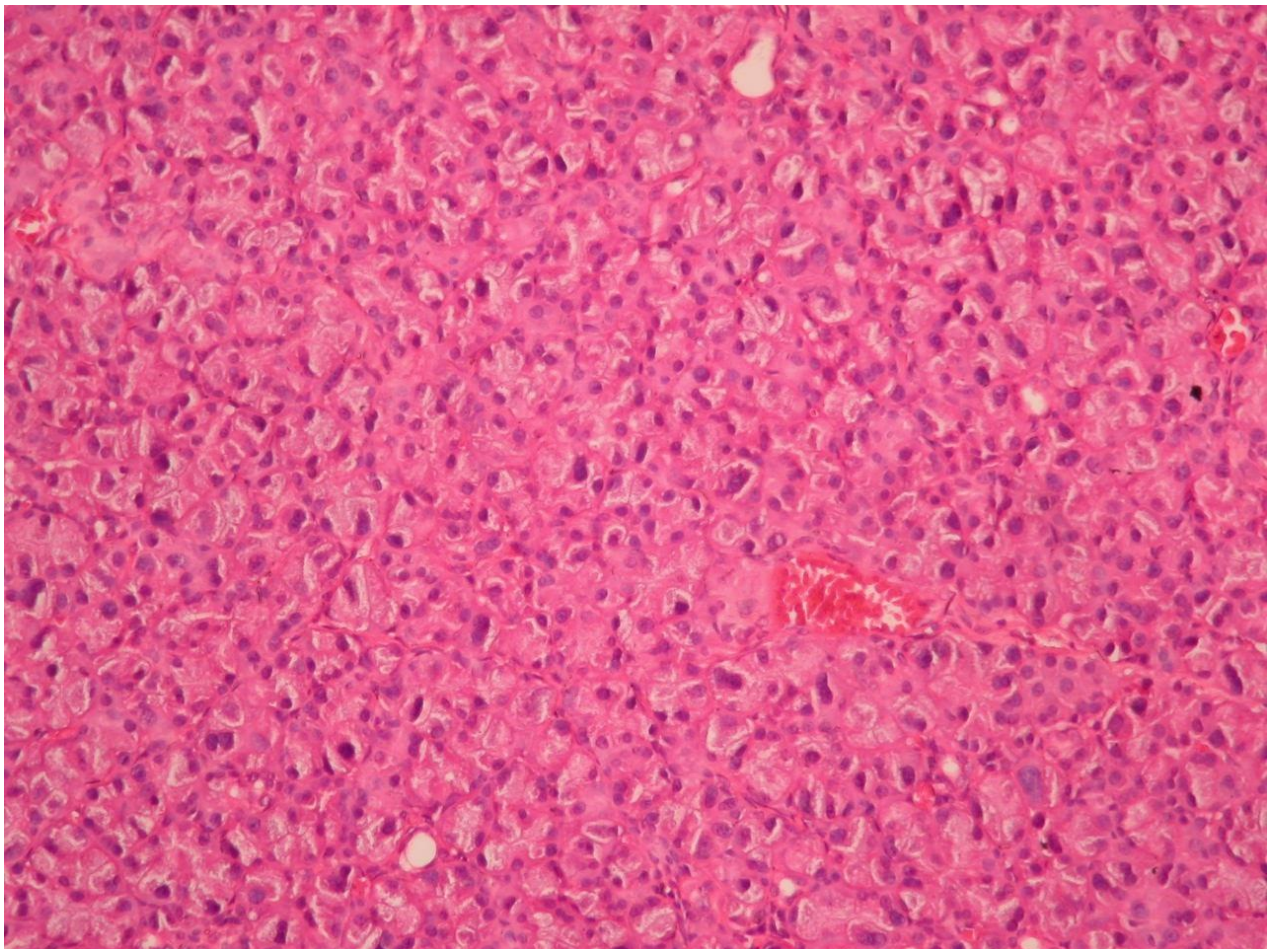


Figure 2. Nonspecific reactive minimal nuclear changes in the parotid glands of PAM group rats (Group III), which received 40 mg/kg/d for 96 h (hematoxylin-eosin x 100).

the nucleus/cytoplasm ratio in favor of the nucleus, hyperchromasia, and pleomorphism. All parotid samples

from the PAM group had a soft, gel-like structure and were sticky like mucus, unlike the parotid samples from

