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Reactivation of brain acetylcholinesterase by monoisonitrosoacetone increases the therapeutic efficacy against nerve agents in guinea pigs

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Abstract

Current oxime therapies do not readily cross the blood–brain barrier to re Activate organophosphorus nerve agent–inhibited cholinesterase (ChE) within the CNS. We investigated the ability of monoisonitrosoacetone (MINA), a tertiary oxime, to reactivate ChE inhibited by the nerve agent sarin (GB), cyclosarin (GF), or VX, in peripheral tissues and brain of guinea pigs and determined whether reactivation in the CNS will enhance protection against the lethal effects of these three agents. In the reactivation experiment, animals were pretreated with atropine methyl nitrate (1.0 mg/kg, i.m.) 15 min prior to subcutaneous (s.c.) challenge with 1.0 × LD₅₀ of GB, GF, or VX. Fifteen minutes later animals were treated intramuscularly (i.m.) with MINA (ranging from 22.1 to 139.3 mg/kg) or 2-PAM (25.0 mg/kg). At 60 min after nerve agent, CNS (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, and striatum) and peripheral (blood, diaphragm, heart, and skeletal muscle) tissues were collected for ChE analysis. MINA reactivated nerve agent–inhibited ChE in the CNS and peripheral tissues in a dose–dependent manner in the following order of potency: GB > GF > VX. In a survival experiment, animals were injected i.m. with atropine sulfate (0.5 mg/kg), 2-PAM (25.0 mg/kg), or MINA (35.0, 60.0, or 100.0 mg/kg) alone or in combination 1 min after challenge with varying s.c. doses of GB, GF, or VX to determine the level of protection. The rank order of MINA’s efficacy in guinea pigs against nerve agent lethality was the same as for reactivation of inhibited ChE in the CNS. These data show that MINA is capable of reactivating nerve agent–inhibited ChE and that the extent of ChE reactivation within the CNS strongly relates to its therapeutic efficacy.

1. Introduction

Organophosphorus nerve agents, such as sarin (GB), cyclosarin (GF), and VX, are potent inhibitors of the enzyme cholinesterase (ChE). Their toxic effects are due to hyperactivity of the cholinergic system as a result of ChE, especially acetylcholinesterase (AChE) inhibition, and the subsequent increase in the level of the neurotransmitter acetylcholine (ACh) in the brain and periphery [1]. Severe inhibition of ChE in the brain initiates seizures, which if not terminated result in neuropathology and contribute to the incapacitating effects of these agents [2,3].

In the event of nerve agent poisoning, immediate medical therapy with atropine sulfate to antagonize the effects of ACh at muscarinic receptors, and an oxime, such as 2-PAM (pralidoxime), P2S, obidoxime (Toxogonin®), or HI-6, to reactivate any unaged, inhibited enzyme [1,4–6] is used to prevent lethality. These oximes possess positively charged quaternary nitrogen structures, cannot readily cross the blood–brain barrier (BBB), and reactivate ChE only in the periphery. Thus, their inability to enter the central nervous system (CNS) and re activate nerve agent–inhibited brain ChE is a major limitation of current oxime therapy. Monoisonitrosoacetone (MINA) is a tertiary oxime that was investigated in the 1950s. It is highly lipid soluble and can readily penetrate the BBB [7], and is able to re activate ChE within the CNS [7,8]. When used alone or in combination with atropine sulfate, MINA was shown to raise the LD₅₀ doses of GB in several animal species [8–13]. Unfortunately, this tertiary oxime was not pursued further, due to reports that quaternary pyridinium oximes (e.g., 2-PAM) were more potent.

