Efecto del Antídoto sobre la toxicidad en ratas del diclorvos

Effect of antidote on dichlorvos toxicity in rats

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Resumen:

Los pesticidas organofosforados inhiben la actividad de la acetilcolinesterasa en mamíferos aún bajo exposición por corto tiempo. Las ratas fueron tratadas intraperitonealmente con diclorvos por 24 y 48 horas, mostrando un 62,2% de inhibición de la actividad de la acetilcolinesterasa a las 24 h del tratamiento con diclorvos. La inhibición de la enzima aumentó a 69,7% a las 48 h de la intoxicación con diclorvos. A otro grupo de ratas se le administró fisostigmina y diclorvos combinados por 24 y 48 h, respectivamente. La actividad de la acetilcolinesterasa aumentó significativamente en ambos grupos. El efecto protector de la fisostigmina contra la toxicidad del diclorvos fue de 1,6 veces después de 24 h y de 1,4 veces a las 48 h. Las ratas tratadas con fisostigmina antes de la administración de diclorvos mostraron un aumento de la actividad de la acetilcolinesterasa de 21,55% y de 13,75 a las 24 h y 48 h, respectivamente. La actividad de la acetilcolinesterasa a las 24 h del tratamiento combinado con diclorvos y fisostigmina parecer ser mayor que a las 48 h.

Palabras claves: diclorvos, antídoto, acetilcolinesterasa

Summary :

Organophosphate pesticides inhibit acetylcholinesterase activity in mammals even in short term exposure. Rats were injected with dichlorvos intraperitoneally for 24 and 48 hours. They showed 65,2% inhibition of acetylcholinesterase activity after 24 hours dichlorvos treatment. Inhibition of enzyme was raised to 69,7%, after 48 hours dichlorvos intoxication. In other two groups of rats physostigmine and dichlorvos were administered in combination for 24 and 48 hours respectively. Acetylcholinesterase activity was increased significantly in both groups. Protective effect of physostigmine from dichlorvos toxicity was 1.6 fold after 24 hours and 1.4 fold after 48 hours. Rats treated with physostigmine prior to dichlorvos administration showed increase of 21,55% in acetylcholinesterase activity after 24 hours and increase of 13,75 % after 48 hours. Remarkably, AChE activity after 24 hours treatment of dichlorvos and physostigmine in combination appeared to be greater than 48 hours after treatment of dichlorvos and physostigmine.

Keywords: dichlorvos, antidote, AChE.

Introduction

Organophosphate (OP) insecticides have the potential to cause behavioral changes and intensive external symptoms of intoxication. These symptoms include nervous paralysis, convulsions, dyspnoea and death. It is now unanimously accepted that, acute neurotoxicity occur due to inhibition of enzyme acetylcholinesterase (AChE) followed by the accumulation of neurotransmitter acetylcholine in nerve terminals. There are several antidotes for cholinesterase inhibitors. Some of the antidote compounds protect *in vivo* and *in vitro* disfunctioning of neuromuscular system remarkably.

Imidazole-pyridinium mono oximes, long chain pyridinium mono oximes, pyridostigmine and phosphorotriesterase are known antidotes against the anticholinesterase action of some known inhibitors. Physostigmine has also been reported to be a protectant for sarin-induced acetylcholinesterase inhibition (Tuovinen *et al* 1999). Therefore, in the present study, an attempt has been made to evaluate the protective effect of physostigmine against the inhibitory action of another OP pesticide dichlorvos on AChE in brain of rat.

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Material and methods

Male albino rats, *Rattus norvegicus* weighing 90-100g procured from Lupin Laboratory, Bhopal were used as experimental animals and kept in plastic cages (12"x9"x9"). Animals were acclimatized to laboratory conditions for about 10 days. Rats were fed with normal pellet diet and supplementary food mixture of rice bran and water *ad libitum*.

OP pesticide (dichlorvos 0,0 dimethyl 0-2,2- dichlorovinyl phosphate, DDVP manufactured by Hindustan Ciba Giegy limited) and physostigmine (Himedia laboratories) were purchased. Rats were divided into five groups of five animals in each. Each dose was injected intraperitoneally with the help of a hypodermic syringe. First group received 7.0 mg/kg b.w. of dichlorvos for 24 hours. Second group received 7.0 mg/kg. b.w. dichlorvos for two successive days and rats were sacrificed after 48 hours. Third group was treated with 0.10 mg/kg physostigmine 30 minutes prior to dichlorvos administration (7.0 mg/kg b.w.) and animals were sacrificed after 24 hours. Fourth group received physostigmine (0.10 mg/kg.b.w.) 30 minutes prior to first dose of dichlorvos and a second dose was given after 24 hours from the first dose. Rats were sacrificed after 48 hours of first dose by cervical dislocation. Fifth group received normal saline solution intraperitoneally (control). None of the animals died during exposure.

Brain tissues were removed and placed in prechilled 0.24M sucrose solution. Each tissue was weighed and a 5% homogenate was prepared. AChE specific activity in brain was assayed according to the technique of Metcalf (1951) using acetylcholine iodide as substrate. Experiments for assaying enzyme activity were conducted at 37°C and increase in absorbance was recorded at 540 nm. Protein contents were estimated by adopting the method of Lowry *et al* (1951). All values are shown as mean ± standard error (N=5). The level of significance was calculated by using students 't' test at P<0.01 and P<0.001.

Results

Dichlorvos intoxicated rats showed acute toxicity symptoms after 24 and 48 hours treatment. Behavioral changes observed due to dichlorvos treatment were salivation, tremors, convulsions and weakness of leg muscles. This condition prevailed till the 48 hours of dose administration. A decrease in intensity of toxicity symptoms was noticed in physostigmine pretreated animals.

