ZAHRANIČNÍ PŘÍSPĚVKY

ASSESSMENT OF THE DRUG COMBINATIONS CONTAINING BIPERIDEN AND CHOLINESTERASE REACTIVATORS AS A PROPHYLAXIS AGAINST SOMAN INDUCED POISONING

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Abstract

Protection against nerve agent-induced convulsive activity is a very important problem which is not solved yet. Pretreatment usually consists of reversible acetylcholinesterase inhibitor, such as pyridostig-mine or physostigmine, alone or associated with centrally acting antimuscarinic compound. Biperiden is a tertiary amine antimuscarinic compound with strong atropine-like bloking effects in the central nervous system. It possess anti-N-methyl-D-aspartate (NMDA) activity as well. It is well known that biperiden is very effective against soman-induced lethality and convulsions. In the present study, biperiden (1.25 mg/kg i.m.) was combined with different types of cholinesterase reactivators – HI-6, VT-89 (2,3) and VT-93 (2,3) (2 x 10^{-4} mol/kg i.m.). The drug combinations were administered 15, 30 and 60 min prior to exposure to 2 LD_{50} soman and their antilethal and anticonvulsive activity were studied in rats. The studies showed that biperiden + HI-6 protected all animals from lethality and convulsive/subconvulsive activity especially when given 15 and 30 min before the challenge. The pretreatment regimen consisting of biperiden and VT-89 (2,3) was not able to prevent soman-induced lethality and convulsions. The third combination tested – biperiden + VT-93 (2,3) provided the same anticonvulsive protection as biperiden + HI-6 when given 60 min prior to exposure to 2 LD_{50} soman.

Key words: Biperiden; Cholinesterase reactivators; Soman; Anticonvulsive pretreatment.

Introduction

The main mechanism of action of highly toxic organophosphorus compounds (OPCs), such as soman, sarin and Vx, is well known – irreversible inhibition of enzyme acetylcholinesterase (EC 3.1.1.7.) and subsequent excessive accumulation of acetylcholine at cholinergic receptor sites which causes a cholinergic crisis in central and peripheral nervous system. As a consequence of these events, different toxic effects including repeated tonic-clonic limbic seizures, convulsions and subsequent brain neuropathological changes are observed. (McLeod et al., 1984; McLeod, 1985). Previous studies have shown a relationship between seizure/convulsive activity and brain lesions (Pazdernik et al., 1985). In addition, the intoxication with OPCs is responsible for a neurological syndrome with long-lasting effects called organophosphate-induced neuropathy (OPIDN) (Abou-Donia et al., 1990). Thus, one of the most important problem which must be resolved by a successful prophylaxis is the elimination or amelioration of convulsions produced by OPCs. Pyridostigmine is a currently fielded compound for pretreatment of nerve agent poisoning. Pyridostigmine pretreatment provides protection against exposure to highly toxic OPCs, however, when combined with postexposure antidot therapy. For this reason it is classified as a pretreatment adjunct (M. Dunn et al., 1998). Centrally acting carbamate pretreatment compounds, such as physostigmine, offer a degree of protection against nerve agent-induced brain injury. Unfortunately, the pretreatment with known brain-protecting compounds such as physostigmine, the benzodiazepine anticonvulsants, and benactyzine are not acceptable because of their decremental effects on the performance. At the same time, some centrally acting cholinolytics, such as scopolamine, trihexyphenidyl and biperiden have also been found efficacious against OPCs-induced convulsions (Capaccio and Shih, 1991; Anderson et al., 1994).

The purpose of the present study is to assess the anticonvulsive efficacy of three drug combinations containing biperiden and cholinesterase reactivators administered prior to intoxication with a highly toxic OPC in rats.

Material and methods

Drug combinations and dose regimen: HI-6 and VT-93 (2,3) are bis-pyridinium oxime rectivators and VT-89 (2,3) is a benzoyl derivate of bis-pyridinium compounds. All compounds were synthezized at MMA, Sofia. They were administered separately i. m. at equimolar doses ($2x10^{-4}$ mol/kg of body weight) in combination with biperiden (Akineton, 5 mg/1 ml, Knoll Pharmaceutical Co.). The dose of biperiden was 1.25 mg/kg ($3.1x10^{-6}$ mol) of body weight. The drug combinations were applied 15, 30 and 60 min prior to intoxication with 2 LD_{50} soman (92% purity). The final concentration of soman was 0.5 mg/kg. Experiments were carried out on 210 male albino Wistar rats.