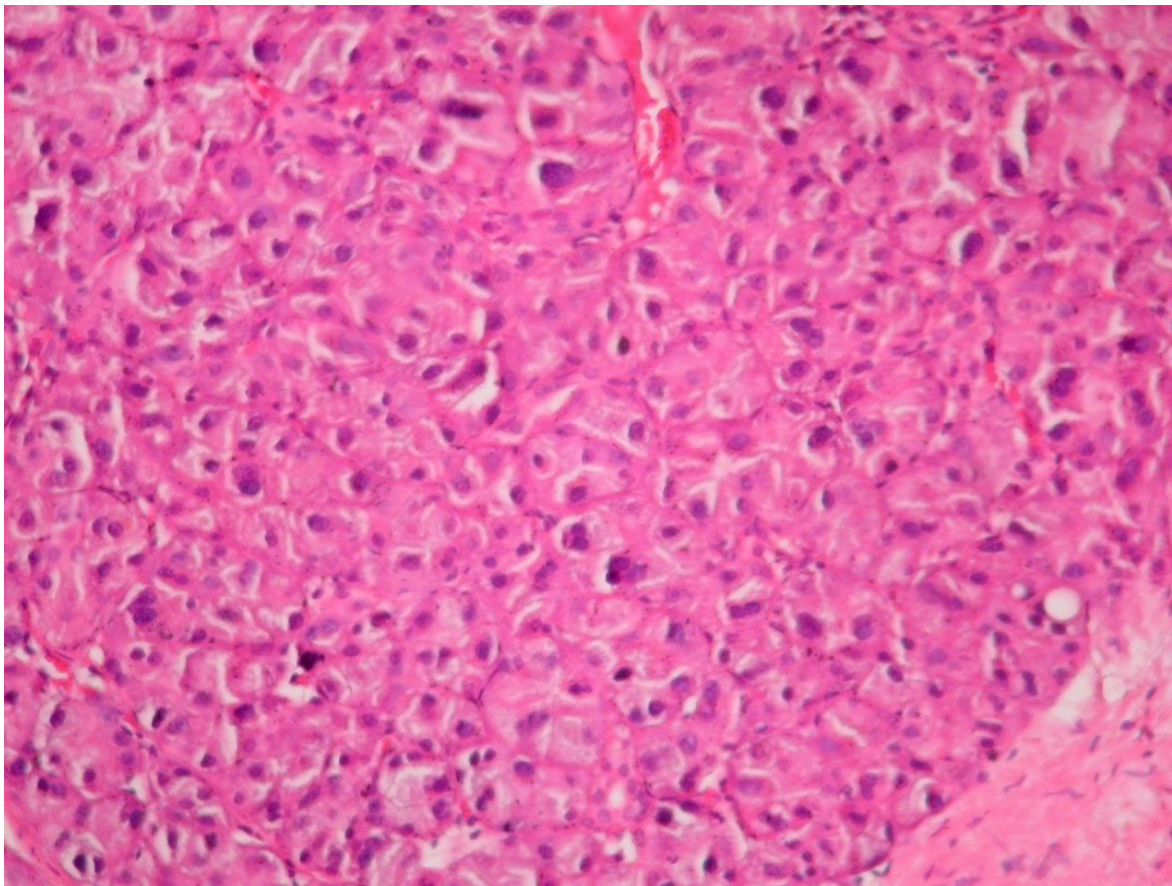


Figure 3. Nonspecific reactive minimal nuclear changes in the parotid glands of PAM group rats (Group III) which received 40 mg/kg/d for 96 h (hematoxylin-eosin x 200).

the controls.

There were no pathological differences due to atropine and PAM between the control tissues and the heart, lungs, liver, spleen, stomach, intestines, and kidneys.

