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# Perturbation Effect of Organophosphate Insecticides on Human Erythrocyte

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The interaction of insecticides with model and natural membranes has been extensively investigated (Omann and Lakowicz 1982, Hijazi,1982). However the recognition and confirmation of human health effects has been difficult.

As most insecticides are highly lipophilic, they are incorporated in the lipidic phase of the membrane, affecting the activity of many membrane-linked functions (Lorusso and Miller 1981, Cherfurka 1983), changing the fluidity of the lipid matrix and the membrane charge (Goormaghtiagh,1981).

We report here the membrane perturbational effect observed in people affected by severe and mild organophosphorous insecticide intoxication. Our results show that these compounds produce the stabilization of the erythrocytes against hypotonic challenge. Minor changes in phospholipids distribution were observed in severe poisoning.

## MATERIALS AND METHODS

The classification of severity of poisoning was done according to the degree of acetylcholinesterase inhibition and clinical manisfestation.We considered under the name of mild intoxication patients with serum or blood cholinesterase inhibition between 40-60% of normal value. In some cases no clinical manifestation was evidenced, but some of them reported headaches, dizziness, abdominal cramps and excesive sweating. We included under the name of severe intoxication patients with more than 60% serum or blood cholinesterase inhibition. The patients had miosis, muscular fasciculations, bronchopharyngeal secretion and in some cases respiratory difficulty and unconsciousness. The preparation of erythrocytes was accomplished with heparinized blood obtained from donors under treatment for organophosphorous insecticide intoxication. Acetylcholinesterase was determined with Voss and Sachsse (1970) technique. Hemolysis was performed according to Dacie et al (1963). Erythrocyte membrane was prepared according to Dodge et al (1963) and quickly frozen and stored at -20ºC. Ghosts obtained from 3 ml of

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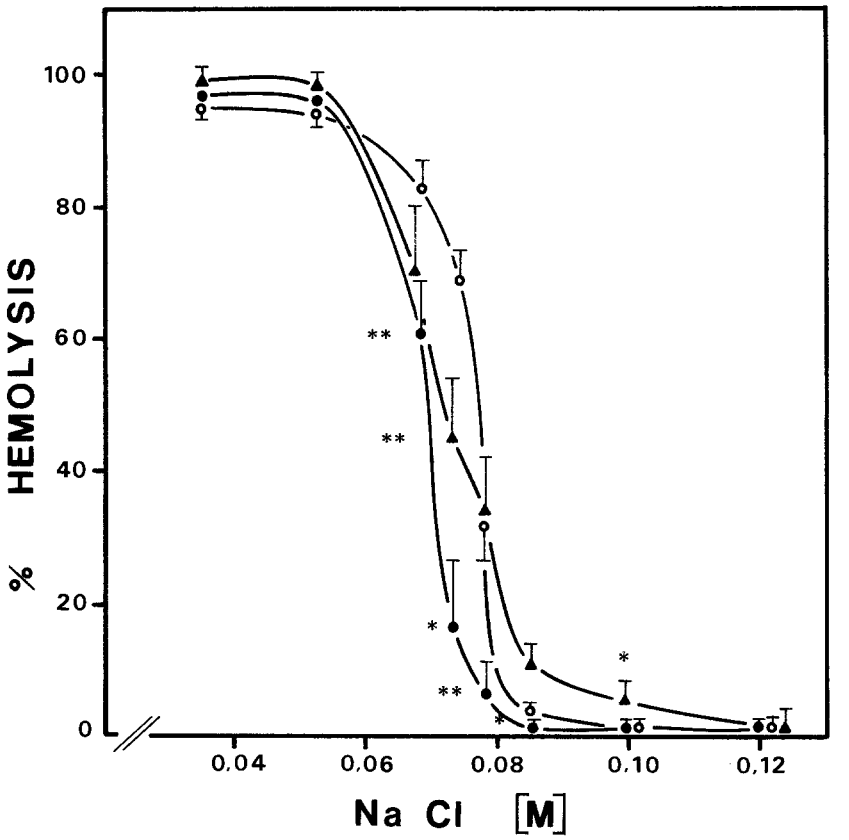


Figure 1. Effect of organophosphorous intoxication on hemolysis curves.

Erythrocytes were incubated for 30 min at 20-22ºC and then centrifugued at 2000 rpm for 5 min. The absorbances of the supernatant were read at 550 nm. A value of 100% hemolysis was assigned to erythrocytes incubated with destilled water. Each point is the mean of 7 samples. Individual values agree within 5% **$p \leq 0.01$, * $p \leq 0.05$ with respect to corresponding control group. O control, ▲ severe intoxication, ● mild intoxication.

Blood was extracted with chloroform methanol 2:1 (Folch et al 1957). Individual phospholipids were separated by bidimensional thin layer chromatography and lipid-phosphorous quantified on each spot (Rouser et al 1970).

## RESULTS AND DISCUSSION

The stabilization of erythrocytes against hypotonic hemolysis in people with mild and severe organophosphorous intoxication is shown in Figure 1. A protecting effect against hypotonic challenge is evidenced at NaCl concentration ranging from 0.055M to 0.085M. However the magnitude of the effect is only significant in mild intoxication.