A seven degree scale for assessment of the toxic signs observed after exposure to 2 LD_{50} soman was used: 0 – none toxic signs,
1 – hyperactivity,
2 – chewing and/or salivation,
3 – fasciculations and/or tremors,
4 – subconvulsive movements;
5 – convulsions
6 – death.

The grade of the most severe signs observed for a given animal was noted at 5, 10, 15, 30, 60, 90, 120, 150, 180 and 240 min and 24 hours after intoxication. The cumulative results presented as a sum of all grades for a given animal obtained across the observation time and mortality were noted as well.

Statistical significance was determined by the use of Student's *t*-test and the differences were considered significant when $P < 0.05$.

Results

The data summarized in Tables 1–3 represent 24 hours survival in the experimental groups pretreated with the drug combinations tested. Data are given in percentage for each group. The results obtained in this study showed that the combination consisting of HI-6 and biperiden provided complete protection from death when was administered 15 and 30 minutes before exposure to 2 LD_{50} soman (Tables 1, 2). At the same time a high level of mortality was observed in the group pretreated by VT-89 (2,3) + biperiden – 24 h after the intoxication the lethality was almost 60 %. The third combination tested provided negligible protection against lethality produced by soman when given 15 min prior to the challenge. Survivors in this group were hardly 30% at the last time of observation. The protective efficacy of the same combination remarkable increased when the time of pretreatment was prolonged to 30 minutes. In this case all of pretreated animals survived an intoxication with 2 LD_{50} soman.

Survival (%) in the experimental groups pretreated with the drug combinations tested: HI-6 + biperiden, VT-89 (2,3) + biperiden and VT-93 (2,3) + biperiden

Table 1

15 min prior to exposure to 2 LD_{50} soman

Time	Groups		
	HI-6 + biperiden	VT-89 (2,3) + biperiden	VT-93 (2,3) + biperiden
60 min	100	66.7	83.3
120 min	100	58.3	83.3
180 min	100	58.3	83.3
240 min	100	58.3	75.0
24 hrs	100	41.7	33.3

Table 2

30 min prior to exposure to 2 LD_{50} soman

Time	Groups		
	HI-6 + biperiden	VT-89 (2,3) + biperiden	VT-93 (2,3) + biperiden
60 min	100	75.0	100
120 min	100	66.7	100
180 min	100	66.7	100
240 min	100	66.7	100
24 hrs	100	41.7	100

Table 3

60 min prior to exposure to 2 LD_{50} soman

Time	Groups		
	HI-6 + biperiden	VT-89 (2,3) + biperiden	VT-93 (2,3) + biperiden
60 min	75.0	66.7	100
120 min	75.0	66.7	100
180 min	75.0	66.7	100
240 min	75.0	66.7	100
24 hrs	50.0	0	50.0

Generally, the effectivenes of the drug combinations tested against soman-induced mortality de-

creased when they were administered 60 min before the exposure (Table 3). The highest level of lethality (100%) was observed in the group pretreated by VT-89 (2,3) and biperiden. The pretreatment with HI-6 + biperiden and VT-93 (2,3) + biperiden provided 75% and 100% survival, respectively, during the initial time of observation. Their protective efficacy decreased to 50% at 24 hours after the exposure.

The anticonvulsive studies showed that HI-6 and biperiden protected animals from the severe toxic signs, including convulsions when the combination was given 15 and 30 minutes before intoxication with 2 LD_{50} soman. The most severe signs observed were fasciculations and tremors. At the last observation time – 24 h after the challenge, animals were in good clinical condition (Figures 1 and 2). Some animals demonstrated hyperactivity and chewing, symptoms graded as 1 or 2 in according to the scale used. The comparative study showed that the combination consisting of VT-89 (2,3) and biperiden was less effective in reducing convulsive activity produced by soman. Some of animals demonstrated subconvulsions and/or convulsions which started 10–15 minutes after the intoxication. Animals did not show recovery 24 hours after the exposure. Significant differences between the effects of both combinations were available at all observation times.