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reactivators of phosphorylated ChE by several orders of magnitude [14]. We recently showed that MINA reactivated ChE in the brain, reduced toxic signs, improved survival, and prevented or terminated seizures following GB intoxication in guinea pigs [15,16]. These findings support previous reports that protection of ChE enzyme activity in the brain as well as in peripheral tissues with centrally acting reversible ChE inhibitors (such as physostigmine) improved survival, prevented seizure occurrence, and reduced neuropathology and behavioral consequences of nerve agent intoxication more effectively than peripherally acting reversible ChE inhibitors, such as pyridostigmine [17–19]. The present study was designed to further evaluate the capability of MINA to reactivate ChE in discrete brain regions when given after the maximal inhibition of this enzyme by the nerve agents GB, GF, and VX was reached [20]. We also conducted a survival experiment, first to see if there was a relationship between CNS reactivation by MINA and survival, and secondly, to examine the effects of combining MINA with 2-PAM in the treatment regimen following exposure to these nerve agents.

2. Materials and methods

2.1. Subjects

Male Hartley guinea pigs (Crl:(HA) BR COPS) weighing 250–300 g were purchased from Charles River Labs (Kingston, NY). They were housed in individual cages in temperature (21 ± 2 °C) and humidity (50 ± 10%) controlled quarters that were maintained on a 12-h light–dark schedule (with lights on at 0600 h). Laboratory chow and filtered tap water were freely available whenever the animals were in home cages.

2.2. Materials

Saline (U.S.P.), Attane™ (Isoflurane, U.S.P.), and heparin sodium were purchased from Braun Medical, Inc. (Irvine, CA), Minrad, Inc. (Bethlehem, PA), and U.S.P., Inc. (Rockville, MD), respectively. 2-PAM was purchased from Ayerst Labs, Inc. (New York, NY). MINA, acetylthiocholine iodide, atropine methylnitrate, and atropine sulfate were purchased from Sigma–Aldrich (St. Louis, MO). Bicinchoninic acid (BCA) Protein Assay Reagent A and Reagent B were purchased from Pierce Biotechnology, Inc. (Rockford, IL). Sarin (GB; isopropyl methylphosphonofluoridate), cyclosarin (GF; cyclohexyl methylphosphonofluoridate), and VX (O-ethyl S-(2-(diisopropylamino)ethyl) methylphosphonothioate) were obtained from the US Army Edgewood Chemical Biological Center (AberdeenProvingGround, MD). Nerve agents were diluted in ice-cold saline prior to subcutaneous (s.c.) injection. In the reactivation experiment atropine methylthionitrate and oxime compounds were prepared in saline individually for intramuscular (i.m.) injection. In the survival experiment, atropine sulfate and 2-PAM were admixed and MINA was injected separately. Injection volumes were 0.50 ml/kg for nerve agent and all treatment drugs.

2.3. Reactivation experiment

One to three days prior to the experiment, control blood samples (~0.5 ml) were drawn using the toenail clip method [21] and collected into a 1.0-ml microfuge tube containing 50 μl of heparin sodium (15 U/ml) to determine baseline ChE activity in whole blood (WB) and red blood cells (RBC). On the day of the study, guinea pigs were pretreated with atropine methylnitrate (1.0 mg/kg, i.m.) 15 min prior to a nerve agent exposure to minimize peripheral toxic effects. Atropine methylnitrate is a peripherally acting muscarinic receptor blocker that does not affect ChE activity. Animals were injected s.c. with either saline (0.5 ml/kg) or a 1.0× LD50 dose of GB (42.0 μg/kg), GF (57.0 μg/kg), or VX (8.0 μg/kg). The severity of toxic signs of each animal was scored at 13 min after nerve agent. Fifteen minutes after nerve agent injection, when the inhibition of ChE activity by these nerve agents reached maximum [20], saline (0.5 ml/kg), 2-PAM (25.0 mg/kg), or MINA (22.1, 35.0, 55.5, 87.9, or 139.3 mg/kg) was given i.m. Control animals received s.c. saline (no nerve agent) and i.m. saline (no oximes). There were eight animals assigned to each treatment group.

