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SHORT COMMUNICATION

Protective Role of Verapamil against Organophosphorus Ester-Induced Delayed Neurotoxicity

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ABSTRACT

The protective efficacy of a well-known calcium channel blocker, Verapamil, against Mipafox, a prototype of nerve gas, has been investigated. Mipafox is a potent organophosphorus ester which has both acute and delayed neurotoxic effects. The results of the present study indicate that as an adjunct to Atropine, Verapamil shows protective action in overcoming the delayed neurotoxic effects. However, it makes either little or no contribution to the recovery of the acute toxic effects of Mipafox.

1. INTRODUCTION

Organophosphorus esters, which include highly toxic nerve agents, viz., Soman (pinacolyl methyl phosphono fluoridate), Sarin (methyl, *O*-isopropyl phosphono fluoridate), Tabun (ethyl *N,N*-dimethyl phosphoramido cyanidate) and Vx [*O*-ethyl S-(2 *N,N*-di isopropyl amino) ethyl methyl phosphono thioate], exert their toxic effects by inhibiting enzyme acetylcholin esterase (AchE) (EC 3.1.1.7)[1,2]. Some of these compounds inhibit irreversibly another enzyme—neurotoxic esterase (NTE), an isoenzyme of carboxyl esterase (EC 3.1.1.1). Symptoms of NTE inhibition appear after 8-14 days of latent period in some of the species, including man. The delayed effect is called organophosphorus ester-induced delayed neurotoxicity (OPIDN)[3].

The treatment commonly adopted for acute effects of organophosphorus ester poisoning is intraparitoneal injection of Atropine for overcoming the muscarinic effects, along with an oxime, such as 2-PAM chloride or obidoxime or toxigonin by intravenous route, with glucose or saline by intramuscular route to counter the nicotinic effects[2]. However, no therapeutic or prophylactic regime is known to overcome the delayed neurotoxic effect of organophosphorus esters.

Calcium plays an important role in the physiology of the nervous system. Regulation of cytosolic calcium in synaptosomes occurs through the high affinity calcium pump. Neurons have different types of calcium channels, which are important in controlling different aspects of nerve activity. In a neuron, different mechanisms may operate simultaneously in different portions of the cell to regulate separate functions. The multiple type of voltage sensitive and receptor-operated calcium channels offer the cell some flexibility in the way it can modulate the entry of calcium ions4. However, increase in intracellular mobilisation of calcium precipitates detrimental events, as calcium is implicated in neurotransmitter release, degradation of neurocytoskeletal muscles, myelin integrity, excitation-contraction coupling, etc. Turnover and degradation of neurofilament has been shown to depend on the activity of calcium-activated or calcium-dependent

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proteases[5]. Inhibition of acetylcholine release by calcium channel blockers in guinea pig colon has been reported[6]. Realising that some of the neurological problems are due to an increase in cytosolic calcium, the present investigation was undertaken to assess the prophylactic efficacy of Verapamil (calcium channel blocker), if any, against acute and delayed neurotoxic effects of Mapifox (*N,N'*diisopropyl phosphorodiamidic fluoride). For this, the inhibition/reactivation of brain acetylcholin esterase (AchE) and NTE in male albino mice, has been investigated.

2. MATERIALS & METHODS

Male albino mice of Wistar strain weighing 30 ± 5g were used. They had free access to Gold Mohar Laboratory, Animal Feed, besides drinking water. The animals were divided into three groups. These groups were subjected to one of the following treatments:

Group I (control) animals received DMSO, the chemical used for dissolving Mipafox.

Group II animals were administered intraparitoneally 2 LD_{50} Mipafox 10 min after 20 mg/kg intramuscular atropine sulphate. LD_{50} of Mipafox in male mice was found[7] to be 14.8 mg/kg.

Group III animals received sign-free dose of Verapamil 10 mg/kg solution prepared in distilled water. It was administered to mice intraparitoneally 10 min after Atropine, but prior to Mipafox, the same way as Group II animals.

Mipafox (99% pure) was synthesised. Its purity was ascertained by gas-liquid chromatography, infrared, ultraviolet and nuclear magnetic resonance. Verapamil, the calcium channel blocker of phenyl alkyl-amine series, was procured from Sigma, USA. All other chemicals used in the study were of analytical grade. The animals were observed at different periods for symptoms of acute and delayed toxicity. The mice, which could withstand 2 LD_{50} dose of Mipafox and survived, were sacrificed at different intervals, i.e. at 24 hr, 48 hr, 96 hr, 1 week, 2 weeks and 3 weeks. At each time interval, six treated animals were compared with an equal number of control animals. Inhibition and reactivation of enzyme acetylcholin esterase[8] and neurotoxic esterase[9] was studied in 10 per cent crude brain homogenate prepared in 0.25 M sucrose. Protein content was estimated using folin phenol reagent[10]. Data analysis was done by significance 't' test[11].

