The benefit of combinations of oximes for the ability of antidotal treatment to counteract sarin-induced brain damage in rats

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Abstract

Background: The aim of our study was to compare the ability of two combinations of oximes (HI-6 + trimedoxime and HI-6 + K203) with atropine to counteract acute sarin-induced brain damage with the efficacy of antidotal treatment involving single oxime (HI-6) and atropin using in vivo methods.

Methods: Brain damage and neuroprotective effects of antidotal treatment were evaluated in rats poisoned with sarin at a sublethal dose (108 μg/kg i.m.; 90% LD50) using histopathological, Fluoro-Jade B and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis 24 h after sarin administration.

Results: Both combinations of oximes reduce the number of rats that died before the end of experiment compared to non-treated sarin poisoning and sarin poisoning treated with HI-6 and atropine. In the case of treatment of sarin poisoning with HI-6 in combination with K203, all rats survived till the end of experiment. HI-6 with atropine was able to reduce sarin-induced brain damage, however, both combinations were slightly more effective.

Conclusions: The oxime HI-6 in combination with K203 and atropine seems to be the most effective. Thus, both tested oxime combinations bring a small benefit in elimination of acute sarin-induced brain damage compared to single oxime antidotal therapy.

Keywords: Sarin, HI-6, Trimedoxime, K203, Rats, Histopathology, FluoroJadeB, TUNEL

Background

Nerve agents are highly toxic organophosphorus compounds representing potential threats to both military and civilian population. These chemical warfare agents were used in the Iraq-Iran (1980s), in the terrorist attack on the Tokyo subway (1995) and more recently in Syria in the suburbs of Damascus resulting in massive casualties [32, 36]. The basic mechanism of nerve agents’ toxicity lies in irreversible inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) leading to the accumulation of the neurotransmitter acetylcholine at cholinergic synapses and neuromuscular junctions which subsequently results in the cholinergic crisis involving multiple organs. In the central nervous system, nerve agents may induce progressive irreversible damage [4]. The brain damage is associated with the dysfunction of irreversibly inhibited AChE and neuronal excitotoxicity and it seems to be largely responsible for persistent profound neuropsychiatric and neurological impairments in the victims of nerve agent exposure [4, 6].

The standard treatment of nerve agent poisoning usually consists of three types of antidotes – anticholinergic agents, oximes and anticonvulsive drugs. Anticholinergic drugs such as atropine sulphate antagonize the muscarinic overstimulation, anticonvulsants such as diazepam are indicated to prevent centrally mediated seizures and secondary brain damage and the oximes disrupt the covalent bond between nerve agent and AChE and restore the physiological function of this enzyme [8]. Among
oximes, monopyridinium oxime pralidoxime and bispyridinium oximes obidoxime, trime-doxime and HI-6 should be mentioned [39]. However, there is no potent broad-spectrum oxime able to sufficiently re-activate AChE inhibited by all nerve agents regardless of their chemical structure. HI-6, for instance, is considered to be a relatively strong reactivator of sarin, soman and cyclosarin inhibited AChE but its ability to re-activate AChE inhibited by tabun and less toxic organophosphorus pesticides is of low to moderate values [7, 17, 21, 26, 39, 41]. Trime-doxime or newly synthesized oxime K203, on the other hand, seem to be effective against tabun and pesticides [22, 24]. Since the effort to develop a potent broad-spectrum reactivator has failed so far, another way how to overcome this issue and possibly to increase the reactivating efficacy of anti-dotes is to combine the oximes in the anti-toxid treatment [19, 41].

The main purpose of this study was to compare the neuroprotective efficacy of the oxime HI-6 with two oxime mixtures containing HI-6 and trime-doxime or the oxime K-203 in combination with atropine in sarin-poisoned rats. The neuroprotective potential was evaluated using histopathological examination with the help of Fluoro-Jade B fluorochrome used in neurosci-ence to label degenerating neurons and terminal deoxy nucleotidyl transferase dUTP nick end labeling (TUNEL) assay detecting DNA fragmentation during apoptosis [25, 37].

Methods

Animals

All experiments in this study were approved by the Eth-ics Committee of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic) and were conducted in accordance with the Animal Protection Law and Ani-mal Protection Regulations.

Wistar rats (6-week-old, 200–230 g; VELAZ, Unetice, Czech Republic) were kept in an accredited animal facility (22 ± 2 °C, 50 ± 10% relative humidity, 12-h day-night cycle) and allowed access to standard food (Velaz) and tap water ad libitum. The rats were acclimatized for 14 days before starting the experiments. For experi-ments, they were randomly divided into groups of 8 animals.

