A COMPARISON OF THE EFFICACY OF NEW MONOPYRIDINIUM OXIMES WITH THE OXIME HI-6 AGAINST MEVINPHOS IN MICE

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Summary: 1. The therapeutic efficacy of three new monopyridinium oximes (2,4-PAEM, 2,5-PAEM, 2,5-PAAM) and the bipyrrolidinone oxime HI-6 was evaluated in combination with benactyzine against acute poisoning with the organophosphorus insecticide mevinphos in mice. 2. When mice were treated two min after mevinphos poisoning, no significant differences in the therapeutic effectiveness of tested oximes were observed. They increased the 24h LD50 values of mevinphos about three times in comparison with non-treated intoxicated animals. 3. On the other hand, there were significant differences in their therapeutic efficacy when they were administered 30 sec following mevinphos administration. The monopyridinium oxime 2,5-PAEM seems to be the most efficacious against mevinphos toxicity. 4. Use of new monopyridinium oxime 2,5-PAEM appears to be the improvement in the antidotal treatment of poisoning with organophosphorus insecticide mevinphos in comparison with HI-6.

Key words: Mevinphos, Monopyridinium oximes, HI-6, Benactyzine, LD50, Mouse;

Introduction

Organophosphorus insecticides (OPI) have become the most widely used class of insecticides in the world. The use of OPI in agricultural fields has replaced more resistant chlorinated hydrocarbon compounds. The choice of OPI is based on their properties of low bioaccumulation and high rate of biodegradation. They are also used in large quantities because of their high potential for insect knockdown ca. (1,5). In spite of relatively low toxicity in comparison with highly toxic nerve agents, they have passed occupational hazards to workers employed in application of theses insecticides. Careless handling of OPI and their voluntary exposure with suicidal intent are the main reasons for intoxication (12,17). One of the most toxic OPI, mevinphos (2-methoxycarbonyl-1-methylvinyl dimethylphosphate), is used for its high efficacy against various insect species (4). The 24h intramuscular (i.m.) LD50 of mevinphos for mice is 0.79 mg/kg body weight (18).

OPI induce clinical signs including salivation, diarrhoea, lacrimation, tremors, convulsions and respiratory distress. Death from exposure to OP compounds is generally due to respiratory failure from excessive airway secretions, constriction of the airways and a loss of central respiratory capability (1,5). In spite of relatively low toxicity in comparison with highly toxic nerve agents, they have passed occupational hazards to workers employed in application of these insecticides. Careless handling of OPI and their voluntary exposure with suicidal intent are the main reasons for intoxication (12,17).

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The increased international concern about the possible occupational hazards to workers employed in application of OPI has prompted us to critically consider the expected value of currently available antidotal treatment of OPI poisoning. Unfortunately, none of currently available oximes can be regarded as a broad spectrum antidote (18). Although the bipyrrolidinone oxime HI-6 (Figure 1) is considered to be the most efficacious oxime against highly toxic OP compounds including soman (7,10,14), its therapeutic effectiveness against OPI poisoning is not quite satisfactory (6,18).
To improve the efficacy of antidotal treatment of acute poisoning with OPI, three new monopyridinium oximes (2,4-PAEtM, 2,5-PAAM, 2,5-PAEtM) have been synthesized at the Institute of Organic Chemistry and Technology of the Czech Academy of Sciences in Prague. The oxime HI-6 has been shown to be very effective against some highly toxic OP compounds not only because of its high reactivating potency but also because of its other more efficacious than other currently available oximes in diminishing acute toxicity of OPI (6,8,18).

The 24hLD50 values of mevinphos in treated mice were increased approximately three times in comparison with the 24hLD50 values in non-treated mice. No significant differences between effectiveness of the oximes tested were observed (Table 2).

Methods

Male mice (20-24g) obtained from Konarovice were housed in an air-conditioned room (20±2°C) on 12h light/12h dark cycles and were allowed access to food and tap water ad libitum. The principles of laboratory animal care were followed and the handling of animals was made under the supervision of the Ethics Committee of the Medical Faculty of Charles University and the Military Medical Academy in Hradec Králové.

The monopyridinium oximes (2,4-PAEtM, 2,5-PAEtM, 2,5-PAAM) and the bispyridinium oxime HI-6 with anticholinergic drug benzydamine against multiple lethal doses of OPI mevinphos in mice.

The monopyridinium oximes (2,4-PAEtM, 2,5-PAEtM, 2,5-PAAM) were prepared by quaternization of tertiary bases by methylidene in the medium of dimethylformamide and potassium hydroxide. Chemical structures of products obtained after synthesis were identified by an elemental analysis and NMR. The chemical purity of products of synthesis assessed by TLC was more than 98%.

Mice were treated i.m. with oximes in equieffective doses (5% or 10% LD50) in combination with benzydamine (BNZ) at a dose 6.4 mg/kg 30 sec after two or five mevinphos injections (Spolana Neratovice) poisoning. LD50 doses of oximes and 95% confidence limits were calculated by probit analysis of death occurring within 24h i.m. administration of oximes at five different doses with six mice per dose (15). The efficacy of antidotal mixtures tested was expressed as protective ratio (LD50 of mevinphos in protected mice/LD50 of mevinphos in unprotected mice).

