EFFICACY OF BIPERIDEN AND HI-6 AS A PROPHYLACTIC COMBINATION AGAINST CONVULSIONS PRODUCED BY A HIGHLY TOXIC ORGANOPHOSPHORUS COMPOUND

Ivan SAMNALIEV
Military Medical Academy, Sofia, Bulgaria

Introduction

Inhibition of enzyme acetylcholinesterase (AChE, EC 3.1.1.7.) at central nervous system by highly toxic organophosphorus compounds (OPC) causes a cholinergic crisis which is the most probable "trigger" mechanism for the convulsive activity observed after intoxication. It is known that the convulsions produced by OPC may cause brain damages and long-term effects with neurobehavioral, cognitive and neuromuscular disturbances (McLeod, 1985; USAMRICD report, 1998). Therefore the abolishment of convulsions plays a very important role in the prophylactic of organophosphorus poisoning. Significant attention has been paid to this problem during last years but it is yet not resolved.

There are different pharmacological approaches to prevent or abolish the convulsive activity. The pretreatment with a potent central acting cholinolytic combined with a cholinesterase reactivator may ameliorate to a great extent the symptoms of OP intoxication and to prevent the delayed neuropathy as well. In previous investigations a relatively high anticonvulsive activity of biperiden have been demonstrated (Anderson et al., Capacio, Shih, 1991).

The **purpose** of current study was to assess the prophylactic efficacy of a drug combination which consist of biperiden and cholinesterase reactivator HI-6 applied 15, 30, 60 and 120 minutes prior to challenge by a highly toxic OP compound.

Material and Methods

Drugs and dosage: Cholinolytics: Biperiden lactate 5.0 mg/ml (Knoll Pharm. Co., Germany) and atropine sulfate substance (Sopharma, Bulgaria). HI-6 - (2-hydroxyiminomethyl-pyridinium-1-methyl) (4-aminocarbonyl-pyridinium-1-methyl) ether dichloride was synthesized at MMA, Sofia. The drug combinations: Biperiden 1.25 mg/kg + HI-6 72 mg/kg and atropine 2.1 mg/kg + HI-6 72 mg/kg were injected i.m. 15, 30, and 60 min prior to intoxication with 2.0 LD_{50} TMPhF (1,1,2 - trimethylpropyl methylphosphonofluoridate, 92 % purity) and 120 min before exposure to 1.5 LD_{50} TMPhF. Biperiden and atropine were applied in equimolar doses - $3.1x10^{-6}$ mol. The volume of injection was 0.1 ml/100 g body weight.

Animals. 210 male albino rats "Wistar" were separated into following groups:
I. Biperiden + HI-6 - 12 rats per each time interval.
II. Atropine + HI-6 - 12 rats per each time interval and
III. Control group - none anticonvulsant pretreatment and poisoned with TMPhF (12 rats per each time interval). 60 rats were used to determine LD_{50} of TMPhF by Litchfield-Wilcoxon's method.

Evaluation of toxic signs. Signs of intoxication were graded from 0 to 6 as follow: 0 - none of the selected signs present; 1 - hyper-activity; 2 -2 chewing or salivation; 3 - fasciculations or body tremor; 4 - subconvulsive movements; 5 - convulsions and 6 - death. The most severe sign observed was noted at 5, 10, 15, 30 min and 1, 1.5, 2, 2.5, 3, 3.5, 4 and 24 hr after intoxication. The grades for a given animal were summed across the observation time to obtain a severity score. For each experiment (15, 30, 60 and 120 min) the anticonvulsive efficacy of biperiden + HI-6 was compared to those for atropine and HI-6 using the Student's t-test. Statistic significance was judged at a probability level of 0.05.

Results

The results obtained are summarized in Figs. 1, 2, 3 and 4.

Pretreatment regimen containing biperiden and HI-6 applied 15, 30 and 60 min before exposure

provided very good protection against the toxic signs induced by 2.0 LD_{50} TMPhF. At the same time the second drug combination tested - atropine + HI-6 demonstrated higher median scores especially when was administered 15 and 30 min prior to the same dose of TMPhF. Comparison of the anticonvulsive efficacy of both combinations showed significant differences ($p < 0.05$ and $p < 0.001$). The pretreatment benefit of atropine and HI-6 increased progressively and was most markedly at 60 min before TMPhF challenge. The date obtained for biperiden + HI-6 demonstrated quite similar effectiveness at all time intervals studied, except 120 min (Figs. 1, 2, 3). At this time point biperiden + HI-6 pretreatment was less effective against convulsive activity caused by 1.5 LD_{50} TMPhF. Generally both drug combinations were found to be ineffective when were injected two hours before 1.5 LD_{50} TMPhF (Fig. 4).