Sixty minutes after s.c. saline or nerve agent administration, the animals were deeply anesthetized with isoflurane and euthanized by decapitation. Blood (~0.5 ml) was collected into a 1.0-ml microfuge tube containing 50 μl of heparin sodium solution (15 U/ml). For the WB samples, 20 μl of blood was diluted 1:25 in 1% Triton-X100 solution. For the RBC samples, the original blood sample was centrifuged for 5 min at 14,000 rpm, and 10 μl of the packed RBC was then diluted 1:50 in 1% Triton-X100 solution. Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord and striatum) and peripheral tissue (diaphragm, heart, and skeletal muscle) were dissected. Brain samples were diluted 1:20, while peripheral samples were diluted 1:5, in 1% Triton-X100 solution and then homogenized. The homogenates were then centrifuged (31,000 × g at 4 °C; 20 min for brain and 30 min for peripheral tissues) and the supernatant decanted and kept frozen at −80 °C until ChE analysis.

2.3.1. Toxic signs test

At approximately 13 min and 58 min after GB, GF, or VX injection, guinea pigs were observed for signs of cholinergic toxicity, including secretions (salivation or lacrimation), motor deficits, and general state (activity and coordination) based on the procedures described earlier [22]. Animals were scored for absence [0] or presence [1] of each of the following signs: salivation, lacrimation, and nystagmus. General motor scores were assessed on the basis of a 0–3 scoring scale: normal = 0, fasciculation = 1, tremor = 2, or convolution = 3. The guinea pig was allowed to walk on the bench top, and general state was also assessed on a 0–3 scoring scale: normal = 0, mild uncoordination = 1, impaired movement/with righting reflex = 2, or prostration/no righting reflex = 3. A cumulative score was then calculated by tabulating the salivation, lacrimation, nystagmus, general motor and general state scores for each subject. The maximal attainable score was 9. A cumulative score was categorized as mild intoxication [0–3.0], moderate intoxication [3.1–6.0] and severe intoxication [6.1–9.0].

2.3.2. ChE activity assay

Processed blood, tissue and brain samples were analyzed for ChE activity and protein concentrations according to the methods described by Shih et al. [21,22], based on the colorimetric method of Ellman et al. [23] and a BCA protein assay (Pierce Biotechnology, Inc.). Briefly, in the modified Ellman method, 7 μl of sample (or enzyme standard), 20 μl H2O, 200 μl DTNB reagent, and 30 μl acetylthiocholine iodide substrate was added to each well of a 96 well microplate (samples run in triplicate), and mean activity was determined by applying Beer–Lambert’s Law, incorporating the observed change in absorbance, known extinction coefficient of the DTNB ion, path-length correction, and dilution factor. All sample values were well within the linear range of the standard curve. Protein concentrations of brain and tissue samples in the BCA assay were determined by interpolating from a standard curve, curve. Protein concentrations of brain and tissue samples in the BCA assay were determined by interpolating from a standard curve, curve. Protein concentrations of brain and tissue samples in the BCA assay were determined by interpolating from a standard curve, curve. Protein concentrations of brain and tissue samples in the BCA assay were determined by interpolating from a standard curve, curve. Protein concentrations of brain and tissue samples in the BCA assay were determined by interpolating from a standard curve, curve.
was normalized as a percentage of each sample's corresponding baseline activity.

2.4. Survival experiments

Guinea pigs were challenged s.c. with various doses of GB, GF, or VX to establish dose–lethality curves and to estimate a 24-h nerve agent LD₅₀ for each treatment regimen. Treatments were injected i.m. in a hind limb 1 min after nerve agent challenge and consisted of one of the following: atropine sulfate (0.5 mg/kg), 2-PAM (25.0 mg/kg), MINA (35.0, 60.0 or 100.0 mg/kg), atropine sulfate + 2-PAM, atropine sulfate + MINA, or atropine sulfate + 2-PAM + MINA. The dose–lethality curves were generated using a sequential, up–down, stage design [24–26]. With this design each stage consisted of 3–6 nerve agent doses with 1–4 animals/dose selected to cover the predicted range of lethality from 0% to 100% for each nerve agent and treatment regimen. Nerve agent doses after the first stage were based on the lethality responses from the previous stage(s). After each stage, probit dose–response models using maximum likelihood were fitted to the combined data for all stages [27]. Three or four stages and 19–42 animals were used to generate each dose–lethality curve.