Chemicals

Sarin was obtained from Military Technical Institute (Brno, Czech Republic). Its purity (98%) was assayed by acidimetric titration. Trimedoxime (1,1′-propane-1,3–diylbis[4-(E)-(hydroxyiminomethyl)pyridinium] dibro-mide), HI-6 (1-[[4(aminocarbonyl)-pyridinio] methyl]-2(hydroxyiminio)pyridinium dichloride) and the oxime K203 [(E)-1-(4-carbamo-ylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide] were synthesized earlier at the Department of Toxicology (Faculty of Military Health Sciences, Hradec Kralove, Czech Republic). Their purities (97.5%) were analyzed using high-performance liquid chromatography techn-ique [11]. All other drugs and chemicals of analytical grade were obtained commercially and used without fur-ther purification. All substances were administered intra-muscularly at a volume of 1 ml/kg of body weight.

In vivo experiments

Animals were divided into 5 groups (8 rats each): 1) a control group (administered with saline), 2) sarin poisoned group (108 μg/kg – 90% LD_{50}), 3) sarin poisoned group treated with atropine sulphate (21 mg/ kg) and HI-6 (39 mg/kg), 4) sarin poisoned group treated with atropine sulphate, HI-6 and trime-doxime (7.5 mg/kg), and 5) sarin poisoned group treated with atropine sulphate, HI-6 and K203 (16.3 mg/kg). In order to develop a broad-spectrum mixture, the combi-nations were selected based on HI-6 being a relatively strong reactivator of sarin, soman and cyclosarin-inhibited AChE, while trimedoxime or K203 are effective against tabun and pesticides. The oximes were administered at equitoxic doses corre-sponding to 5% of their LD_{50} values. These doses are sufficiently safe. The antidotes were administered 1 min after sarin challenge. Surviving rats were anesthetized by ether vapor 24 h after intoxication and euthanized by decapitation. Their brains were rapidly removed and fixed with a 10% neutral buffered formalin (Chemapol, Prague, Czech Republic). Samples were subsequently embedded into paraffin (Paramix, Holice, Czech Republic) and 6 μm thick coronary brain sections were cut (Microtome model SM2000 R, Leica) at level between 3 mm and 4.2 mm from bregma according to Paxinos stereotactic brain atlas [33]. Three sections for every staining method were evalu-ated by 2 experts. In a section, damage was scored in 6 regions, including amygdaloid body, cortex, hippocampus, hypothalamus, piriform cortex and thalamus with relevance to sarin-induced neuropa-thology [13].

Hematoxylin-eosin staining and histopathology evaluation

Selected brain sections were fixed in 10% buffered formalin (Kulich, Hradec Kralove, Czech Republic), pro-cessed through conventional histological techniques, and stained with hematoxylin and eosin (both Merck, Kenilworth, NJ, USA). The histological changes were scored using a BX-51 microscope (Olympus, Tokyo, Japan) and following semi-quantitative criteria published previously [20]:
(0) no pathology,
(1) mild damage: 1–2 neurons with nucleus margination, chromatolysis and/or vacuolar degeneration,
(2) moderate damage: ≥ 3 neurons with changes described in (1) and/or ≥ 1 shrunken eosinophilic neurons in 1 nucleus/subregion,
(3) severe damage: focal damage or multiple changes described in (2) present in ≥2 nuclei/subregions and/or hemorrhage
(4) very severe damage: diffuse damage of the region and/or multiple hemorrhages

**Fluoro-jade B staining and evaluation**

For Fluoro-Jade B staining, paraffin sections were dewaxed and rehydrated through xylene and an alcohol series. Afterwards, the sections were immersed in 0.06% potassium permanganate (Sigma-Aldrich, St. Louis, MO, USA) for 10 min, rinsed in distilled water for 2 min and dyed by 0.0002% solution of the Fluoro-Jade B (Merck) for 20 min in dark. Washed (3 distilled water washes, 1 min each) and dried slides were mounted with DPX (Sigma-Aldrich) in dark. Fluorescent cells were evaluated using a BX-51 microscope (Olympus) with green excitation fluorescence filter and following semi-quantitative criteria published previously [20]:

(0) no fluorescent cells,
(1) 1–2 fluorescent cells in 1 nucleus/subregion,
(2) ≥ 3 in 1 nucleus/subregion or 1–2 fluorescent cells in ≥2 nuclei/subregions,
(3) multiple fluorescent cells in ≥2 nuclei/subregions and/or hemorrhage,
(4) diffuse fluorescent positivity and/or multiple hemorrhages