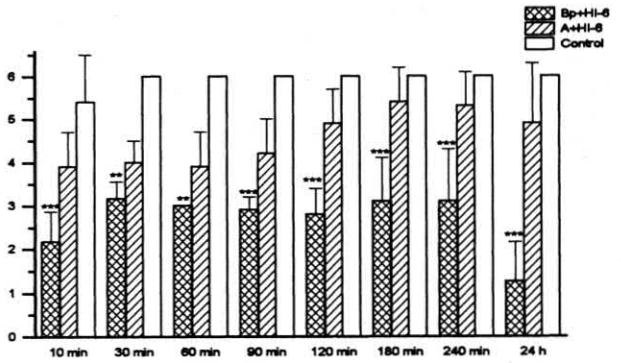

Fig. 1. Anticonvulsive activity of the examened drug combinations applied 15 min before exposure to 2 LD50 TMPhF
Bp+HI-6 : A+HI-6 - ** - p<0.01; *** - p<0.001

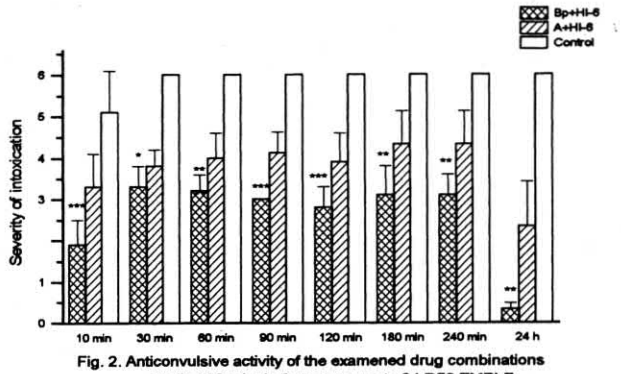

Fig. 2. Anticonvulsive activity of the examened drug combinations applied 30 min before exposure to 2 LD50 TMPhF
Bp+HI-6 : A+HI-6 - * - p<0.05; ** - p<0.01; *** - p<0.001

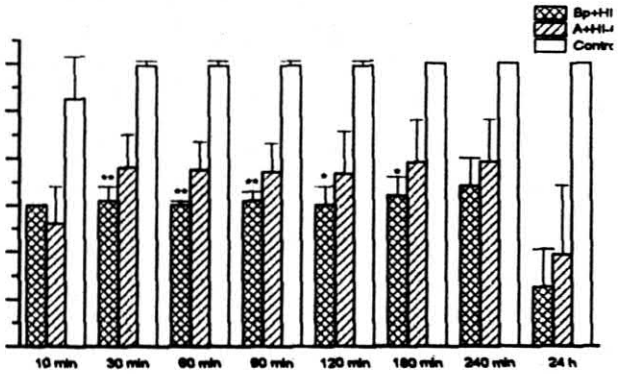

Fig. 3. Anticonvulsive activity of the examened drug combinations applied 60 min before exposure to 2 LD50 TMPhF.
Bp+HI-6 : A+HI-6 - * - p<0.05; ** - p<0.01

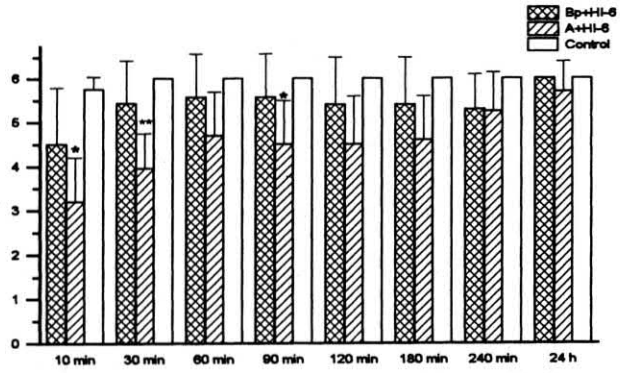

Fig. 4. Anticonvulsive activity of the examened drug combinations applied 120 min before exposure to 1.5 LD50 TMPhF
Bp+Hi-6 : A+HI-6 - * - p<0.05; ** - p<0.01

Discussion

In the work presented here a prophylactic drug combination containing biperiden, as an anticholinergic and HI-6 as a power cholinesterase reactivator demonstrated strong anticonvulsive efficacy against TMPhF-induced convulsions. It is important to be noted that the pretreatment doses of HI-6 and biperiden used in this study were moderate - 72 mg/kg and 1.25 mg/kg respectively. Instead of the combination tested provided for very good protection against TMPhF intoxication including convulsions.

The most likely explanation to this fact are both central antimuscarinic effects of biperiden and peripheral effects of HI-6.

Biperiden is a synthetic, tertiary antimuscarinic compound. It has strong atropine-like blocking effects in the CNS, and on parasympathetic structures. Biperiden is used for the treatment of par-

kinsonian syndrome (Anderson et al., 1994). Anti-parkinsonian anticholinergic drugs, like biperiden, have been reported to have anti-N-methyl-D-aspartate (ant-NMDA) activity (Olney et al., 1987). Thus, the anticonvulsant properties of biperiden may be related to the multiple action characteristics.

In summary, the drug combination biperiden and HI-6 is more effective than atropine and HI-6 applied 15, 30 and 60 min before exposure to 2.0 LD_{50} TMPhF. Further work is needed to determine the protective properties of this combination at higher doses of biperiden and HI-6.

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Correspondence: Ivan Samnaliev
Military Medical Academy
Sofia
Bulgaria

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