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THE APOPTOSIS EXPRESSION IN STOMACH, BRONCHI AND LUNGS IN RATS POISONED WITH SUBLETHAL DOSES OF SOMAN OR MEVINPHOS - A PRELIMINARY STUDY

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Summary

The features of organophosphate-induced injuries in the respiratory and gastro-intestinal tracts were investigated. The rats were poisoned i. m. with highly toxic organophosphate soman at 48 μg/kg (0.8 LD_{50}) or organophosphorus insecticide mevinphos at 0.4 mg/kg (0.8 LD_{50}). On the third or fifth day following the exposure to soman or mevinphos, the rats were killed using cervical dislocation and the apoptotic changes in stomachs, bronchi and lungs were demonstrated using immunochemical detection of apoptotic cells with the M30 Cytodeath kit. While very few apoptotic cells in the stomach were observed regardless of the type of organophosphorus compound and the time following the exposure, the detection of apoptotic cells in the bronchi and lungs showed a significant increase in their numbers in bronchi in the case of mevinphos exposure and in bronchi as well as lungs in the case of soman exposure compared to the control values. Therefore, not only necrotic but also apoptotic injury can be present in the organism poisoned with high doses of organophosphorus compounds.

Key words: Soman; Mevinphos; Apoptosis; Stomach; Lungs; Bronchi; Rat.

Introduction

Organophosphorus (OP) compounds are widely used as OP insecticides in increasing quantities for the control of insects affecting agriculture. Suicidal, accidental or occupational exposure to OP is common especially in developing countries (8). Moreover, there are highly toxic OP, called nerve agents, produced for military or terroristic purposes. They represent potential neurotoxic threats to both military and civilian populations as evidenced in recent terroristic attacks in Japan (14). OP toxicity results from the irreversible binding to and inactivation of acetylcholinesterase (AChE, EC 3.1.1.7), the enzyme that normally catalyzes the hydrolysis of neurotransmitter acetylcholine (ACh) at central as well as peripheral cholinergic synapses. The inhibition of this enzyme leads to signs and symptoms characteristic for the overstimulation of the cholinergic nervous system because of accumulation of ACh in the synaptic clefts (12, 19).

Recent pathophysiological studies have revealed that OP compounds, expecially highly toxic nerve agents, may result in CNS and myocardial lesions. CNS damage usually began as microvacuolation of the neuropil and was followed by astrocytic degeneration and neuronal necrosis culminated in liquefaction necrosis and multifocal haemorrhage. They have been reported to be prominent in frontal and entorhinal cortex, amygdala and thalamic nuclei, substantia nugra and hippocampus (2, 20). Myocardial damage began with myocytolysis and contraction bands and evolved into coagulative myocytolysis and replacement fibrosis with a transient recruitment of acute inflammatory cells. The left ventricle, especially its free wall and papillary muscles, was consistently affected (20). Myonecrotic lesions in soleus and diaphragm following the exposure to highly toxic OP compounds were also observed (7).

Moreover, damage of respiratory tract were described in OP compound poisoned animals. Lungs of exposed rats revealed an increased cellular proliferation with progressive diffused interstitial thickening and subsequent loss of alveolar space and con-

solidation of large area of all lobes. Respiratory bronchioles were also damaged (15).

Apoptosis, a programmed cell death, is an important process for cellular homeostasis in various tissues. The mechanisms regulating apoptosis are complex and they involve the interaction of nuclear and cytoplasmic proteins. Recently, the influence of various chemical substances including some toxic compounds on the apoptosis development has been described (1, 4, 8, 9, 11, 13, 17). Nevertheless, the importance of apoptotic mechanism for their toxic efficacy has not been still sufficiently understood.

The purpose of the present study was to find whether morphogenetic changes caused by OP compounds may also be accompanied by changes characterized for apoptosis. The representatives of highly toxic OP compounds (soman) and OP insecticide (mevinphos) were administered intramuscularly (i.m.) and the respiratory and gastro-intestinal systems were chosen to follow apoptotic changes.