**TUNEL immunostaining and evaluation**

TUNEL staining was carried out to identify the apoptotic cells according to the manufacturer's instructions using in situ cell death detection kit (Roche, Mannheim, Germany) as published previously [34]. TUNEL positive cells were evaluated using a BX-51 microscope (Olympus) and following semi-quantitative criteria:

(0) 0–1 TUNEL positive cells in 1 nucleus/subregion
(1) ≥ 2 TUNEL positive cells in 1 nucleus/subregion
(2) multiple TUNEL positive cells in ≥2 nuclei/subregions
(3) diffuse TUNEL positivity

Due to verified false positivity of TUNEL assay and a very slight positivity (1 cell) in all control samples, only 3 levels of damage were recognized compared to the histopathological and Fluoro-Jade B analysis.

| Table 1 | Survival of rats following exposure to sarin |
|---------|-------------------------------------------|
| group   | survival in the time of sample collection (survived/total) | time of death (min) |
| control | 8/8 | 28, 42, 63 |
| GB      | 5/8 | 33, 125 |
| GB + A + H | 6/8 | 59 |
| GB + A + H + T | 7/8 |  |
| GB + A + H + K | 8/8 |  |

**Fig. 1** Sample of dentate gyrus from control group (hematoxylin-eosin, 600fold original magnification). The microphotograph displays a boundary between granular (upper) and polymorphic (lower) layer. The arrow indicates an example of a neuronal vacuole surrounded with condensed cytoplasm.

**Fig. 2** Sample of dentate gyrus from sarin-poisoned rat receiving no treatment (hematoxylin-eosin, 600fold original magnification). The microphotograph displays a boundary between polymorphic (upper) and granular (lower) layer. Sarin-induced damage dominates in polymorphic layer and includes multiple neuronal vacuoles surrounded with condensed chromatin and red cells with condensed nuclei (acute eosinophilic necrosis).
Individual animal brain damage score
Finally, individual animal brain damage scores were calculated representing a sum of histopathological, Fluoro-Jade B and TUNEL scores from all tested brain subregions in one animal.

Data analysis
We used non-parametric Kruskal-Wallis rank sum test with multiple pairwise comparisons to evaluate statistical differences between all groups within particular brain nucleus/subregion, which were considered significant when \( p \leq 0.05 \). All analyses were performed in IBM SPSS Statistics version 22.0 software (IBM Corp., Armonk, NY, USA).

Results
In the case of sarin poisoning without antidotal treatment, three sarin-poisoned rats did not survive until the end of experiment. Antidotal treatment eliminated or at least reduced the mortality of sarin-poisoned rats. Two rats did not survive till the end of experiment if HI-6 in combination with atropine was used for the antidotal treatment and one rat did not survived till the end of experiment if HI-6 and trimedoxime in combination with atropine were used for the antidotal treatment of sarin poisoning. Only HI-6 and K203 in combination with atropine were able to eliminate sarin-induced death of poisoned animals (Table 1).

Histopathological findings
The most extensive alterations were observed in sarin-poisoned animals receiving no antidotal treatment. Compared to the control group, significantly increased histopathological damage scores were measured in cortex, hypothalamus and piriform cortex (\( p = 0.023, 0.001 \) and \( 0.020 \), respectively). In affected animals, cortex and piriform cortex displayed pseudolaminar necrosis in 1 and 3 cases, respectively. Hemorrhage was present in one case in hypothalamus. Single cell damage characterized by multifocal degenerative/necrotic changes accompanied with oedema was observed in the remaining positively scored rats. Especially, basolateral nucleus of amygdaloid body, the third and fifth layer of cortex (pyramidal cells), neurons in CA1 (> dentate gyrus > CA3 > CA2) zone of hippocampus (Figs. 1 and 2), supramamillary hypothalamic nuclei, the third layer of piriform cortex, and medial and dorsolateral thalamic nuclei were markedly impaired.