A tendency to decrease the anticonvulsive efficacy of HI-6 and biperiden was noted when the time of pretreatment was prolonged to 60 minutes. Some of animals demonstrated subconvulsive or convulsive activity, especially 2 h after the exposure. Mortality was observed as well. No recovery to the normal behavior 24 hours after the intoxication was observed (Figure 3). The second combination tested was not able to protect the animals from the severe toxic signs. Convulsions were available in this group during all the time of observation. The comparison of the effectiveness of both combinations showed that HI-6 and biperiden was superior in reducing soman-induced convulsions especially at the beginning of observation.

The third combination tested consisting of VT-93 (2,3) and biperiden demonstrated moderate anticonvulsive activity. The most severe signs observed in the experimental groups pretreated by this combination were tremors or fasciculations. Some animals showed subconvulsions as well. The most manifested anticonvulsive efficacy was noted when the combination was administered 30 min before the exposure to 2 LD_{50} soman (Figures 4 and 5). In this case, a recovery to the normal behaviore, was available 24 hours after the challenge. The comparison of the effectivenes of VT-93 (2,3) + biperiden with HI-6 + biperiden showed that the second combination was somewhat more effective against convulsive activity produced by soman when it was given 15 and 30 minutes before to the exposure.

Significant differences were noted at some observation times. The efficacy of both combinations was quite similar when the time of pretreatment was prolonged to 60 minutes prior to the intoxication (Figure 6). Generally the prolongation of the term of pretreatment led to decrease the anticonvulsive activity.

The date obtained for 24 hours severity scores of the drug combinations tested are summarized in Table 4. The severity scores give important information for the extent of the intoxication for each experimental group. The results obtained in this study showed that the severity scores for groups pretreated by HI-6 and biperiden at 15 and 30 minutes before the intoxication with 2 LD_{50} soman are quite similar and lower than those obtained for the other combinations tested. The highest scores among studies were found for the groups pretreated with VT-89 (2,3) + biperiden. The third combination tested consisting of VT-93 (2,3) and biperiden provided a medial scores ranging from 55.50 to 61.80. Equal severity scores were obtained for the groups pretreated with HI-6 + biperiden and VT-93 (2,3) + biperiden when given 60 min prior to the challenge.

Table 4

24 hrs severity scores of experimental groups*

Time of pretreatment	Experimental Groups		
	HI-6 + biperiden	VT-89 (2,3) + biperiden	VT-93 (2,3) + biperiden
15 min	48.81	72.65	61.76
30 min	48.62	74.17	55.52
60 min	60.95	77.20	60.59

* Severity score for each group is represented as a sum of all average values determined at 11 obs. times (5, 10, 15, 30, 60, 90, 120, 150, 180 min, 4 and 24 hrs).

♣ The drug combinations tested were applied 15, 30 and 60 min before exposure to 2 LD_{50} soman.

