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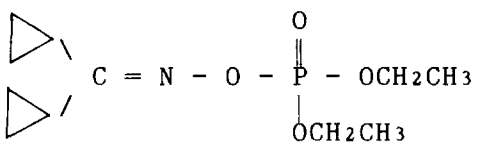
Anti-Cholinesterase Action of a New Organophosphorus Ester, Diethylphosphoric Ester of the Dicyclopropyloketoxime, on Wistar Rats

K. Machera[1] and E. Kostakis[2]

[1]Laboratory of Pesticide Toxicology, Benaki Phytopathological Institute, 8 Stephanou Delta Street, GR-145 61 Kiffissia, Athens, Greece and [2]Department of Pharmaceutical Chemistry, University of Athens, 104 Solonos Street, GR-106 80 Athens, Greece

Organophosphorus (OP) compounds have been widely used as important insecticides in hygiene and plant protection, although the number of OP insecticides that can be used shortly before harvest is very limited. The OP molecule diethylphosphoric ester of the dicyclopropyloketoxime (I) combines a good insecticidal action and



(I)

low persistence. Studies on the stability and degradation have shown that it is very rapidly hydrolyzed through the formation of oxime, which is gradually rearranged to amide (Michailides 1986). Three days after foliar application no residues were detected on treated crops (Michailides 1986). Toxicity studies showed that it is highly toxic to mammals with acute oral LD_{50} : 10.86 mg/kg for male and 7.85 mg/kg for female rats (Machera et al. 1991). The toxicity of OP compounds to mammals and insects is mainly attributed to cholinesterase (ChE) inhibition in central and peripheral nervous system. The level of erythrocyte ChE is an excellent indicator of acetycholinesterase activity on nerve synapses (Vandekar 1980). Besides the anticholinesterase activity of the OP compounds, there are other important factors concerning their toxicity, such as stability in the organism, recovery rate of inhibited enzyme and degree of cummulative inhibition of ChE in cases of repeated intoxication.

The objective of the presented investigation was the determination of a) the degree of inhibition of blood

Send reprint requests to K.Machera at the above address

and brain ChE in cases of single oral administration of lethal doses, b) the time of maximum ChE inhibition and the time of recovery in erythrocytes and plasma after a single administration, c) the rate of cummulative inhibition of erythrocyte and plasma ChE after consecutive oral administrations of the test compound.

MATERIALS AND METHODS

Adult Wistar rats were selected from the colony of our laboratory having the same age and weight (±10% for each sex). The hematocrit values were 49±1 for male and 47±1 for female rats.

The test compound, diethylphosphoric ester of the dicyclopropyloketoxime was synthesized at the Department of Pharmaceutical Chemistry, University of Athens (Rouman 1984). Corn oil aliquots of the test compound were freshly prepared before each administration. Acetylthiocholine iodide, dithiobisnitrobenzoic acid and bovine erythrocyte cholinesterase were purchased from Sigma. Measurements on ChE activity were made using a photometric method (Ellman et al. 1961) as modified by Voss et al. (1970), with minor adaptations. According to the method activity differences higher than 10% were considered as significant.

Whole blood and brain ChE inhibition was measured after the single oral administration of lethal doses of 20 and 16 mg/kg body weight (b.w.) to male and female rats, respectively. Blood samples were taken from the tails of the animals immediately after the development of severe toxic symptoms, usually observed 15 min after the administration of lethal doses. ChE measurements were performed on whole blood. Brain ChE measurements were performed immediately after the death of the animals.

Erythrocyte and plasma ChE inhibition was measured after the administration of test compound to 10 male rats, single oral administration at dose 0.5 acute LD_{50}'s, 5.0 mg/kg b.w. Four animals were used as control to check normal values. After the administration, blood samples were taken every 15 min, until ChE inhibition reached a maximum (tmax) and began to recover. Thereafter daily samples were taken until the erythrocyte and plasma ChE were restored to the normal values.

Erythrocyte and plasma cummulative ChE inhibition was measured after the daily administration of the test compound to 10 female rats, at a dose of 2.5 mg/kg b.w. which was the maximum tolerated dose in the case of chronic administration of the compound to female rats