The doses of atropine sulfate and 2-PAM used in this study were human relevant doses of these drugs. The atropine sulfate dose (0.5 mg/kg) was the human equivalent of three 2-mg atropine autoinjectors (MARK I Kit®) based on inter–species body surface area scaling formulas recommended by the Food and Drug Administration [28]. The 2-PAM dose (25.0 mg/kg) was the human
received atropine methylnitrate (1.0 mg/kg, i.m.) 15 min prior to nerve agent exposure. Efficacy of MINA treatment against nerve agents in guinea pigs. Table 3A

Summary of the significant ChE reactivation by oxime treatments in brain regions, peripheral tissues and blood components following exposure to GB, GF, and VX. a.

<table>
<thead>
<tr>
<th>GRP#</th>
<th>Treatment (mg/kg)a</th>
<th>GB LD50 ratiosb (95% CI)</th>
<th>GF</th>
<th>VX</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-PAM (25.0)b</td>
<td>MINA (22.1)</td>
<td>MINA (35.0)</td>
<td>MINA (55.5)</td>
<td>MINA (87.9)</td>
</tr>
<tr>
<td>(A) Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, stratum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>GF</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>VX</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>(B) Peripheral tissues (diaphragm, heart, skeletal muscle)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GF</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VX</td>
<td>**</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(C) Blood (red blood cells and whole blood)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>**</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GF</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VX</td>
<td>**</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

a For each of the brain regions (A), peripheral tissues (B), or blood components (C), an oxime treatment that significantly reactivated a nerve agent-inhibited ChE activity was assigned a “+” sign. When an oxime treatment did not significantly reactivate a nerve agent-inhibited ChE in any tissue, it was assigned a “−” sign.

b Numbers inside the pairs of parentheses indicated the dosages (mg/kg, i.m.) of oxime administered 15 min after challenge with 1.0 × LD50 dose of GB, GF, or VX. Animals received atropine methylnitrate (1.0 mg/kg, i.m.) 15 min prior to nerve agent exposure.

2.5. Data analysis

In the reactivation experiment, statistical analysis of toxic sign scores was performed using a Kruskal–Wallis test to compare across treatments. A Dunn test was then performed as a post-test for multiple comparisons. Differences in the incidence of toxic signs between treatment groups were evaluated using Fisher’s exact test. A Mann–Whitney test was performed to compare toxic sign scores between the 13-min and 58-min scoring times.

For ChE activity statistical analysis of enzymatic activities was performed using a one-way ANOVA to compare across treatments. A post hoc Tukey test was performed for multiple comparisons. Statistical significance for all tests was defined as p < 0.05.

We divided the ChE reactivation data into three compartments: the brain regions, the peripheral tissues, and the blood components. A “+” sign was assigned for each of the brain regions, peripheral tissues, or blood components, when an oxime treatment significantly reactivated nerve agent-inhibited ChE. For example, if an oxime treatment significantly reactivated ChE in all seven brain regions its cell received “++++++++” maximum, in all three peripheral tissues its cell received “++++” maximum, or in the two components of blood its cell received “+++” maximum. When an oxime treatment did not significantly reactivate a nerve agent-inhibited ChE in any tissue, it was assigned a “−” sign.

In the survival experiment, custom-designed probit models (Battelle Memorial Institute, Columbus, OH) and SAS NLIN (V 6.12) were used to calculate LD50s, 95% confidence intervals (CI), and for comparison of the LD50s. LD50 ratio (or protective ratio [PR]) was defined as the nerve agent LD50 for a particular treatment regimen divided by the nerve agent LD50 for atropine sulfate treatment. LD50 ratios were considered significantly different (p < 0.05) from each other, by definition, if their 95% CI did not include the number 1.0.