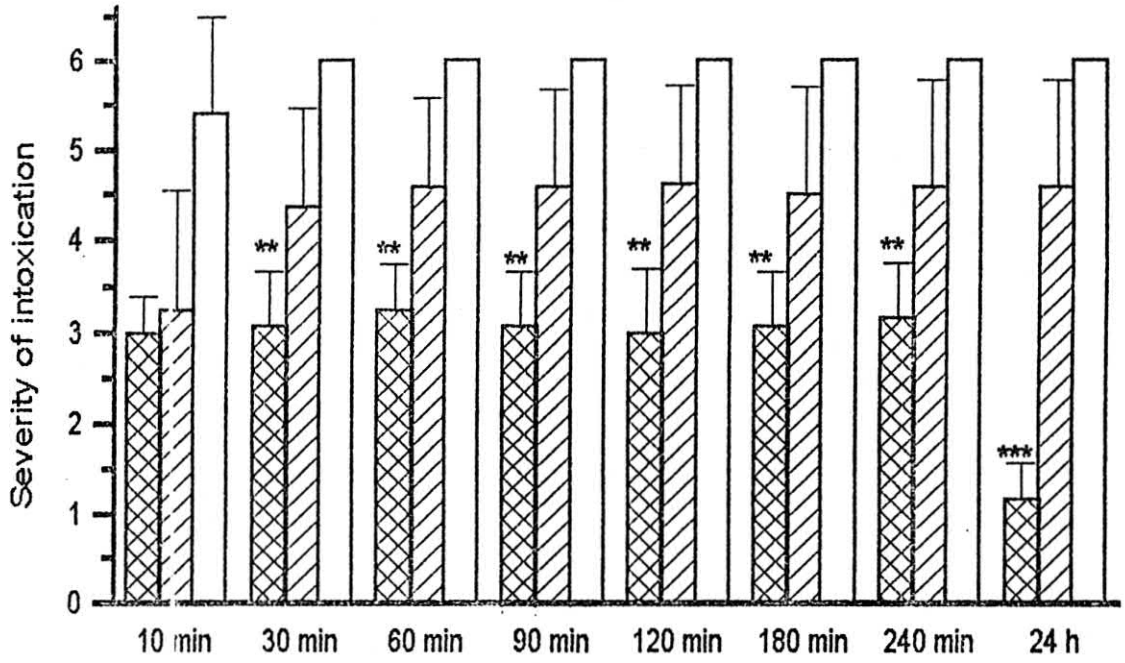

Fig. 1. Anticonvulsive activity of the examened drug combinations applied 15 min. before exposure to 2 LD50 Soman

HI-6+BP : VT89 (2,3)+BP - ** - p<0.01;

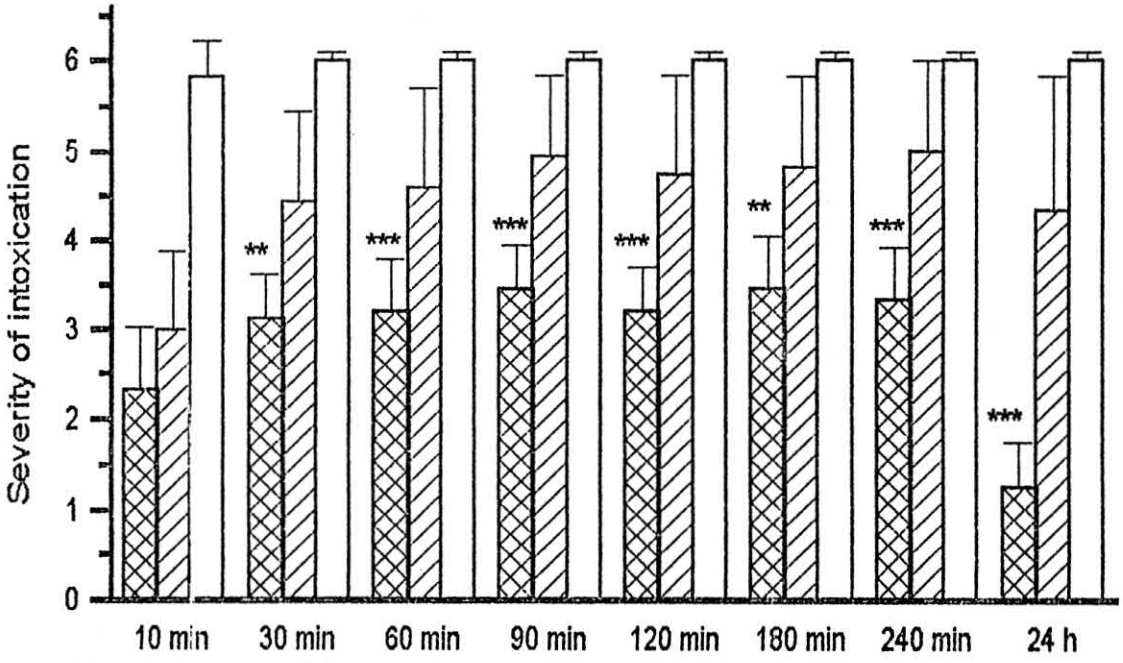

Fig. 2. Anticonvulsive activity of the examened drug combinations applied 30 min. before exposure to 2 LD50 Soman