DISCUSSION

OP poisoning continues to be a problem, especially in agricultural areas of developing countries. OP compounds irreversibly bind to AChE, causing phosphorylation and inactivation of AChE. Clinical findings occur due to the aggregation of ACh at cholinergic junctions and neuromuscular junctions, and in autonomic ganglia and the central nervous system (Goel et al., 2007; Tuncok and Aksay, 2006). Atropine is traditionally used to treat OP intoxication due to its anticholinergic effect. PAM is used to reactivate AChE in the nicotinic and central nervous systems; however, its adverse effects are controversial (Tuncok and Aksay, 2006; Saydam et al., 2006). Clinicians have sometimes needed to use high doses to treat patients, but the effects of these agents on tissues remain unknown (Gokel et

al., 2002; Guven et al., 2004). In addition to essential doses, the duration of treatment and the effects of atropine and 2-PAM are still controversial (Eyer, 2003; Eddleston et al., 2008). Complications have been reported during the OP treatment stage, including pancreatitis, parotitis, necrosis, sepsis, and multi-organ deficiency syndrome (Goel et al., 2007; Gokel et al., 2002; Gulle et al., 2010; Lankisch et al., 1990; Leuwer et al., 1990; Ozucelik et al., 2004; Panieri et al., 1997; Weizman and Sofer, 1992). However, many of the complications were associated with the organophosphates in the literature. The high doses of drugs, such as atropine and PAM, required for therapy could be the one of the actual causes of these complications. There are no data in the literature showing the pathological effects of high doses of drugs, such as atropine and PAM, on tissues; thus, in our study, unpoisoned rats were treated with the drugs.

Studies in the literature have explained the tissue findings, especially in poisoning cases. It was reported that atropine, even at high doses, was not sufficient to prevent pancreatitis following fenthion poisoning (Ela et al., 2008). A previous study found no renal ultra-structural

difference between rats with methamidophos poisoning and poisoned rats treated with atropine and 2-PAM (Satar et al., 2005). The same treatment agents were described as preventing the toxic effects of methamidophos in the rat liver (Satar et al., 2004). It was reported that atropine caused damage to the tracheal epithelium cells; this effect was dose-dependent (Konradova et al., 1996). ICD-467, an oxime which reactivates cholinesterase suppressed by organophosphates, was found to have a toxic interaction with soman (Shih, 1993). It was related to the high risk of mortality to hepatotoxicity and AChE inhibition due to a high dose of PAM (Güven et al., 2004).

In our study, non-specific nuclear reactive changes, especially in the secretory glands, were identified in the atropine group. These changes could be due to the increase in the nucleus/cytoplasm ratio in favor of the nucleus, hyperchromasia, and pleomorphism. The non-specific nuclear reactive changes may have resulted from the inhibition of secretion and the accumulation of glandular ingredients in the secretory glands following administration of high-dose atropine. In a previous study, OP intoxication was treated with atropine and PAM for four days, and major changes—parotid nuclear hyperchromasia, enlargement, irregularity, and binuclear cells—were found in rats. These changes could create a cellular environment prone to malignancy. In our study, the changes were nonspecific but significant at the end of the short 96-h period; they could be the initial form of a definite pathological change (Gulalp et al., 2007). In our previous study, probable preliminary malignant cells were identified in the parotids during treatment. Macroscopically, the parotid tissues of all the rats treated with PAM resembled a gel substance, unlike the tissues of the control group rats. We could not identify or better characterize these changes with the light microscope. Only one case of acute parotitis in humans, that of a 67-year-old male who ingested parathion, has been documented so far (Gokel et al., 2002). In our study, parotid tissues in the PAM group had nonspecific reactive changes, suggesting that PAM may increase changes in parotid tissue, especially during OP treatment.

One limitation of this study was the use of a light microscope and pathological evaluation instead of an electron microscope and histological examination. The latter combination could have been used to identify and detail the different nonspecific changes in the above mentioned tissues with electron microscope.

Conclusion

Nonspecific nuclear changes were observed in the secretory glands of atropine group rats and in the parotids of rats treated with PAM, using doses administered in our previous study to treat OPs, during the 96 h period. Complications and vague nuclear

changes have been previously associated with OPs. This study was unable to clarify the mechanism underlying non-specific reactive nuclear changes in the parotid glands using a light microscope. No studies exist in the literature which could explain the observed macroscopic effects of 2-PAM on the parotid glands. Atropine could have contributed to nonspecific reactive effects during OP poisoning without any significant tissue changes. No harmful effects on healthy tissues were attributed to atropine or PAM, even at high doses, in this murine model conducted over a short 96 h period. New models studied for periods longer than four days are needed to identify changes using advanced techniques.

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