Administration of antidotal treatment reduced the extent of damage. Any diffuse changes and/or hemorrhage in all groups treated with antidotes was not observed. However, significantly decreased histopathological scores were found in cortex (\( p = 0.009 \)) of animals administered with atropine and HI-6 and in cortex, hippocampus (Fig. 3), hypothalamus, and piriform cortex (\( p = 0.023, 0.010, 0.009, \) and \( 0.020 \), respectively) in rats treated with atropine, HI-6 and K203 (Table 2).

| Table 2 | Score of sarin-induced histopathological damage in rat brain and its modulation by different therapeutic approaches |
|---------|---------------------------------------------------------------------------------------------------------------|
|         | AMG | CTX | HIPP | HYPOTH | PIRI | TH |
| score scale | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| control | 6 1 1 0 0 | 1 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| GB | 2 0 0 3 0 | 1 1 1 1 1 | 1 0 0 0 0 | 2 0 0 0 0 | 3 0 0 0 0 | 0 0 0 0 0 |
| GB + A + H | 6 0 0 0 0 | 2 0 0 0 0 | 3 0 0 0 0 | 1 0 0 0 0 | 4 0 0 0 0 | 0 0 0 0 0 |
| GB + A + H + T | 5 0 2 0 0 | 3 0 0 0 0 | 3 0 0 0 0 | 1 0 0 0 0 | 6 0 0 0 0 | 1 0 0 0 0 |
| GB + A + H + K | 6 2 0 0 0 | 7 0 0 0 0 | 8 0 0 0 0 | 0 0 0 0 0 | 6 1 1 0 0 | 0 0 0 0 0 |

Each number represents a number of animals suffering from particular damage score in the group

Bold: statistically significant compared to control group (\( p \leq 0.05 \))

Bold italic: statistically significant compared to sarin-poisoned group (\( p \leq 0.05 \))

Abbreviations: A atropine, AMG amygdaloid body, CTX cortex, GB sarin, H oxime HI-6, HIPP hippocampus, HYPOTH hypothalamus, K oxime K203, PIRI piriform cortex, T trimedoxime, TH thalamus
**Fluoro-jade B**

Significantly increased Fluoro-Jade B positivity in amygdaloid body, cortex, hypothalamus, piriform cortex, and thalamus ($p = 0.029, 0.032, < 0.001, 0.032$, and $0.029$, respectively) was found in non-treated, sarin-poisoned rats. Diffuse fluorescent patterns were present in 3 of 5 surviving rats. In all six regions, maximal Fluoro-Jade B positivity correlated with histopathological findings (Figs. 4 and 5).

The antidotal treatment markedly reduced brain degeneration marked by Fluoro-Jade B. We found significantly decreased Fluoro-Jade B positivity in hippocampus ($p = 0.017$) of atropine and HI-6 treated rats and in amygdaloid body and hippocampus ($p = 0.038$ and $0.026$, respectively) of animals administered with atropine, HI-6 and trimedoxime. No hemorrhages were observed in both groups. In atropine, HI-6 and K203 treated group, Fluoro-Jade B fluorescence significantly decreased in cortex, hippocampus (Fig. 6), piriform cortex, and thalamus ($p = 0.032, < 0.001, 0.032$, and $0.029$, respectively). On the other hand, a small hemorrhage in the hypothalamic region was found. However, Fluoro-Jade B positivity in this area was scored as 1 (Table 3).

**TUNEL**

TUNEL positivity was significantly increased in amygdaloid body, hippocampus and piriform cortex ($p = 0.012, 0.003$ and $0.041$, respectively) of sarin-poisoned animals without antidotal treatment. The distribution of TUNEL staining pattern corresponds to the histopathological and Fluoro-Jade B findings except for hippocampal dentate gyrus (Figs. 7 and 8).

Atropine and HI-6 treatment did not significantly influence the outcome of sarin intoxication. In atropine, HI-6 and trimedoxime treated group, a decreased amount of TUNEL positive cells was only observed in amygdaloid body ($p = 0.016$). The combination of atropine, HI-6 and K203 decreased positivity in amygdaloid body, hippocampus (Fig. 9) and piriform cortex ($p = 0.012, 0.003$ and $0.041$, respectively) (Table 4).

**Individual animal brain damage score**

Compared to the control group, a significantly increased individual animal brain damage scores in
sarin-poisoned group without antidotal treatment was found \((p < 0.001)\). Sarin-induced damage was significantly reduced only in atropine, HI-6 and K203 treated rats \((p = 0.019)\) (Table 5).

**Discussion**

In this study, the neuroprotective effects of single oxime (HI-6) or two oxime mixtures (HI-6 and trimedoxime or oxime K203) in combination with atropine in sarin-poisoned rats was evaluated. The model used in this study follows our previous study, in which the benefit of oxime mixtures for the neuroprotective efficacy of antidotal treatment of nerve agent poisoning was demonstrated using a functional observatory battery [19].

