

## **67. NEW APPROACHES TO TREATMENT OF POISONING BY SOMAN**

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### **SUMMARY**

The acute toxicity of organophosphates (OPs) in mammals is primarily due to their irreversible inhibition of acetylcholinesterase in the nervous system, which leads to increased synaptic acetylcholine levels. However, the toxic effect of some OPs is not limited to inhibition of cholinesterase: following the cholinergic crisis, changes in non-cholinergic neurotoxic parameters, such as specific damage to cell membranes, are observed. One of the major problems in assessing the role of lipid peroxidation in any chemical toxicity is to resolve whether this pathogenic cascade is a cause or a consequence of damage. The present study was undertaken to elucidate the relations between lipid peroxidation, OPs toxicity and delayed, long lasting, non-cholinergic changes. We studied the influence of OP intoxication on lipid peroxidation in rat cerebral hemispheres. The level of lipid peroxidation was measured as the amount of common phospholipids, peroxidate lipids and malondialdehyde (MDA) in reaction with thiobarbituric acid. Results were compared to those with pre-treatment with atropine and reversible cholinesterase inhibitor - galanthamine alone or together with different antioxidants. OPs caused a rapid, dose-dependent increase of peroxidate lipids and MDA 15-30 days after intoxication. The level of lipid peroxidation correlated with the rate of conditioned reflex reaction. With paraoxon and sarin, pre-treatment with atropine and galanthamine totally prevents all the symptoms of intoxication and changes in lipid peroxidation. Comparatively such type of prophylaxis in soman poisoned rats did not normalize the biochemical and physiological parameters. The protective effect of antioxidants against soman - induced lipid peroxidation was shown. Therefore soman - associated lipid peroxidation is likely to arise mainly as a primary change that may, however, play a significant role in delayed neurotoxicity and conditioned reflex activity.

### **INTRODUCTION**

Organophosphates (OPs) are widely used as pesticides and many have thus become environmental contaminants. In addition some OPs, like sarin, soman, Vx and tabun are important warfare agents. Although their production and testing have been forbidden by international agreements, their manufacture still continues in some countries. In the treatment of OPs poisonings, the combination of muscarinic cholinergic antagonists like atropine and some oximes (obidoxime, pralidoxime, HI-6 etc.) has been used (1). The efficiency of oximes is not, however, satisfactory in the case of soman, cyclosarin and tabun poisonings, on account of the rapid dealkylation ("aging") of acetylcholinesterase (AChE). The resulting methylphosphonyl-AChE is resistant against the nucleophilic attack of oximes (2, 3). It was found that pretreatment with certain cholinesterase reversible inhibitors such as physostigmine in conjunction with atropine and oximes, gave appreciable protection against poisonings by any OPs, including soman (4,5). Nevertheless the delayed neurotoxic effect was shown to occur in animals

poisoned by soman, even after usage of different effective OPs antagonists.

The acute toxicity of organophosphates in mammals is primarily due to their irreversible inhibition of acetylcholinesterase in the nervous system, which leads to increased synaptic acetylcholine levels. However, the mechanism of toxic effect of some OPs is not limited to inhibition of cholinesterase: following the cholinergic crisis, there are observed changes of non-cholinergic neurotoxic parameters, e.g., specific damage of cell's membranes (6). One of the major problems in assessing the role of lipid peroxidation in any chemical toxicity is to resolve whether this pathogenetic cascade is a cause or a consequence of damage (7,8).

Lipid peroxidation is the reaction of oxidative deterioration of polyunsaturated lipids. Peroxidation involves the direct reaction of oxygen and lipid to form free radicals. The latter, produced during lipid peroxidation, are similar to the chemically damaging radicals emanated by radiation. Tissues most susceptible to lipid - peroxidation appear to be those with low mitotic rates such as a brain. It has also been reported by different investigators that of the different tissues from the normal rat, the brain showed a considerably higher rate of lipid peroxidation than liver, kidney, spleen and heart homogenates (9).

## **MATERIALS AND METHODS**

Male albino rats weighting 150 - 200 g (60-90 days old) were used throughout this study. They received a pellet diet and water until the time sacrifice. We studied the influence of OPs intoxication (1.25 DL50 - 1.75 DL50 ) by paraoxon, sarin and soman on lipid peroxidation in rat's cerebral hemispheres. The level of lipid peroxidation was measured as the amount of common phospholipids, peroxidate lipids and as amount of malondialdehyde (MDA) in reaction with thiobarbituric acid (10, 11). The level of lipid peroxidation was also analysed by chemoluminescence method (12). Results were compared to those with pre-treatment with atropine (20 mg/kg) and reversible cholinesterase inhibitor - galanthamine (8 mg/kg). The influence of different antioxidants (a-tocopherol and oxymetacyl) on lipid peroxidation and toxicity of OPs was estimated also. The rate of reaction of conditioned reflex of active avoidance was measured. As a conditional stimulus was switching of electric lamp, as nonconditional stimulus was the irritation of skin by the current.

## **RESULTS**

As shown in [Tables 1](#) and [2](#), MDA and peroxidate lipids contents in rat's cerebral hemisphere increased markedly 1.5 hours after poisoning with paraoxon, sarin and soman, as compared to normal values.

The results indicated a rapid, dose-dependent increase of peroxidate lipids and MDA and decrease of common phospholipids in rat's brain during 15-30 days after injection of soman (1 LD50). The high degree of correlation between the level of MDA and common phospholipids (0.89) and between the MDA and the level of conditional reflex of active avoidance (0.93) in rats poisoned by soman was shown.