HI-6+BP : VT89 (2,3)+BP - ** - p<0.01; *** - p<0.001

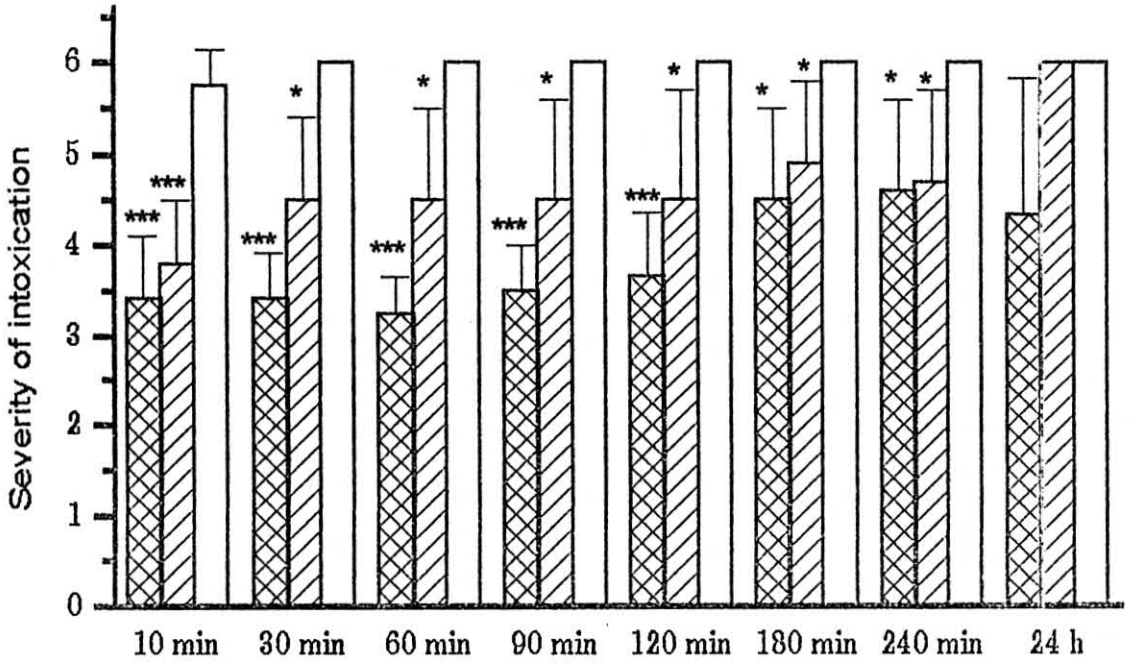

Fig.3. Anticonvulsive activity of the examened drug combinations applied 60 min. before exposure to 2 LD50 Soman