3. Results

3.1. Reactivation experiment

3.1.1. Signs of toxicity

About 60 min after exposure to a 1.0 × LD50 s.c. dose of GB, GF, or VX, guinea pigs not receiving any oxime (saline-treated group) therapy showed a high incidence of cholinergic toxic signs (GB 92%; GF 100%; and VX 78%). The average toxic sign scores for GB- and VX-exposed untreated animals significantly increased from the 13-min to the 58-min scoring time (from 2.62 to 5.00 and from 0.33 to 3.44, respectively) and rated in the moderate range. Toxic sign scores for GF-exposed untreated animals also rated in the moderate

Table 3A

<table>
<thead>
<tr>
<th>GRP#</th>
<th>Treatment (mg/kg)a</th>
<th>LD50 ratiosb (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Atr (0.5)</td>
<td>GB: 1.0 (1.3–13.3)</td>
</tr>
<tr>
<td>2</td>
<td>2-PAM (25)</td>
<td>GB: 2.0 (1.0–1.4)</td>
</tr>
<tr>
<td>3</td>
<td>Atr + 2-PAM</td>
<td>GB: 3.3 (1.0–1.4)</td>
</tr>
<tr>
<td>4</td>
<td>MINA (35)</td>
<td>GB: 3.6 (1.1–1.8)</td>
</tr>
<tr>
<td>5</td>
<td>MINA (60)</td>
<td>GB: 7.3 (1.3–1.6)</td>
</tr>
<tr>
<td>6</td>
<td>MINA (100)</td>
<td>GB: 7.1 (1.3–1.6)</td>
</tr>
<tr>
<td>7</td>
<td>Atr + MINA (35)</td>
<td>GB: 4.4 (1.1–1.9)</td>
</tr>
<tr>
<td>8</td>
<td>Atr + MINA (60)</td>
<td>GB: 7.8 (1.6–2.8)</td>
</tr>
<tr>
<td>9</td>
<td>Atr + MINA (100)</td>
<td>GB: 7.8 (2.0–2.7)</td>
</tr>
<tr>
<td>10</td>
<td>Atr + 2-PAM + MINA (35)</td>
<td>GB: 16.7 (6.3–44.1)</td>
</tr>
<tr>
<td>11</td>
<td>Atr + 2-PAM + MINA (60)</td>
<td>GB: 14.2 (9.1–22.2)</td>
</tr>
<tr>
<td>12</td>
<td>Atr + 2-PAM + MINA (100)</td>
<td>GB: 14.2 (9.1–22.2)</td>
</tr>
</tbody>
</table>

a Treatment was given i.m. 1 min after s.c. challenge with nerve agent.
b The LD50 ratio was defined as the nerve agent LD50 for a particular treatment regimen divided by the nerve agent LD50 for atropine sulfate (Atr) treatment.
c The LD50 doses for agent alone are 42.0, 57.0, and 8.0 μg/kg, and the LD50 doses for agent plus Atr treatment are 40.2, 52.1, and 9.0 μg/kg for GB, GF, and VX, respectively.
range, but did not significantly increase from the 13-min to the 58-
min scoring time (from 4.50 to 5.50). Administration of a high dose
(139.3 mg/kg) of MINA alone (without nerve agent) produced mild
toxic signs (average score = 0.67) in the guinea pigs.

Following GB or GF, animals treated with 2-PAM exhibited a
similar incidence (100%) of cholinergic toxic signs as that of the
saline-treated controls, and had average toxic scores of 3.25 and
4.63, respectively. Guinea pigs exposed to VX and treated with
2-PAM showed a low incidence (38%) of toxic signs and scored sig-
ificantly lower than saline-treated control animals (0.75 ± 0.67) in the
guinea pigs.

The number of GB-exposed animals treated with MINA showing
signs of nerve agent intoxication (48%) was significantly smaller
(Fisher’s exact test, p < 0.05) than that of the saline-treated
controls (92%). The number of VX-exposed animals treated with MINA
showing toxic signs (47%) was not found to be significantly differ-
ent from saline-treated controls (78%). Animals treated with MINA
following GB exposure showed a similar incidence (95%) of toxic
signs as that of the saline-treated controls (100%). Additionally,
animals treated with MINA at doses of 35.0 mg/kg and above dis-
played significantly lower toxic sign scores following GB exposure
than did the saline-treated controls. Animals treated with MINA
at doses of 55.5 and 87.9 mg/kg also displayed significantly lower
toxic sign scores following GB and VX exposure, respectively, than
did saline-treated controls. No animal treated with 2-PAM or MINA
died within 60 min of GB, GF or VX exposure.