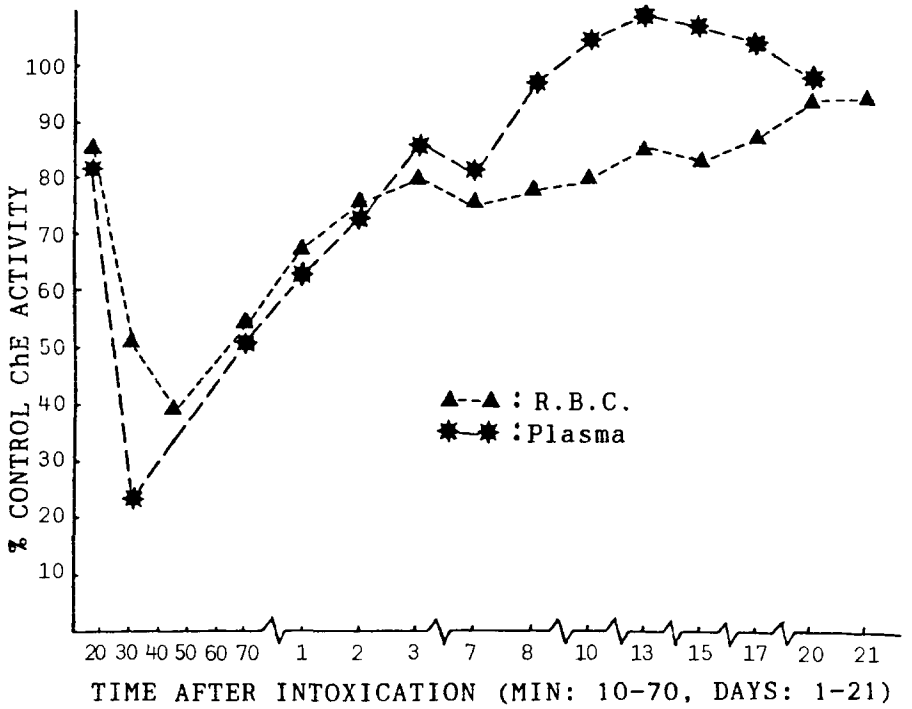


Figure 1. Inhibition and recovery of erythrocyte and plasma ChE in male rats after single oral administration of diethylphosphoric ester of the dicyclopropylketoxime at the dose of 5 mg/kg b.w.

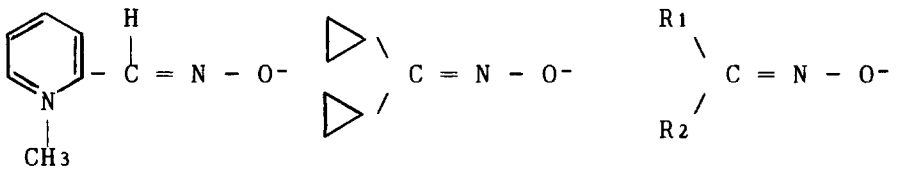
(Machera 1990). After each daily administration of the test compound to the animals, blood samples were taken at a time equal to tmax determined in the previous test. The administration of the OP ester and the respective measurements lasted until the degree of inhibition reached an equilibrium.

RESULTS AND DISCUSSION

The whole blood ChE inhibition after oral administration of lethal doses was over 90%, at the time of observation of toxic symptoms, approximately 15 min after intoxication. The animals died 15 to 20 min after administration, in terminal convulsions. No significant brain ChE inhibition was detected on these animals thus the death was not related to brain ChE inhibition. Similarly lack of brain ChE inhibition has also been reported by other investigators in cases of single oral administration of lethal doses (Frawley et al. 1952). Death appears to be directly related to peripheral paralysis in respiratory muscles, leading to anoxic

changes in the blood resulting in central stimulation, convultions and death.

When a single dose of 5.0 mg/kg b.w. was administered to male animals, significant erythrocyte ChE inhibition was observed 15 min after the intoxication and in 45 min a maximum inhibition of 61% was observed (Fig. 1). During the first 24 hrs, about 50% of the inhibited enzyme was recovered and the respective value was approximately 33%. In the following days recovery was more gradual and reached the normal values on the 21st day after the intoxication. It has been reported by other investigators that erythrocyte ChE activity recovers to normal about 4 weeks after intoxication, coinciding with the production of new rat erythrocytes (Smith et al. 1959). In the case under study, recovery rate was higher than the erythrocyte production rate. Apparently another mechanism exists which accelerates reactivation rate. This observation could be attributed to formation of an oxime anion during the OP hydrolysis and phosphorylation reaction (Michailides 1986), which may support the reactivation of the inhibited enzyme in a way similar to the oxime reactivators. As it can be shown from the structural formulae of pralidoxime (I), the most commonly used ChE reactivator and the dicyclopropyloketoxime (II), which is formed during the OP ester hydrolysis or phosphorylation reaction, are very similar molecules and can be represented by the same structural formula (III)



(I) (II) (III)

Thus the formation of oxime anion even in relatively low concentration compared to pralidoxime dose, administered in cases of intoxication, would allow us to expect a significant reactivating action on the inhibited ChE of the survived animals. Similar action on the reactivation rate has been reported by other investigators, for the case of formation of other nucleophilic ions except water (Health 1961, O'Brien 1960). This effect is more enhanced during the first hours of inhibition, when no aging and transformation of the inhibited enzyme to a stable form has occured. Another parameter that recovery rate depends on is the continued presence of the inhibitor having as a result the inhibition of reactivated or newly synthesized enzyme (Vandekar 1980). In the studied case the OP compound is hydrolysed very fast ($t^1/_2$ = 0.75 hr, EtOH,

