THE INFLUENCE OF ANTICHOLINERGIC DRUG SELECTION ON THE EFFECTIVENESS OF OXIMES AGAINST SOMAN-INDUCED SUPRALETHAL POISONING IN MICE

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Summary: 1. The influence of anticholinergic drugs (atropine, benactyzine, biperiden) on the efficacy of monopyridinium and bispyridinium oximes (HI-6, BI-6, obidoxime, pralidoxime, methoxime) on soman-induced supralethal poisoning was studied in mice. 2. While methoxime combined with benactyzine or biperiden seems to be more efficacious in the elimination of toxic effects of supralethal dose of soman than its combination with atropine, the efficacy of the other oximes studied against soman-induced toxic effects is not significantly influenced by the anticholinergic drug selection. 3. On the other hand, there are big differences in the effectiveness of oximes tested as to their ability to eliminate toxic effects of soman at supralethal doses. 4. The findings support the fact that the choice of acetylcholinesterase reactivator is more important than the anticholinergic drug selection for the effectiveness of antidotal mixture in the case of prophylactic administration of antidotes.

Key words: Soman, Oximes, Anticholinergic drugs, Acute toxicity, Mice
Methods
Mice male weighing between 19 and 23 g were obtained from Konarovie. The animals were maintained in an air-conditioned room with light from 07.00 to 19.00 and were allowed free access to standard food and tap water. The principles of laboratory animal care were followed and the handling of animals was made under the supervision of the Ethics Committee of Medical Faculty of Charles University and Parkyn Military Medical Academy in Hradec Králové.

Soman of 95% purity was purchased from Zemianske Kostolany (Slovak Republic). Its purity was assayed by acidi-metric titration. The oximes Hi-6 and Bi-6 of 98.5% purity were synthesized at the Department of Toxicology of Parkyn Military Medical Academy in Hradec Králové. All other chemicals and drugs of analytical grade were obtained commercially and used without further purification.

The efficacy of oximes against soman administered intra-muscularly (i.m.) at a supralethal dose (240 mg/kg, 2 x LD50) was determined by the evaluation of their medium efficacy doses (ED50 values) and their 95% confidence limits using probit analysis of death occurring within 24 h following soma-n poisoning in at least four groups of six experimental animals (21). In these experiments, the oximes were injected i.m. in combination with one of the anticholinergic drugs used (atropine, benactyzine, biperiden) at equie-fective doses (5% LD50) 5 min before challenge of soman. Finally, the safety of oximes administered at the efficacious doses was determined by the calculation of safety ratio (LD50/ED50). The acute toxicity of all oximes tested in mice (LD50 values) was also evaluated using probit-logarithmic analysis (21).

Statistical significance was determined by the use of Student’s t-test and differences were considered significant when p < 0.05.

Results
The LD50 values of all oximes tested are shown in Tab. 1. These values were used for the calculation of the safety of administration of oximes at efficacious doses.

The efficacy of each oxime in combination with various anticholinergic drugs is presented in Table 2. Pralidoxime as well as obidoxime appear to be ineffective against toxic effects of soman administered at supralethal dose regardless of the choice of anticholinergic drug in the case of the administration of obidoxime at therapeutic doses (below 25% LD50). The effectiveness of H oximes (Hi-6 and Bi-6) does not change significantly when they are combined with various anticholinergic drugs. On the con-trary, the prophylactic efficacy of methoxime depends on the selection of the anticholinergic drug. The combination of methoxime with benactyzine or biperiden is significantly more efficacious in antagonizing toxic effect of soman than the combination of methoxime with atropine (p < 0.05).

To compare the efficacious doses of oximes studied, ca-pable of elimination of toxic effects of soman at a supra-lethal dose, H oximes seem to be significantly more effective in antagonizing lethal effects of soman than other oximes tested regardless of the choice of anticholi-nergic drugs (p < 0.05) (Tab. 2).

Tab. 1: LD50 values of oximes following i.m. administration in mice

| OXIME | Pralidoxime | LD50 (mg/kg) 95% confidence limits | 263.6 (253.7-273.8) |
|-------|-------------|-----------------------------------|---------------------|
| Atropine | 5.7 (4.0 - 8.4) | > 4.0 |
| Benactyzine | 7.0 (4.2 - 11.2) | 95.6 |
| Biperiden | 25.8 (19.6 - 34.9) | * 26.0 |

Tab. 2: The prophylactic antidotal potency (ED50 value) and safety ratio (SR) of oximes in combination with various anticholinergic drugs in soman-poisoned mice. Statistical significance: * p < 0.05.

| OXIME | Cholinolytic drug | ED50 (mg/kg) 95% confidence limits | SR (LD50/ED50) |
|-------|------------------|-----------------------------------|----------------|
| PRAF | Atropine | 5.7 (4.0 - 8.4) | 4.0 |
| DOM | Benactyzine | 7.0 (4.2 - 11.2) | 95.6 |
| BIP | Biperiden | 25.8 (19.6 - 34.9) | * 26.0 |
| METH | Atropine | 105.3 (59.5 - 117.1) | 61 |
| HOX | Benactyzine | 50.5 (33.9 - 73.0) | 18.7 |
| BIP | Biperiden | 25.8 (19.6 - 34.9) | 26.0 |

Discussion
Nerve agents are still considered to be the most impor-tant chemical warfare agents. With the existing threat of the use of chemical weapons not only in military conflicts but also in terrorist attacks, the search for effective protection is in the central concern of different laboratories both civi-lian and military (4,16).