Herein, we focus on structural brain changes using three different approaches, including histopathological, Fluoro-Jade B and TUNEL analysis.

Based on our results, the established scoring system resulted in positivity of histopathological method in 5 (of 8) control animals due to the presence of cytoplasmic vacuoles. The clinical significance of vacuolation is not known. Neuronal vacuoles can appear spontaneously, especially in aging animals, without having any obvious clinical relevance [23, 40].

After sarin poisoning, a widespread brain damage was observed. The alterations were particularly profound in cholinceptive subregions and/or subregions sensitive to hypoxic/ischemic stress [3, 27, 29, 35]. This observation is in accordance with generally accepted mechanism of nerve agent-induced acute brain injury and with previously published findings [6, 13, 28]. The distribution of histopathological changes correlated with Fluoro-Jade B and TUNEL staining pattern with one exception. In gyrus dentatus (hippocampus), histopathological findings and Fluoro-Jade B fluorescence showed the highest positivity in the polymorphic layer, whereas TUNEL positivity prevailed in the granular layer. Although both layers display different sensitivity to various stress

### Table 3 Score of sarin-induced degeneration (Fluoro-Jade B positivity) in rat brain and its modulation by different therapeutic approaches

|                | AMG | CTX | HIPP | HYPOTH | PIRI | TH |
|----------------|-----|-----|------|--------|------|----|
| score scale    | 0   | 1   | 2    | 3      | 4    | 0  |
| control        | 8   | 0   | 0    | 0      | 0    | 0  |
| GB             | 2   | 0   | 0    | 2      | 2    | 0  |
| GB + A + H     | 5   | 0   | 0    | 1      | 5    | 0  |
| GB + A + H + T | 7   | 0   | 0    | 1      | 5    | 2  |
| GB + A + H + K | 7   | 1   | 0    | 0      | 8    | 0  |

Each number represents a number of animals suffering from particular damage score in the group

**Bold**: statistically significant compared to control group \((p \leq 0.05)\)

**Bold Italic**: statistically significant compared to sarin-poisoned group \((p \leq 0.05)\)

**Abbreviations**: A atropine, AMG amygdaloid body, CTX cortex, GB sarin, H oxime HI-6, HIPP hippocampus, HYPOTH hypothalamus, K oxime K203, PIRI piriform cortex, T trimedoxime, TH thalamus

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Fig. 7 Sample of dentate gyrus from control group (TUNEL assay counterstained with methyl green, 600-fold original magnification). The microphotograph displays a boundary between granular (upper) and polymorphic (lower) layer with no TUNEL positivity.

Fig. 8 Sample of dentate gyrus from sarin-poisoned animal receiving no treatment (TUNEL assay counterstained with methyl green, 600-fold original magnification). The microphotograph displays a boundary between granular (upper) and polymorphic (lower) layer. Three TUNEL positive cells were detected in the granular layer.
stimuli [15, 38], the reason of this discrepancy remains uncertain. An explanation may lie in the limitations of TUNEL method. The method is used to detect DNA fragmentation in apoptotic and necrotic cells [2]. On the other hand, DNA fragmentation occurs also in S phase of cell cycle and the method was reported to produce false positivity in proliferating tissues [9]. Since the subgranular zone of dentate gyrus is considered to be one of the two main neurogenic zones in the adult brain [5], TUNEL positivity observed in this subregion could possibly represent the damage as well as its regeneration.

Antidotal treatment plays a pivotal role in acute phase of nerve agents’ toxicity [6]. In our model, we utilized bispyridinium oximes. Although the bispyridinium oximes poorly penetrate across the blood–brain barrier, they are able to re Activate sarin-inhibited AChE not only at the peripheral compartment but also in the brain when they are administered at equitoxic doses corresponding to 5% of their LD₅₀ values at 1 min after the administration of sarin [14, 18, 19]. According to our results, all three therapeutic regimes mitigated the extent of sarin-induced brain injury. Even though we did not find any statistically significant differences among groups treated with oximes, the neuroprotective efficacy of oxime mixtures was slightly higher compared to the group treated with a single oxime. Particularly, the combination of HI-6 and K203 appears to be the most effective to protect experimental animals from acute sarin-induced neuropathological changes in surviving animals. This conclusion corresponds to the previously published results demonstrating the benefit of combinations of oximes for the reactivating and therapeutic efficacy of antidotal treatment of sarin poisoning in rats and mice [18]. Based on the described results, both trimedoxime and K203 do not interfere with HI-6 bioavailability but rather support its action. Therefore, combining the oximes in the antidotal treatment could be a promising step towards a broad-spectrum antidotal treatment of acute nerve agent-exposure regardless of the chemical structure of nerve agent. Such approach may also help to overcome similar issue regarding organophosphorus pesticides. HI-6 has only limited reactivation efficacy towards chlorpyrifos, dichlorvos, methamidophos, and paraoxon [1, 12, 30, 31]. Its combination with trimedoxime or K203 might not only improve these results but may also broaden the spectrum of reactivation towards leptophos-oxon and possibly other pesticides [12]. On the other hand, even in HI-6 and K203 group, animals displayed signs of neuronal damage, which tends to progress overtime [13]. Limited therapeutic efficacy is probably related to low blood-brain barrier (BBB) penetration of bispyridinium oximes [10, 16]. Thus, the development and usage of oxime mixtures with better BBB penetration may further increase their ability to counteract the acute toxicity of organophosphorus compounds.