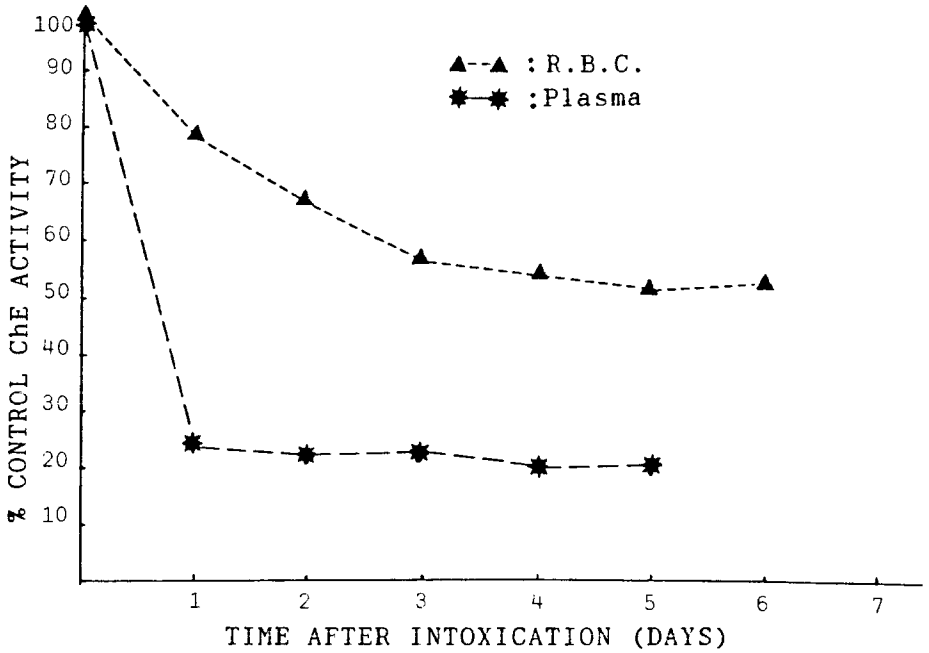


Figure 2. Cummulative inhibition of erythrocyte and plasma ChE in female rats after daily administration of diethylphosphoric ester of the dicyclopropyloketoxime at the dose of 2.5 mg/kg b.w.

T = 70°C) in the presence of polar solvents through the formation of oxime. Thus no prolonged presence in the animal organism is expected due to metabolism to more polar molecules, followed by excretion.

Significant plasma ChE inhibition was observed 15 min after intoxication of the experimental animals. The maximum inhibition of 77% was observed after 35 min (Fig. 1). During the first 24 hrs about 50% of the inhibited enzyme had been recovered and the respective value was 34%. On the third day plasma ChE was completely recovered. On the 10th to 13th day after intoxication, we observed an increase in plasma ChE activity above the normal values which gradually returned to normal. Plasma ChE inhibition and recovery is a faster process than erythrocyte ChE. Similar observations on plasma ChE, concerning inhbition, recovery rate and final higher activity in the intoxicated animals, have been reported by other investigators, after the intoxication with several OP compounds (Heath 1961, Frawley et al. 1952).

When the test compound was daily administered to female rats at a dose of 1/3 acute LD_{50}'s value, we observed a high plasma ChE inhibition, 76% and relative low erythrocyte ChE inhibition 22%, after the first administration (Fig. 2). However no toxic symptoms were developed. As it has been reported, plasma ChE inhibition is not related to toxic symptoms (Sawyer et al. 1946). After the second and third day of administration, a significant increase in erythrocyte ChE inhibition was observed. The rate of inhibition became constant after the 5th administration. On plasma ChE we did not observe significant increase of inhibition after the second administration (Fig. 2). This finding is in agreement with the previous study concerning the higher rate of inhibition and reactivation of plasma ChE compared to erythrocyte ChE. This study shows that we have a relatively limited cummulative ChE inhibition, that reaches an equilibrium during the 5th day of administration, at a dose equal to 1/3 of acute LD_{50} value. Higher cummulative inhibition has been reported in the case of diethyl phosphorylated esters. The relative low cummulative ChE inhibition of the studied OP compound is probably associated with the oxime anion formation during hydrolysis and phosphorylation reactions which facilitates the reactivation of the inhibited enzyme. Additionally the very fast decomposition of the free OP molecule in the organism of the experimental animals minimizes the cummulative ChE inhibition.

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