3. RESULTS & DISCUSSION

Inhibition and reactivation patterns of mice brain AchE at different time intervals after Mipafox treatment, with and without Verapamil, are presented in Fig. 1. In the Verapamil-treated animals, AchE reactivation is more compared to nonVerapamil-treated animals, though this is not statistically significant.

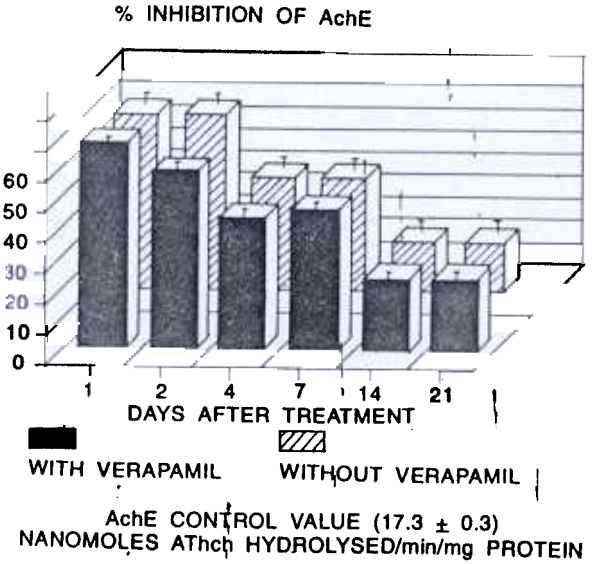

Figure 1. Effect of 2LD_{50} mipafox on brain AchE of mice

The recovery in enzyme activity may be spontaneous or due to dephosphorylation of the phosphorylated enzyme. There may be some contribution from fresh *de novo* synthesised AchE also. Therefore, it cannot be postulated whether Verapamil has any role in overcoming the acute toxic effects of Mipafox. However, increase in protective index in organophosphorus toxicity with Nifedipine, another calcium channel blocker, had been reported when it was used as an adjunct to Atropine and Obidoxime[12].

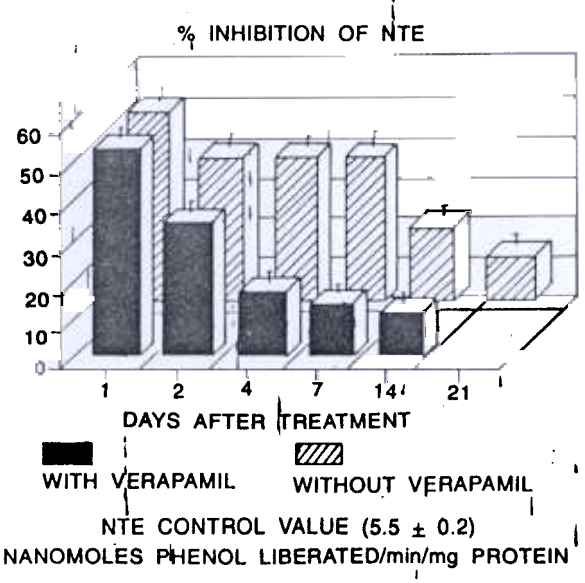

Figure 2. Effect of 2LD$_{50}$ mipafox on brain NTE of mice

The neurotoxic esterases inhibition and reactivation, at 24 hr level of NTE inhibition with and without Verapamil is almost identical. But, at subsequent intervals, from 48 hr onward up to 21 days, the recovery in enzyme activity of Verapamil-treated animals is significantly faster, with complete recovery on 21st day (Fig. 2). This indicates that Verapamil has some contribution in the recovery of NTE activity. One of the causes for the delayed neurotoxicity due to Mipafox may be the increased intracellular calcium level. Intracellular calcium has been reported to have some role in the development of OPIDN[13,14] We consider that the Verapamil, at the dose level used, has served to prevent calcium entry into cytosol at least to a certain extent so as not to allow progression of symptoms of OPIDN. Besides this, dephosphorylation or *de novo* synthesis of the new enzyme at later stages might also have contributed to recovery of the enzyme level, though this aspect needs further investigation.

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