Table 1. Phospholipid distribution of ghost in control and intoxicated patients by organophosphorous insecticides

| Phospholipid | PC | PE | Sph | PS |
|---|---|---|---|---|
| Control | $27.09 \pm 0.51$ (N=7) | $26.00 \pm 0.56$ (N=7) | $24.51 \pm 0.55$ (N=7) | $14.44 \pm 0.62$ (N=7) |
| Mild intoxication | $27.88 \pm 0.48$ (N=7) [a] | $25.71 \pm 0.52$ (N=7) [a] | $24.16 \pm 0.32$ (N=7) [a] | $13.89 \pm 0.52$ (N=7) [a] |
| Severe intoxication | $30.22 \pm 0.91$ (N=6) [b,c] | $26.04 \pm 0.65$ (N=6) [a] | $23.12 \pm 0.41$ (N=6) [a] | $14.02 \pm 0.68$ (N=6) [a] |

Phospholipids are expressed as percent of total phospholipid. Data represent mean $\pm$ standard deviation.
N: Number of samples. PC: phosphatidylcholine, PE:phosphatidylethanolamine. Sph: sphingomyelin, PS: phosphatidylserine
a: non significant
b: $P \leq 0.01$ with respect to control
c: $P \leq 0.05$ with respect to mild intoxication

Acetylycholinesterase inhibition above 60% facilitated the hemolysis at NaCl concentrations from 0.085M to 0.10M. This observation provides evidence for difference in the structural consequences of mild and severe intoxication.

The antihemolytic effect is not associated with changes in phospholipid distribution (Table 1), but a significant increase in phosphatidylcholine is evidenced in patients with more than 60% inhibition of acetylcholinesterase.

The antihemolytic behaviour of organophosphorous insectide has previously been tested in vitro with pig erythrocytes (Antunes Madeira et al 1981). However the authors could not find any correlation between the anti hemolytic action and the toxicity of the compound. A higher hypotonic challenge is needed to obtain the same rate of hemolysis in human than in pig erythrocytes.

Many surface active compounds have a biphasic action on erythrocyte stability (Godin et al 1979, Seeman,1972) according to their concentrations. Higher concentrations produce gross membrane damage with leakage of cellular constituents, while lower concentrations are membrane stabilizers. The effect could be due to an alteration of the density of fixed charges on the surface of the membrane with fluidization and expansion. These actions make the pore of the membrane impermeable to electrolytes and hemoglobin, but when the drug accumulation increases the membrane begins to be destroyed with the existence of hemolysis.

Following Yasuhara's et al (1985) correlation between the production of hemolysis and enzyme leakage, subclinical hepatic damage associated with changes in membrane structure is suggested, although no abnormalities in liver function due to organophosphorous intoxication has been previously reported (Namba et al, 1971).

REFERENCES

Antunes Madeira MC, Carvalho AP, Madeira VMC (1981) Interactions of insecticides with erythrocyte membranes. Pest Biochem Physiol 15: 79-89

Cherfurka W (1983) The effect of DDT and related insecticides on the mitochondrial ATPase of houseflies. Comp Biochem Physiol 74C: 259-266 (1983)

Dacie JV, Lewis SM (1963) Practical hematology 3rd Ed.J. A.Churchill. Ltd. London

Dodge JT, Mitchell C, Hanahan DJ (1963) The preparation and chemical characteristics of hemoglobin free-ghost of human erythrocytes. Arch Biochem Biophys 100: 100-119

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues J Biol Chem 226:497-509

Godin DV, Au T, Garnett M (1979) Membrane perturbation effects of antiarrythmic drugs. J Molec Cell Cardiol 11:261-274

Goormaghtiagh EL, Caspers J, Ruysschaert JM (1981) Lipid-drug interaction in model membranes Colloid Interface Sci 80:163-170

Hijazi A AH (1982) Use of the fluorescent probe 1-anilino -1 naphthalene sulfonate, to monitor the interaction of pesticide chemicals with mitochondrial membranes. Comp Biochem Physiol 73C: 369-375

Lorusso DJ, Miller TL (1981) Hexachlorophene induced changes in erythrocyte membrane ATPase activity. Res Comm Chem Pathol Pharmacol 31: 205-216

Namba T, Holte C, Jackrel J, Grob D (1971) Poisoning due to organophosphate insecticides Am J Med 50: 475-491

Omann, GM, Lakowicz J (1982) Interaction of chlorinated hydrocarbon insecticides with membranes. Biochem Biophys Acta 684: 83-95

Rouser G, Fleischer S, Yamamoto A (1970) Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorous analysis of spots. Lipids 5:494-496

Seeman P (1972) The membrane actions of anesthetics and tranquilizer. Pharmacol Rev 24: 584-655

Voss G, Sachsse K (1970) Red cell plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a modified acetylthiocholine/DTNB procedure. Toxicol Appl Pharmacol 16 764-772

Yashuara H, Tnooka M, Kamei D,Sakamoto K (1985) Membrane effects of various drugs on isolated rat hepatocytes and erythrocytes. Toxicol Appl Pharmacol 79:453-460

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