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In conclusion, our data indicate that the choice of AChE reactivators is more important for the survival of so-man-poisoned experimental animals than the selection of anticholinergic drugs in the case of prophylactic adminis-tration of antidotes.

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To compare the efficacious doses of oximes studied, capable of elimination of toxic effects of soman at a super lethal dose, H oximes seem to be significantly more effective in antagonizing lethal effects of soman than other oximes tested regardless of the choice of anticholinergic drugs (p < 0.05) (Tab. 2).

| Tab. 1: | LD50 values of oximes following i.m. administration in mice | | | |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|         | LD50 (mg/kg) ± 95% confidence limits | Statistical significance | p < 0.05 | | | | | | | | | | | |
| **OXIME** | **Pralidoxime** | **LD50 (mg/kg) ± 95% confidence limits** | | | | | | | | | | | | | |
|         | 263.3 (253.7 - 273.8) | | | | | | | | | | | | | |
|         | 188.4 (156.3 - 208.0) | | | | | | | | | | | | | |
|         | 641.8 (590.5 - 716.0) | | | | | | | | | | | | | |
|         | 671.3 (627.4 - 718.5) | | | | | | | | | | | | | |
|         | 266.3 (246.5 - 285.4) | | | | | | | | | | | | | |
| **BI-6** | **Hi-6** | **OXIME** | **Cholinolytic drug** | **LD50 (mg/kg) ± 95% confidence limits** | **SR** | | | | | | | | | | |
|         | **Pralidoxime** | | | | | | | | | | | | | |
|         | Atropine | 70 | 4.0 | | | | | | | | | | | |
|         | Benactyzine | 70 | 4.0 | | | | | | | | | | | |
|         | Biperiden | 70 | 4.0 | | | | | | | | | | | |
|         | **DOXIME** | **LD50 (mg/kg) ± 95% confidence limits** | | | | | | | | | | | | |
|         | Atropine | 50 | 4.0 | | | | | | | | | | | |
|         | Benactyzine | 50 | 4.0 | | | | | | | | | | | |
|         | Biperiden | 50 | 4.0 | | | | | | | | | | | |
|         | **METHO** | **LD50 (mg/kg) ± 95% confidence limits** | | | | | | | | | | | | |
|         | Atropine | 105 (79.5 - 137.1) | 61 | | | | | | | | | | | |
|         | Benactyzine | 50 (33.9 - 73.0) | 127 | | | | | | | | | | | |
|         | Biperiden | 28.8 (19.6 - 34.9) | 24.0 | | | | | | | | | | | |
|         | **HI-6** | **LD50 (mg/kg) ± 95% confidence limits** | | | | | | | | | | | | |
|         | Atropine | 72 (61.4 - 84.1) | 93.2 | | | | | | | | | | | |
|         | Benactyzine | 70 (4.2 - 112.5) | 95.6 | | | | | | | | | | | |
|         | Biperiden | 5.9 (3.7 - 9.5) | 113.0 | | | | | | | | | | | |
|         | **Bi-6** | **LD50 (mg/kg) ± 95% confidence limits** | | | | | | | | | | | | |
|         | Atropine | 11.7 (10.1 - 13.5) | 22.8 | | | | | | | | | | | |
|         | Benactyzine | 10.4 (8.3 - 12.8) | 18.8 | | | | | | | | | | | |
|         | Biperiden | 5.6 (2.8 - 11.5) | 47.4 | | | | | | | | | | | |

**Discussion**

Nerve agents are still considered to be the most important chemical warfare agents. With the existing threat of the use of chemical weapons not only in military conflicts but also in terrorist attacks, the search for effective protection is in the central concern of different laboratories both civilian and military (4,16). The effectiveness of oximes against soman administered intramuscularly (i.m.) at a supralethal dose (240 µg/kg, 2 x LD50) was determined by the evaluation of their medium efficacious doses (ED50 values) and their 95% confidence limits using probit analysis of death occurring within 24 h following soman poisoning in at least four groups of six experimental animals (21). In these experiments, the oximes were injected i.m. in combination with one of the anticholinergic drugs used (atropine, benactyzine, biperiden) at equieffective doses (5 % LD50 ± 5 min before challenge of soman. Finally, the safety of oximes administered at the efficacious doses was determined by the calculation of the ratio of LD50 (ED50 value, SR). The acute toxicity of all oximes tested in mice (LD50 values) was also evaluated using probit-logarithmic analysis (21).

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