Comparison to control group - * - p<0.05; *** - p<0.001

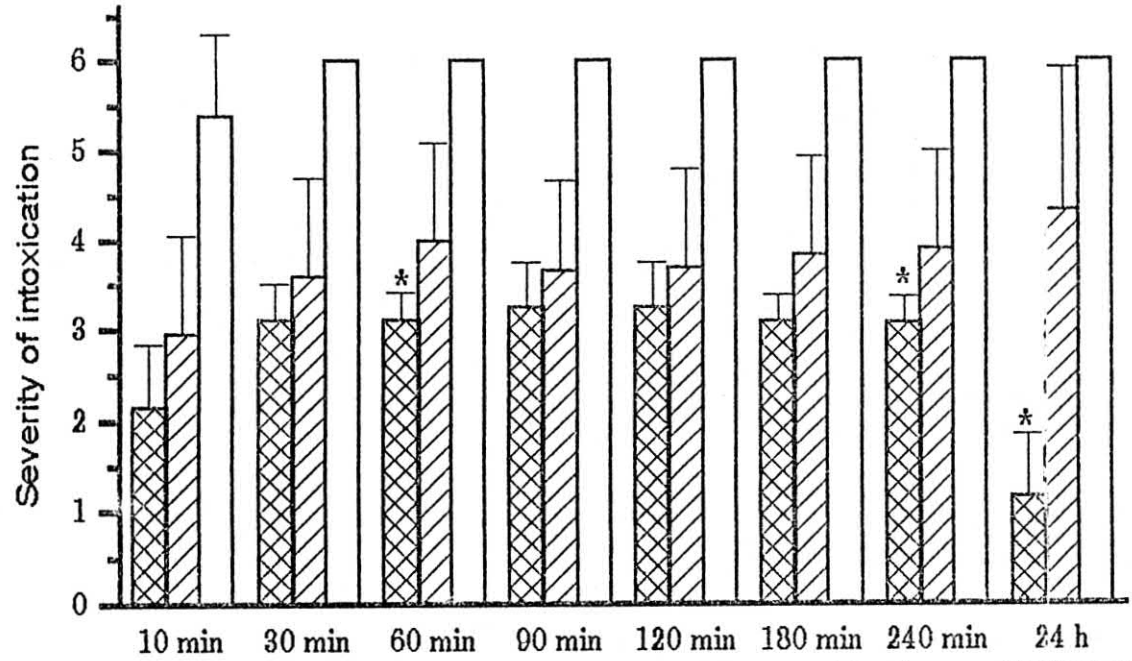

Fig. 4. Anticonvulsive activity of the examened drug combinations applied 15 min. before exposure to 2 LD50 Soman

HI-6+BP : VT93 (2,3)+BP - * - p<0.05;

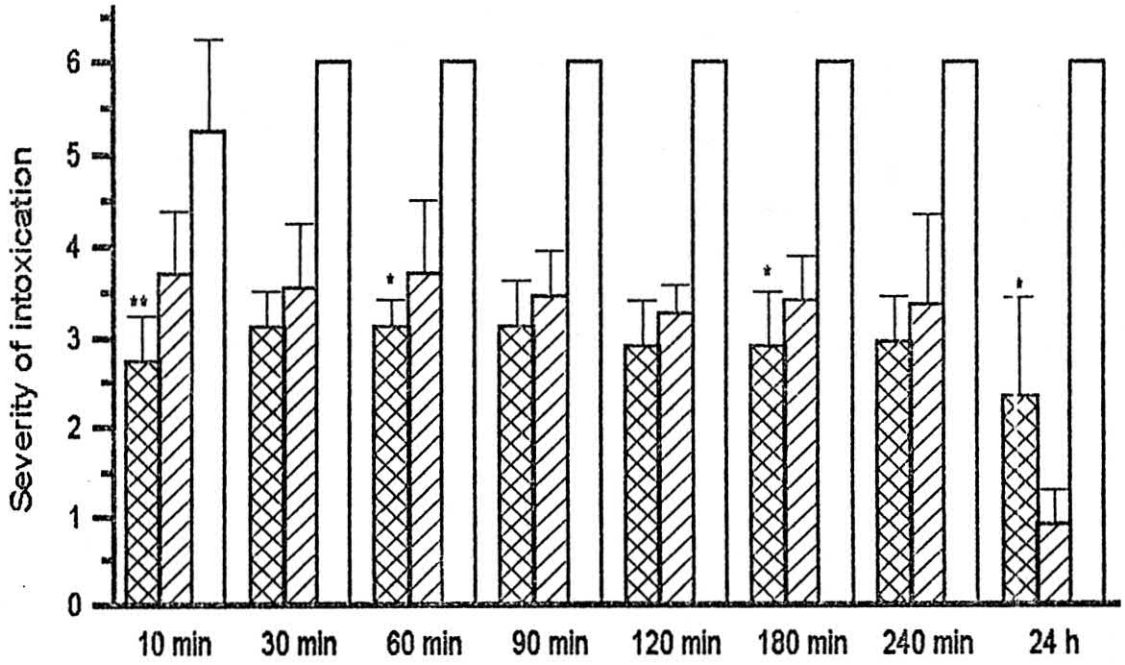

Fig. 5. Anticonvulsive activity of the examened drug combinations applied 30 min. before exposure to 2 LD50 Soman
HI-6+BP : VT93 (2,3)+BP - * $p < 0.05$; ** $p < 0.01$