AChE specific activity was observed to be 6.96 ± 0.558 μm substrate hydrolyzed/mg protein/hour in control rat brain. It became 2.42 ± 0.143 μm and 2.18 ± 0.173 μm due to 24 and 48 hours toxicity of dichlorvos, respectively (Figure-1). After 24 hours of acute dichlorvos treatment a significant inhibition (65.2%) of AChE specific activity was observed (Figure-2). Forty eight hours exposure of dichlorvos caused an inhibition of 69.7% in AChE activity. Results showed that AChE enzyme inhibition in both groups was significant (P<0.001). Furthermore, after 24 hours dichlorvos treatment in physostigmine pretreated animals showed AChE activity as 3.92 ± 0.432 μm (P<0.01). After 48 hours dichlorvos and physostigmine, AchE activity became 3.10 ± 0.376 μm(P<0.01).

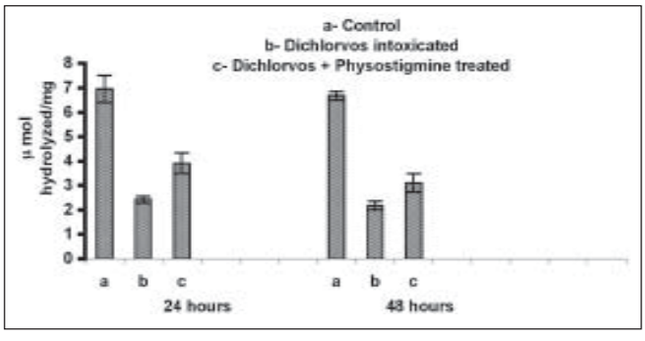

Figure 1

Comparative AChE specific activity in brain of *Rattus norvegicus* subjected to dichlorvos toxicity, dichlorvos and physostigmine treatment for 24 and 48 hours. Specific activity is expressed as μm substrate hydrolyzed/mg protein/hour. All values were indicated as mean ± standard error (N=5).

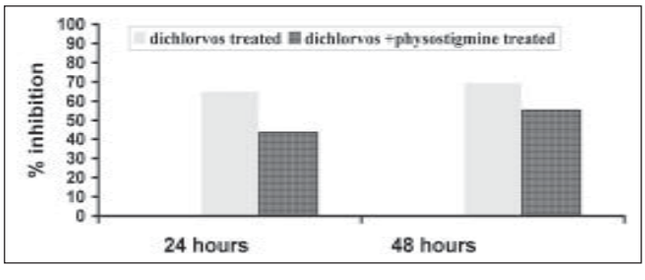

Figure 2

Comparative percent inhibition of AChE specific activity in brain of *Rattus norvegicus* subjected to dichlorvos toxicity, with and without physostigmine treatment for 24 and 48 hours.

Discussion

AChE specific activity is significantly inhibited in rats after the dichlorvos intoxication. Concomitantly, behavioral changes are indicated as a result of the disturbance of cholinergic system (Parveen & Kumar 2001). It is now well established that AChE is inhibited in rat's brain to the exposure to numerous organophosphates (Broberger *et al* 2000; Carr *et al* 2001). In the present studydichlorvos inhibited AChE specific activity in a treatment duration dependent manner. Repeated dosing of dichlorvos caused more inhibition as compared to that of single dose.

In rats treated with physostigmine before dichlorvos administration, AChE inhibition was decreased, showing

1.6 and 1.4 fold protective effect of physostigmine, similar to results of Tuovinen *et al* (1999).

This compound is useful in protective therapy of cholinergic dysfunction as it's affinity is associated with AChE esteric site. Physostigmine and pyridostigmine have been previously reported to be capable of increasing tolerance level in OP intoxicated mammals. (Tuovinen *et al* 1999 and Dube *et al* 2000). These results did not include a chronic follow-up, but this outcome does not exclude the enzyme effects from longer exposures. Inhibitory results of the dichlorvos and protective results of physostigmine in the present study reflect early response of the brain to these compounds. Inhibition observed in the *in vivo* exposures varied from approximately 40-70% inhibition of acetylcholinesterase specific activity.

This inhibition of AChE was observed at both exposures, and did exhibit a strong exposure-response relationship. Kinetics evaluations of AChE inhibition in rat brain induced by OP showed temperature dependence. (Dave *et al* 2000). AChE enzyme shows a combined molecular dynamics and its diffusion occurs through neuromuscular junctions making it widely distributed and circulated enzyme in the body (Kua *et al* 2002, Tai *et al* 2003). AChE specific activities were examined in this study as a possible mechanistic rationale for reduced AChE–dichlorvos interaction in presence of physostigmine. Additional isoforms of AChE may exist, but they may also be sensitive to inhibition by dichlorvos. This possibility could be further explored by the use of other esterase inhibitors to examine the inhibition of the remaining activity.

Results suggests the importance of studying the diversity of isoenzyme abundance and physical characteristics of rat AChE. Antidotes of AChE inhibitors have medical application in the treatment of disorders such as organophosphate induced toxicity. Physostigmine seems to be a ptotective agent against Ops intoxication and suggested to be a beneficial antidote against dichlorvos intoxication. Utility of understanding the biochemical mode of action for OPs in presence of physostigmine is that it may lead to the development of a new tool to account for the presence of OP induced toxicity, thereby allowing for the elucidation of toxicity caused by these pesticides. We may have a clear explanation to these findings in further studies by understanding the kinetic mechanisms after evaluation of AChE kinetics.

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