### Table 4 Score of sarin-induced apoptotic activity (TUNEL positivity) in rat brain and its modulation by different therapeutic approaches

|            | AMG | CTX | HIPP | HYPOTH | PIRI | TH |
|------------|-----|-----|------|--------|------|----|
| score scale| 0   | 1   | 2    | 3      | 0    | 1  |
| control    | 8   | 0   | 0    | 0      | 8    | 0  |
| GB         | 2   | 2   | 1    | 0      | 0    | 5  |
| GB + A + H | 5   | 1   | 0    | 4      | 1    | 1  |
| GB + A + H + T | 7 | 0   | 0    | 6      | 1    | 0  |
| GB + A + H + K | 8 | 0   | 0    | 8      | 0    | 0  |

Each number represents a number of animals suffering from particular damage score in the group

**Bold:** statistically significant compared to control group (p ≤ 0.05)

**Bold Italic:** statistically significant compared to sarin-poisoned group (p ≤ 0.05)

**Abbreviations:** A atropine, AMG amygdaloid body, CTX cortex, GB sarin, H oxime HI-6, HIPP hippocampus, HYPOTH hypothalamus, K oxime K203, PIRI piriform cortex, T trimedoxime, TH thalamus
Table 5 Individual animal brain damage score

| animal No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|------------|---|---|---|---|---|---|---|---|
| control    | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 3 |
| GB         | 5 | 10| 47| 48| 51|
| GB + A + H | 0 | 3 | 5 | 6 | 7 | 36|
| GB + A + H + T | 0 | 3 | 3 | 4 | 4 | 5 | 28|
| GB + A + H + K | 0 | 1 | 1 | 3 | 3 | 3 | 3 | 3 |

Each number represents a number of animals suffering from particular damage score in the group

Bold: statistically significant compared to control group (p ≤ 0.05)
Bold Italic: statistically significant compared to sarin-poisoned group (p ≤ 0.05)

Abbreviations: A: atropine, GB: sarin, H: oxime HI-6, K: oxime K203, T: trimedoxime

Conclusion

Our results show that neuroprotective potency of both oxime combinations (HI-6 with trimedoxime and HI-6 with K203) is slightly higher than using HI-6 alone in sarin poisoned model. Particularly, the oxime HI-6 in combination with K203 seems to be the most effective. Using oxime combinations seems to be an effective approach to develop a potent broad-spectrum reactivator.

Abbreviations

AChE: Acetylcholinesterase; EC: Enzyme commission number; i.m.: Intramuscularly; LD50: Median lethal dose; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling

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Availability of data and materials

All tested compounds as well as the datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

FC and JK conceived and planned the experiments. FC, JP and JM carried out the experiments. FC and JP participated in sample evaluation. FC, JP, JM, JK, MW, and KK contributed to the interpretation of the results. FC took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and both have read and approved the manuscript.