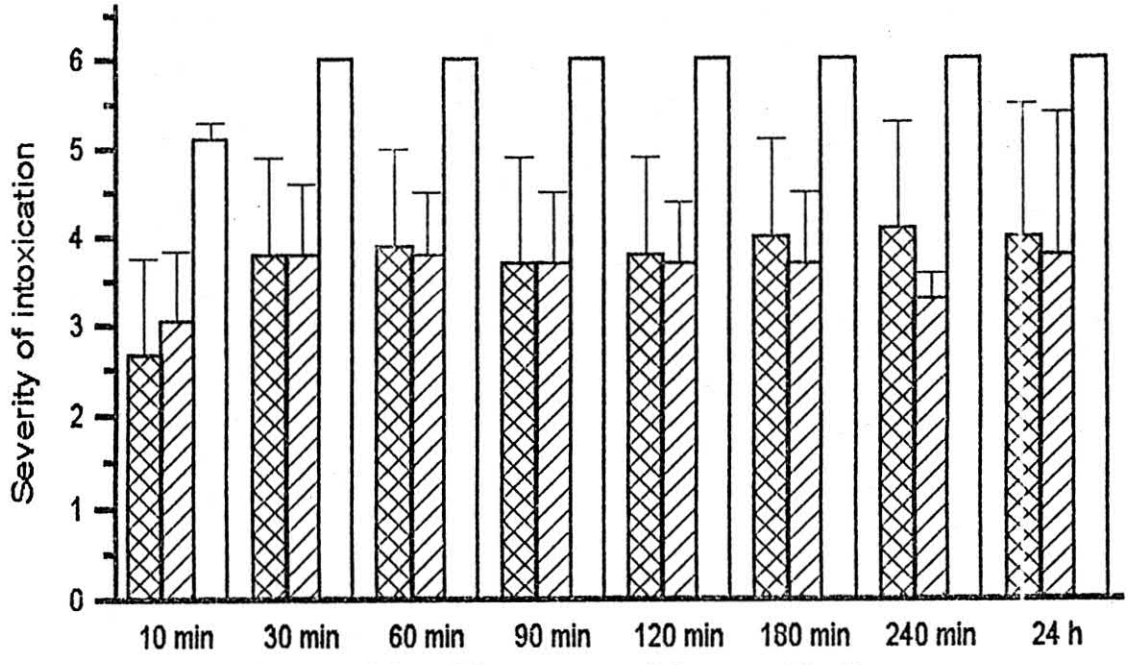

Fig. 6. Anticonvulsive activity of the examened drug combinations applied 60 min. before exposure to 2 LD50 Soman
HI-6+BP : VT93 (2,3)+BP - $p > 0.05$;

Discussion

The cholinesterase reactivators tested in the current study belong to the group of bis-pyridinium oximes. Each compound has an oxime group in the first pyridinium ring. In the second pyridinium ring, they possess different chemical groups as mentioned above. It is well known that the antidotal activity of cholinesterase reactivators including interaction with cholinesterase receptors depends on the type and disposition of the functional groups. Many studies have shown that HI-6 is one of the most efficacious oximes against highly toxic OPC's. The effectiveness of this compound can be explained by its chemical structure which includes two different chemical groups: an oxime group on the second place in the first pyridinium ring and a carboxamide group on the fourth place in the second pyridinium ring. Obviously HI-6 possesses an optimal chemical structure which ensures a strong antidote activity against highly toxic organophosphorus agents. This suggestion is reinforced by the QSAR studies performed by Binenfield et al. (1984) and Dishovsky (1989).

Biperiden is a synthetic, tertiary amine antimuscarinic compound. It has strong atropine-like blocking effects in the CNS, and on parasympathetic-innervated peripheral structures. In addition to the anticholinergic properties, biperiden has anti-N-methyl-D-aspartate (NMDA) activity (Olney et al., 1987). It is known that excitatory amino acid antagonists with anti-NMDA activity are effective anticonvulsants when given before or after initiation of soman-induced seizures (McDonough et al., 1993; Shih et al., 1991). Previously studies have shown that biperiden is very effective against soman-induced lethality and convulsive activity (Shih et al., 1991; Capacio, Shih, 1991; Anderson et al., 1994).

Since biperiden is available in all combination tested the beneficial effect demonstrated by HI-6 + biperiden against soman-induced lethality and convulsive activity compared to the lack of efficacy of VT-89 (2,3) + biperiden can be attributed to HI-6. When VT-89 (2,3) is replaced by the bis-pyridinium bis-oximes VT-93 (2,3), an increase in antilethal and anticonvulsive activity is observed.

In summary, the drug combination consisting of biperiden and HI-6 is effective against soman-induced lethality and convulsive activity in rats, especially when it is administered 15 and 30 min prior to the challenge. The results herein suggest that the same combination is superior to the other two combinations tested – biperiden + VT-89 (2,3) and biperiden + VT-93 (2,3).

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