3.1.3. ChE activity in peripheral tissues and blood

Following exposure to GB, ChE activities in the diaphragm, heart,
and skeletal muscle were inhibited to about 20%, 11%, and 23% of
control, respectively, and in RBC and WB to about 9% and 11% of
control, respectively. In the peripheral tissues and blood, MINA did
not produce any reactivation of GB-inhibited ChE at the lower doses
(22.1 and 35.0 mg/kg), but did produce significant increases in ChE
activity in these tissues at higher doses. MINA significantly reac-
tivated GB-inhibited ChE activity at 55.5, 87.9, and 139.3 mg/kg in the
heart, RBC, and WB, and at 87.9 and 139.3 mg/kg in the diaphragm.
MINA at doses 55.5 and 139.3 mg/kg also significantly reactivated
skeletal muscle ChE. 2-PAM was readily able to reactivate signifi-
cantly GB-inhibited ChE in all three peripheral tissues (up to 60% of
control) and in RBC and WB (to above 60% of control).

Following exposure to GF, ChE activities in the diaphragm, heart,
and skeletal muscle were inhibited to about 39%, 24%, and 46% of
control, respectively, and in RBC and WB to about 6% and 10% of
control, respectively. In the diaphragm, MINA did not produce any
reactivation of GB-inhibited ChE at the lower doses (22.1 and 35.0 mg/kg),
but did produce significant increases in ChE activity in these tissues at higher doses. MINA significantly reac-
tivated GB-inhibited ChE activity at 55.5, 87.9, and 139.3 mg/kg in the
heart, RBC, and WB, and at 87.9 and 139.3 mg/kg in the diaphragm.
MINA at doses 55.5 and 139.3 mg/kg also significantly reactivated
skeletal muscle ChE. 2-PAM was readily able to reactivate signifi-
cantly GB-inhibited ChE in all three peripheral tissues (up to 60% of
control) and in RBC and WB (to above 60% of control).

Following exposure to VX, ChE activities in the diaphragm, heart,
and skeletal muscle were inhibited to about 28%, 23%, and 26% of
control, respectively, and in RBC and WB to about 13% and 26% of
control, respectively. In the diaphragm, RBC, and WB, MINA
produced significant increases in ChE activity only at the highest
dose (139.3 mg/kg). In the heart MINA at doses 55.5, 87.9, and
139.3 mg/kg significantly reactivated VX-inhibited ChE. MINA at

3.2. Survival experiments

Table 3A shows the 24-h LD₅₀s and LD₅₀ ratios for various treatment regimens after GB, GF, or VX intoxication, while Table 3B displays the statistical significance comparisons among treatment groups. Against all three nerve agents, treatment with atropine sulfate plus 2-PAM displayed the statistical significance comparisons among treatment regimens after GB, GF, or VX. It is striking to notice that MINA was capable of reactivating ChE inhibited by all three nerve agents in brain regions (Table 2A). Following GB exposure, doses of MINA at 35.0 mg/kg and above were able to significantly reactivate inhibited brain ChE. As the dose of MINA increased the ChE activity was reactivated in more brain regions. At the highest dose of 139.3 mg/kg, all seven brain regions displayed significant ChE reactivation. MINA began to show its ability to reactivate ChE when the dose reached 55.5 mg/kg and above following GB exposure. At 55.5 mg/kg ChE activity was significantly reactivated in three brain regions and spinal cord and at 139.3 mg/kg two additional regions showed significant ChE reactivation. In the case of VX, MINA at doses of 55.5 and 87.9 mg/kg significantly reactivated ChE only in the hippocampus. When the dose reached 139.3 mg/kg, four additional brain regions then displayed significant ChE reactivation. The relative ChE reactivating potency of MINA against these nerve agents was GB > GF > VX. 2-PAM did not show any ChE reactivation in the brain.