Ethics approval

Handling with the experimental animals was approved by the Ethics Committee of the Faculty of Military Health Sciences in Hradec Králové (Czech Republic) by approval number: 57–10/2011–3696.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Antonijevic E, Musilek K, Kuca K, Djukic-Cosic D, Vucinic S, Antonijevic B. Therapeutic and reactivating efficacy of oximes K027 and K020 against a direct acetylcholinesterase inhibitor. Neurotoxicology. 2016;55:33–9.
2. Baille V, Clarke PG, Brochier G, Dorandeu F, Verna JM, Four E, Lallement G, Carpentier P. Soman-induced convulsions: the neuropathology revisited. Toxicology. 2005;215:1–24.
3. Ben-Ari Y, Zigmond RE, Shute CC, Lewis PR. Regional distribution of choliner and acetyltransferase and acetylcholinesterase within the amygdaloid complex and stria terminalis system. Brain Res. 1977;120:435–44.
4. Brown MA, Brix KA. Review of health consequences from high-, intermediate- and low-level exposure to organophosphorus nerve agents. J Appl Toxicol. 1998;18:393–408.
5. Cyre M, Carol P, Goldman JE. Cell migration in the normal and pathological postnatal mammalian brain. Prog Neurobiol. 2009;88:41–63.
6. Chen Y. Organophosphate-induced brain damage: mechanisms, neuropsychiatric and neurological consequences, and potential therapeutic strategies. Neurotoxicology. 2012;33:391–400.
7. Clement JC. Central activity of acetylcholinesterase oxime reactivators. Toxicol Appl Pharmacol. 1992;112:104–9.
8. Geoghegan J, Tong JL. Chemical warfare agents. Contin Educ Anaest Crit Care Pain. 2006;6230–40.
9. Ito Y, Shibata MA, Kusakabe K, Otsuki Y. Method of specific detection of apoptosis using formamide-induced DNA denaturation assay. J Histochem Cytochem. 2006;54:683–92.
10. Jossen MJ, van der Schans MJ, van Dijk CG, Kuijpers WC, Wortelboer HM, van Helden HP. Increasing oxime efficacy by blood-brain barrier modulation. Toxicol Lett. 2011;206:67–71.
11. Jun D, Kuca K, Stodulka P, Koleckar V, Dolezal B, Simon P, Veverka M. HPLC analysis of HI-6 dichloride and dimethanosulfonate – antidotes against nerve agents and organophosphorous pesticides. Anal Lett. 2007;40:2785–7.
12. Jun D, Musilova L, Musilek K, Kuca K. In vitro ability of currently available oximes to reactivate organophosphate pesticide-inhibited human acetylcholinesterase and butyrylcholinesterase. Int J Mol Sci. 2011;12:2077–87.
13. Kadar T, Shapira S, Cohen G, Sahar R, Alkalay D, Raveh L. Sarin-induced neuropathology in rats. Hum Exp Toxicol. 1995;14:252–9.
14. Kalasz H, Nulalain SM, Veress G, Artsus S, Darvas F, Adeghate E, Adem A, Hashemi F, Tekes K. Mini review on blood-brain barrier penetration of pyridinium aldoximes. J Appl Toxicol. 2015;35:116–23.
15. Kaler S, Dhar P, Bhattacharya A, Mehra RD. Preliminary morphological and immunohistochemical changes in rat hippocampus following postnatal exposure to sodium arsenite. Toxicol Int. 2013;20:160–9.
16. Karasova JZ, Pohanka M, Musilek K, Zemek F, Kuca K. Passive diffusion of acetylcholinesterase oxime reactivators through the blood-brain barrier: influence of molecular structure. Toxicol in Vitro. 2010;24:1839–44.
17. Kassa J, Karasova J, Bajgar J, Kuca K, Musilek K. A comparison of the therapeutic and reactivating efficacy of newly developed bisporydinium compounds (K206, K269) with currently available oximes against tabun in rats and mice. J Enzyme Inhib Med Chem. 2008;23:776–80.
18. Kassa J, Karasova JZ, Sepsova V, Caisberger F. The benefit of combinations of oximes for the reactivating and therapeutic efficacy of antidotal treatment of sarin poisoning in rats and mice. Basic Clin Pharmacol Toxicol. 2011;109:30–4.
19. Kassa J, Kunesova G. The benefit of combination of oximes for the neuroprotective efficacy of antidotal treatment of sarin-poisoned rats. Toxicol Mech Methods. 2012;22:260–7.
20. Kassa J, Misik J, Hatlapatkova J, Zdarova Karasova J, Sepsova V, Caisberger F, Pejchal J. The evaluation of the reactivating and neuroprotective efficacy of two newly prepared Bispyridinium oximes (K305, K307) in Tabun-poisoned rats—a comparison with Trimedoxime and the oxime K203. Molecules. 2017;22:1152.

21. Kassa J, Sepsova V, Matouskova L, Horova A, Musilek K. A comparison of the reactivating and therapeutic efficacy of two novel bispyridinium oximes (K727, K733) with the oxime Hi-6 and obidoxime in sarin-poisoned rats and mice. Basic Clin Pharmacol Toxicol. 2015b;116:367–71.