Materials and methods

Male albino rats weighing 180-200g were purchased from Konárovice (Czech Republic). They were kept in an air-conditioned room and allowed to access to standard food and tap water ad libitum. The rats were divided into groups of six animals.

Handling of the experimental animals was done under the supervision of the Ethics Committee of the Medical Faculty of Charles University and the Military Medical Academy in Hradec Králové.

Soman (48 μg/kg) as well as mevinphos (0.4 mg/kg) were administered i. m. at a sublethal, convulsive dose (0.8 LD_{50}). At the third or fifth day following the intoxication, the rats were killed using cervical dislocation and the chosen organs (stomachs, lungs with bronchi) were removed. Instead of soman or mevinphos, saline administered by the same way was used for control rats.

Immunohistochemical examination

The removed organs were carefully fixed in 10% neutral buffered formalin, embedded into paraffin. Then, 4 μm sections were cut off and stained using Gramm's stain to evaluate bacterial infection. The M30 Cytodeath kit (Boehringer Mannhein, Germany) was used to detect apoptotic cells. After 20 min blocking, the tissue sections were incubated with individual monoclonal antibody for 1 hour at room temperature and wasted three times in phosphate-buffered saline (PBS, pH 7.2). Then, the slides were incubated with a horseradish peroxidase-coupled mouse secondary antibody for 30 minutes at room temperature. Excessive antibody was washed off with PBS. Finally, 0.05% 3,3-diaminobenzidine tetrahydrochloride chromogen solution (Sigma Prague, Czech Republic) in PBS containing 0.02% hydrogen peroxide was added for 10 minutes at room temperature to visualize the formed antigen-antibody complexes. This examination makes possible to detect apoptotic cells of epithelial origin only.

Measurement of apoptotic cells

Immunohistochemical samples were evaluated using IMT-2 light microscope (Olympus Company Prague, Czech Republic) and a computer image analysis - Image Pro (Media Cybernetics, MD USA). The range of positive staining cells were determined as red (112-170), green (76-149) and blue (0-147) in the lung tissue and bronchial epitelial samples; as red (76-150), green (67-150) and blue (39-104) in the stomach tissue. The percentage of apoptotic cells in 500 bronchial epithelial cells per examined rat was counted. In the lung tissue, the number of apoptotic cells in 20 randomly selected fields of 10 744.32 μm^2 without bronchi and large vessels were evaluated in each preparation at a 400 fold original magnification. The same approach was used for the counting of positive cells in stomach samples. The obtained numbers of apoptotic cells were compared to the control values.

Data analysis

The diameter ± SEM for each group of data was calculated and Mann-Whitney's test was used to determine a statistical significance. Differences were considered significant when $P < 0.05$.

Results

The numbers of epithelial apoptotic cells in stomachs, lungs or bronchi were shown in the Table 1. While no significant differences between the number of apoptotic cells in experimental and control rats were found in the stomachs regardless of the type of OP compound used or time interval following the exposure, a significant increase in the number of apoptotic cells in lungs as well as bronchi

Table 1

The number of apoptotic cells in bronchi, lung or stomach following the exposure of rats to soman or mevinphos

TISSUE	BRONCHI (% of cells)	LUNG (No of cells/field)	STOMACH (No of cells/field)
Saline	1.59 ± 1.23	0.13 ± 0.05	2.76 ± 0.40
Soman - the third day	16.77 ± 2.38*** c	0.56 ± 0.08***	3.96 ± 0.71
Soman - the fifth day	6.02 ± 1.26	0.44 ± 0.08*	3.69 ± 0.77
Mevinphos - the third day	8.03 ± 1.77*** b	0.31 ± 0.06	2.69 ± 0.54
Mevinphos - the fifth day	5.18 ± 1.40	0.28 ± 0.06	3.94 ± 0.56

Statistical significance between control and experimental values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
Statistical significance between time intervals following the exposure to soman or mevinphos: [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$.

was observed. Both OP compounds studied were able to cause the apoptosis in bronchial epithelial samples but only soman caused the significant apoptosis in the lung at the third day following soman exposure of rats. On the other hand, the significant increase in the number of apoptotic cells at the fifth day following the soman exposure of rats was only detectable in lungs.