Table 1: Toxicity parameters of oximes tested.

| OXIMES | LD50 (mg/kg) |
|--------|-------------|
| HI-6   | 673.3       |
| 2,4-PAEtM | 150.3 (1187.2 - 2050.5) |
| 2,5-PAEtM | 1381.6 (1267.6 - 1510.9) |
| 2,5-PAAM | 1264.4 (1100.3 - 1377.8) |

The protective efficacy of the monopyridinium oximes and HI-6 as presented in Table 1. Generally, the monopyridinium oximes are significantly less toxic for mice than the oxime HI-6.

On the other hand, when mice were treated 30 sec following mevinphos intoxication, the efficacy of all tested oximes was significantly increased and there were some differences in their therapeutic effects. The 10% LD50 values of mevinphos in mice protected with monopyridinium oxime 2,4-PAEtM or 2,5-PAEtM in combination with BNZ were increased 12 - 20 times in comparison with the 24hLD50 values in unprotected mice while the 24hLD50 values of mevinphos in mice protected with 2,5-PAAM plus BNZ were increased 6 - 8 times in comparison with the 24hLD50 values of mevinphos in unprotected mice.

Discussion

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The therapeutic efficacy of the monopyridinium oximes as well as the oxime Hf-6 is presented in Table 2 and 3. When the oximes in combination with BNZ were administered two min after mevinphos poisoning, the 24h LD50 values of mevinphos in treated mice were increased approximately three times in comparison with the 24h LD50 values in non-treated mice. No significant differences between effectiveness of the oximes tested were observed (Table 2).

Table 2: Therapeutic effect of oximes administered at 2 min after poisoning on the LD50 value of mevinphos.

| TREATMENT       | DOSE OF OXIME | LD50 (mg/kg) | Protective ratio |
|-----------------|---------------|--------------|-----------------|
| Hf-6 + BNZ      | 5% LD50       | 2.44 (2.03 - 2.83) | 3.1             |
|                 | 10% LD50      | 2.35 (2.15 - 2.56) | 3.4             |
| 2,4-PAEtM + BNZ | 5% LD50       | 2.40 (2.11 - 2.65) | 3.0             |
|                 | 10% LD50      | 2.43 (2.19 - 2.67) | 3.1             |
| 2,5-PAEtM + BNZ | 5% LD50       | 2.36 (2.07-2.70)  | 3.0             |
|                 | 10% LD50      | 2.36 (2.07-2.70)  | 3.0             |
| 2,5-PAAM + BNZ  | 5% LD50       | 2.30 (1.99 - 2.61) | 2.9             |
|                 | 10% LD50      | 2.30 (1.99 - 2.61) | 2.9             |

On the other hand, when mice were treated 30 sec following mevinphos intoxication, the efficacy of all tested oximes was significantly increased and there were some differences in their therapeutic effect. The 24h LD50 values of mevinphos in mice protected with monopyridinium oxime 2,4-PAEtM or 2,5-PAEtM in combination with BNZ were increased 12 - 20 times in comparison with the 24h LD50 values in unprotected mice while the 24h LD50 values of mevinphos in mice protected with 2,5-PAAM plus BNZ were increased 6 - 8 times in comparison with the LD50 values of mevinphos unprotected mice only. The effectiveness of the bispyridinium oxime Hf-6 in combination with BNZ varied between them. The monopyridinium oxime 2,5-PAEtM seems to be the least efficacious oxime according to the 24h LD50 values (Table 3).

Table 3: Therapeutic effect of oximes administered at 30 sec after poisoning on the LD50 value of mevinphos.

| TREATMENT       | DOSE OF OXIME | LD50 (mg/kg) | Protective ratio |
|-----------------|---------------|--------------|-----------------|
| Hf-6 + BNZ      | 5% LD50       | 9.73 (9.10-10.40) | 12.2             |
|                 | 10% LD50      | 9.35 (9.00-10.10) | 12.2             |
| 2,4-PAEtM + BNZ | 5% LD50       | 10.09 (9.35-10.84) | 10.5             |
|                 | 10% LD50      | 10.08 (9.40-10.84) | 10.5             |
| 2,5-PAEtM + BNZ | 5% LD50       | 13.85 (12.80-14.90) | 17.5             |
|                 | 10% LD50      | 13.85 (12.80-14.90) | 17.5             |
| 2,5-PAAM + BNZ  | 5% LD50       | 13.09 (12.30-14.53) | 17.6             |
|                 | 10% LD50      | 13.09 (12.30-14.53) | 17.6             |
| 2,5-PAAM + BNZ  | 5% LD50       | 16.05 (15.09-17.04) | 20.4             |
|                 | 10% LD50      | 16.05 (15.09-17.04) | 20.4             |

Following antitodal treatment of mevinphos-poisoned mice at two min after intoxication, the similar intensity of clinical signs and symptoms attributable to ACh accumulation at cholinergic sites (salivation, lachrymation, convulsions of skeletal muscles and respiratory depression) were found. When antitodal treatment was administered 30 sec following mevinphos challenge, a slight clinical improvement of mevinphos-poisoned mice treated with monopyridinium oxime 2,5-PAEtM or 2,4-PAEtM in comparison with the other oximes tested was observed.