22. Kassa J, Sepsova V, Tumova M, Horova A, Musilek K. A comparison of the reactivating and therapeutic efficacy of two newly developed oximes (K727 and K733) with oxime K203 and trimedoxime in tabun-poisoned rats and mice. Basic Clin Pharmacol Toxicol. 2015b;116:367–71.

23. Kinka G. Neuronal vacuolation. Toxicol Pathol. 2011;39:1140.

24. Kuca K, Hrabinova M, Jun D, Musilek K, Penhaker M, Krejcar O, Soukup O. Universality of oxime K203 for reactivation of nerve agent-inhibited AChE. Med Chem. 2015;11:683–6.

25. Labat-Moleur F, Guillermet C, Lorimier P, Robert C, Lantuejoul S, Brambilla E, Negoescu A. TUNEL apoptotic cell detection in tissue sections: critical evaluation and improvement. J Histochem Cytochem. 1998;46:327–34.

26. Lundy PM, Hansen AS, Hand BT, Boulet CA. Comparison of several oximes against poisoning by soman, tabun and GF. Toxicology. 1992;72:99–105.

27. Lysakowski A, Wainer BH, Bruce G, Hersh LB. An atlas of the regional and laminar distribution of choline acetyltransferase immunoreactivity in rat cerebral cortex. Neuroscience. 1989;28:291–196.

28. McDonough JH Jr, Shih TM. Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology. Neurosci Biobehav Rev. 1997;21:559–79.

29. Mesulam MM, Mufson EJ, Wainer BH, Levey AI. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience. 1983;101:185–201.

30. Musilek K, Holas O, Jun D, Dohnal V, Gunn-Moore F, Dolezal M, Kuca K. Monooxime reactivators of acetylcholinesterase with (E)-but-2-ene linker: preparation and reactivation of tabun- and paraoxon-inhibited acetylcholinesterase. Bioorg Med Chem. 2007;15:6733–41.

31. Musilova L, Kuca K, Jung YS, Jun D. In vitro oxime-assisted reactivation of paraoxon-inhibited human acetylcholinesterase and butyrylcholinesterase. Clin Toxicol (Phila). 2009;47:545–50.

32. Okumura T, Seto Y, Fuse A. Countermeasures against chemical terrorism in Japan. Forensic Sci Int. 2013;227:2–6.

33. Paxinos G, Watson C. The rat brain in stereotactic coordinates. 4th ed. San Diego: Academic Press; 2006.

34. Pejchal J, Novotny J, Marak V, Osterreicher J, Tichy A, Vavrova J, Sinkorova Z, Zarybricka L, Novotna E, Babicova A, Kubelkova K, Kuca K. Activation of p38 MAPK and expression of TGF-ß1 in rat colon enterocytes after whole body y-irradiation. Int J Radiat Biol. 2012;88:348–58.

35. Riudavets MA, Aronica-Pollak P, Troncoso JC. Pseudolaminar necrosis in cyanide intoxication: a neuropathology case report. Am J Forensic Med Pathol. 2005;26:189–91.

36. Rosman Y, Eisenkraft A, Milik N, Shiyovich A, Opfrin N, Shrot S, Kreiss Y, Kassirer M. Lessons learned from the Syrian sarin attack: evaluation of a clinical syndrome through social media. Ann Intern Med. 2014;160:644–8.

37. Schmued LC, Albertson C, Slikker W Jr. Fluoro-Jade: a novel fluorochrome for the sensitive and reliable histochemical localization of neuronal degeneration. Brain Res. 1997;751:37–46.

38. Valente T, Domínguez MI, Bellmann A, Journot L, Ferrer I, Auladell C. Zac1 is up-regulated in neural cells of the limbic system of mouse brain following seizures that provoke strong cell activation. Neuroscience. 2004;128:323–36.

39. Wilhelm CM, Snider TH, Babin MC, Jett DA, Platoff GE Jr, Yeung DT. A comprehensive evaluation of the efficacy of leading oxime therapies in Guinea pigs exposed to organophosphorus chemical warfare agents or pesticides. Toxicol Appl Pharmacol. 2014;281:254–65.

40. Wohlfart P, Deschl U, Baumgarthner W. Nonleisons, unusual cell types, and postmortem artifacts in the central nervous system of domestic animals. Vet Pathol. 2013;50:122–43.

41. Worek F, Aubert N, Thiemann H. Reactivation of organophosphate-inhibited human AChE by combinations of obidoxime and Hi 6 in vitro. J Appl Toxicol. 2007;27:582–8.