Photomicrographs of lung and bronchi tissues clearly documented the extent of apoptosis in the respiratory tract in soman or mevinphos exposed organisms in comparison with control animals where the number of apoptotic cells is very low (see color suppl. p. III, Fig. 1-5). On the other hand, the photomicrographs of stomach tissue in soman or mevinphos exposed rats did not differ from the control animals (see color suppl. p. IV, Fig. 6-7).

Discussion

Apoptosis, a programmed cell death, is the important process for cellular homeostasis in various tissues. Recently, the sensitivity of tissues to apoptosis, induced by various external stimuli, has been closely linked to the intracellular concentrations of cell-death regulators including Bcl-2 and CD95 (6). While a high level of Bcl-2 protects the cells from apoptosis, the main function of CD95 appears to be the induction of apoptosis in sensitive cells triggered by CD95 ligand (6). Generally, the cells with a high mitotic activity seem to be very sensitive to apoptosis. Therefore, gamma-irradiation of the radiosensitive cells leads to increasing in CD95 expression which initiates the activation of caspases, the key enzymes of apoptosis (6, 16). In addition, the drugs capable of radiosensitizing various tissues such as pimozide (a neuroleptic drug) and gemcitabine (an inhibitor of DNA synthesis) can enhance the process of apoptosis (13, 18). Moreover, nitric oxide whose function as a signalling or cytotoxic molecule (4, 11) has been reported to induce apoptotic death of a variety of cells including neural (3) and immune (5) cells.

It was demonstrated that the exposures to OP compounds can also induce the process of apoptosis especially in the neuronal tissue via the increase in the level of nitric oxide (1). The apoptotic brain injury was observed to start much later than the necrotic brain injury following the exposure to diisopropylfluorophosphate (10). Our study demonstrated that OP-induced apoptosis can also appear in the peripheral tissue, especially in the respiratory tract that seems to be the key system for the prognosis of poisoning because OP-induced acute respiratory insuficiency usually leads to the death (12, 19). The apoptosis process in the respiratory tract could be caused by the increase in the level of nitric oxide as in the case of apoptotic brain injury or by alteration of nucleic acid metabolism (9, 17).

In conclusion, not only neuronal tissue but also some peripheral systems can be damaged via apoptotic process following severe exposures of mammals to highly toxic nervous agents as well as OP insecticides.

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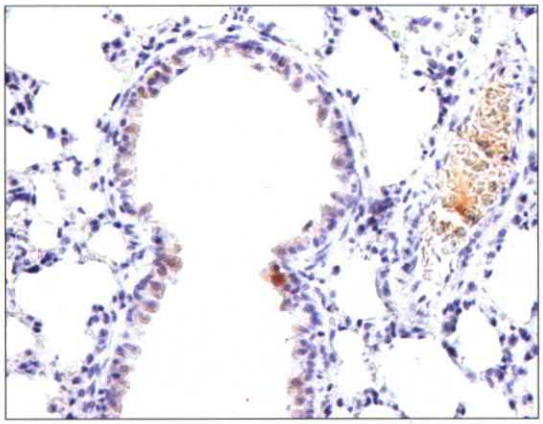

Fig. 1: Photomicrograph of 4 μm section of bronchi from control group. Original magnification x 400, apoptosis detection. The presence of apoptotic cells was observed occasionally.