Pre-treatment of rats with acute poisonings of paraoxon and sarin by atropine and galanthamine totally prevents the all symptoms of intoxication and changes in lipid peroxidation and in conditioned reflex reaction. Comparatively such type of prophylaxis in soman poisoned rats did not normalize the biochemical and physiological parameters. The protective effect of antioxidants (a-tocopherol and oxymetacyl) against soman - induced lipid peroxidation and conditioned reflex reaction was shown ([Table 3, 4](#)). In

experiments on rats, cats and dogs poisoned by soman, the efficiency of prophylactic antidotes increased in the presence of different antioxidants. The antioxidant oxymetacyl as a component of antidote, normalized the rate of lipid peroxidation in cat's cortex during 24 hours after injection of soman (1.0 - 1.5 LD50). Taken together, the present data show that the of lipid peroxidation in rat's brain poisoned by OPs increased with direct (soman) prooxidant action and non-direct (seizures, hypoxia) prooxidant action of OPs type of paraoxon and sarin.

The protective effect of antioxidants against soman - induced lipid peroxidation appears to result mainly as a primary change, which may play a significant role in delayed neurotoxicity and conditioned reflex activity.

#### REFERENCES:

1. Heilbronn, E. and Tolagen, B. *Biochem. Pharmacol.*, 1965, 14, 73-77.
2. Wolthuis, O.L., Berends, F. and Meeter E. *Fundam.Appl.Toxicol.*, 1981, 1, 183-192.
3. Dunn, M.A. and Sidell, F.R. *JAMA*, 1989 262, 649-642.
4. Berry, W.K. and Davies, D.R *Biochem. Pharmacol.*, 1970, 19, 927-934.
5. Somani, S.M. and Dube, S.N. *Int.J.Clin.Pharmacol. Ther.Toxicol.*, 1989, 27, 367-387.
6. Bajgar, J. *Proceedings of CBMTS*, 1996, Spiez, 200-210.
7. Ahmad, M. and Glees, P. *Acta Anatomica*, 1971, 78, 91-98.
8. Kappus, H. *Chem.Phys.Lipids*, 1987, 45, 105-115.
9. Karatha, V.M.R. and Krishnamurty, S. *Ind. J. Phys Pharmacol.*, 1978, 22, 44-52.
10. Utley, H. G., Bernheim, F. and Hochtein, P. *Arch. Biochem. Biophys.* 1967, 118, 29-32.
11. Yoshioka, T. and Mori, M. *Am. J. Obstet. Gynecol.*, 1979, 135, 372-375.
12. Zimakov, G.A. and Gukasov, V.M. *Pharmacol. Toxicol.*, 1983, 46, 20-27.

#### FIGURES AND TABLES

**Table 1.: Content of malondialdehyde (MDA) in rat's cerebral hemisphere after injection of OPs.**

| Experiments | Dose (mg/kg) | MDA (nm/1g tissue) |
|-------------|--------------|--------------------|
| Control     | -            | 7.02±0.16          |

|           |       |            |
|-----------|-------|------------|
| Paraoxon  | 0.75  | 8.2±0.15   |
| Sarin     | 0.140 | 9.59±0.20  |
| Soman     | 0.055 | 8.33±0.20  |
| - « - « - | 0.075 | 11.50±0.15 |
| - « - « - | 0.110 | 12.16±0.61 |

**Table 2.: Content of peroxidate lipids (PL) in rat's cerebral hemisphere after injection of OPs (1LD50)**

| Experiments | Dose (mg/kg) | Content of PL (% from control) |
|-------------|--------------|--------------------------------|
| Control     | -            | 100.0±4.14                     |
| Paraoxon    | 0.54         | 140.5±7.5                      |
| Sarin       | 0.140        | 141.2±8.3                      |
| Soman       | 0.075        | 152.3±10.1                     |

**Table 3.: Influence of pretreatment by OPs antagonists and antioxidants on rate of lipid peroxidation in rat's cerebral hemisphere.**

| Experiments   | Dose (mg/kg)                 | MDA (nM/1g tissue) |
|---|------------------------------|--------------------|
| Control   | -                            | 7.02±0.16          |
| Sarin   | 0.140                        | 9.59±0.20          |
| Sarin<br>Atropine+<br>galanthamine                  | 0.140<br>20.0<br>8.0         | 6.76±0.20          |
| Soman   | 0.110                        | 12.16±0.61         |
| Soman<br>Atropine+<br>galanthamine                  | 0.110<br>20.0<br>8.0         | 12.77±0.40         |
| Soman<br>Atropine+<br>galanthamine+<br>α-tocopherol | 0.110<br>20.0<br>8.0<br>10.0 | 7.32±0.71          |
| Soman<br>Atropine+<br>galanthamine+<br>Oxymetacyl   | 0.110<br>20.0<br>8.0<br>8.0  | 6.50±0.61          |

**Table 4.: The rate of reaction of active avoidance of conditioned reflex in rats after 5 days of poisoning by soman (1.75 LD50).**

| <b>Experiments</b>                                  | <b>Dose of drugs (mg/kg)</b> | <b>Quantity of stimulus combinations</b> |
|---|------------------------------|--|
| Control   | -                            | 8.0±1.0                                  |
| Soman<br>Atropine+<br>galanthamine                  | 0.140<br>20.0<br>8.0         | 21.0±3.0                                 |
| Soman<br>α-tocopherol+<br>atropine+<br>galanthamine | 0.140<br>15.0<br>20.0<br>8.0 | 5.1±1.0                                  |
| Soman<br>Oxymetacyl+<br>atropine+<br>galanthamine   | 0.140<br>10.0<br>20.0<br>8.0 | 7.5±2.0                                  |