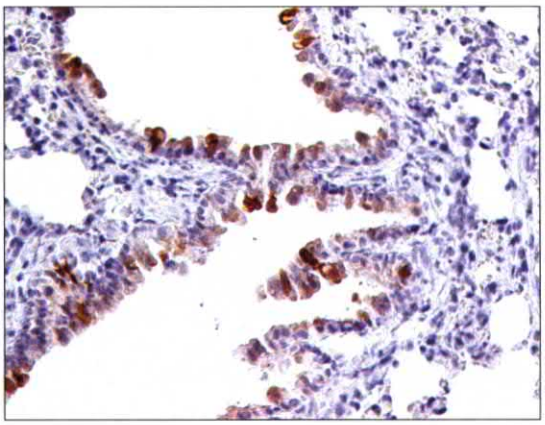

Fig. 2: Photomicrograph of 4 μm section of bronchi from soman-exposed rat at the third day following the exposure. Original magnification x 400, apoptosis detection. The number of apoptotic cells was highly increased in comparison with the control group with the statistical significance.

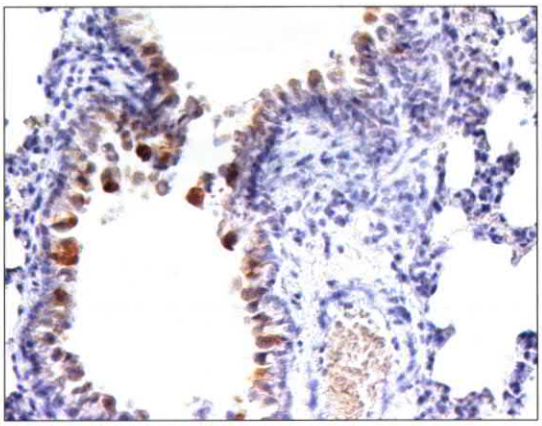

Fig. 3: Photomicrograph of 4 μm section of bronchi from mevinphos-exposed rat at the third day following the exposure. Original magnification x 400, apoptosis detection. The number of apoptotic cells was significantly increased in comparison with the control group.

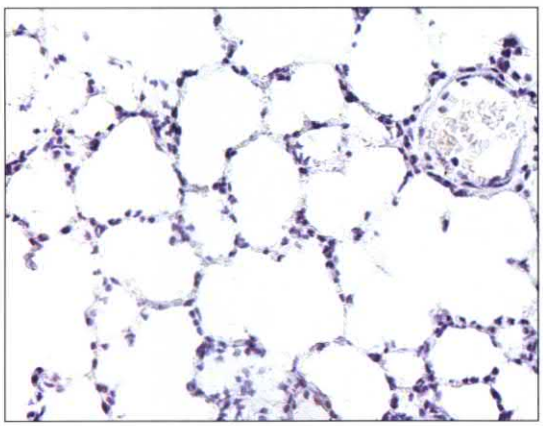

Fig. 4: Photomicrograph of 4 μm section of subpleural area of lung from control group.
Original magnification x 400, apoptosis detection. The presence of apoptotic cells was rare.

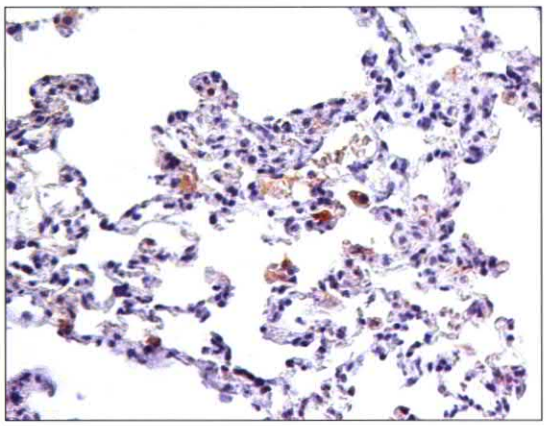

Fig. 5: Photomicrograph of 4 μm section of subpleural area of lung from soman-exposed rat at the third day following the exposure. Original magnification x 400, apoptosis detection. The number of apoptotic cells was significantly increased in comparison with the control group.