In three peripheral tissues (Table 2B) MINA reactivated ChE inhibited by GB and GF in at least two peripheral tissues when the dose reached 55.5 mg/kg and above. MINA reactivated VX-inhibited ChE in one peripheral tissue at 22.1, 55.5, and 87.9 mg/kg and all three tissues at 139.3 mg/kg. 2-PAM reactivated GB- and VX-inhibited ChE, but not GF-inhibited ChE, in the peripheral tissues. In the blood components (RBC and WB) as shown in Table 2C MINA reactivated GB-inhibited ChE in the blood at doses 55.5 mg/kg and above, while only reactivating VX-inhibited ChE in the blood at 139.3 mg/kg. At 35.0 mg/kg and above, MINA reactivated GF-inhibited ChE only in the WB. 2-PAM reactivated GB- and VX-inhibited ChE in the RBC and WB, but only reactivated GF-inhibited ChE in the WB.

Overall, the capability of MINA to reactivate ChE activity in peripheral tissues and brain inhibited by these three nerve agents was in the order of GB > GF > VX.

3.2.1. Summary on reactivation of ChE activity

Table 2A–C summarizes the statistically significant ChE reactivation by oxime treatments in the brain regions, the peripheral tissues, and the blood components, respectively, following exposure to GB, GF, and VX. It is striking to notice that MINA was capable of reactivating ChE inhibited by all three nerve agents in brain regions (Table 2A). Following GB exposure, doses of MINA at 35.0 mg/kg and above were able to significantly reactivate inhibited brain ChE. As the dose of MINA increased the ChE activity was reactivated in more brain regions. At the highest dose of 139.3 mg/kg, all seven brain regions displayed significant ChE reactivation. MINA began to show its ability to reactivate ChE when the dose reached 55.5 mg/kg and above following GB exposure. At 55.5 mg/kg ChE activity was significantly reactivated in three brain regions and spinal cord and at 139.3 mg/kg two additional regions showed significant ChE reactivation. In the case of VX, MINA at doses of 55.5 and 87.9 mg/kg significantly reactivated ChE only in the hippocampus. When the dose reached 139.3 mg/kg, four additional brain regions then displayed significant ChE reactivation. The relative ChE reactivating potency of MINA against these nerve agents was GB > GF > VX. 2-PAM did not show any ChE reactivation in the brain.

In three peripheral tissues (Table 2B) MINA reactivated ChE inhibited by GB and GF in at least two peripheral tissues when the dose reached 55.5 mg/kg and above. MINA reactivated VX-inhibited ChE in one peripheral tissue at 22.1, 55.5, and 87.9 mg/kg and all three tissues at 139.3 mg/kg. 2-PAM reactivated GB- and VX-inhibited ChE, but not GF-inhibited ChE, in the peripheral tissues. In the blood components (RBC and WB) as shown in Table 2C MINA reactivated GB-inhibited ChE in the blood at doses 55.5 mg/kg and above, while only reactivating VX-inhibited ChE in the blood at 139.3 mg/kg. At 35.0 mg/kg and above, MINA reactivated GF-inhibited ChE only in the WB. 2-PAM reactivated GB- and VX-inhibited ChE in the RBC and WB, but only reactivated GF-inhibited ChE in the WB.

Overall, the capability of MINA to reactivate ChE activity in peripheral tissues and brain inhibited by these three nerve agents was in the order of GB > GF > VX.
have the advantage of reaching the blood and neuromuscular and synaptic junctional ChE pools, placing it in prime position to react with and degrade VX when it arrives. However, this was not the case in the present study. The reason for weaker action of MINA in reactivating VX-induced ChE activity was unclear, but could be due to several factors. The most likely explanation is that MINA has less affinity and reactivity as a reactivator of VX-inhibited ChE than it has as a reactivator of GB-inhibited ChE.

It is unclear why MINA reactivated GF-inhibited ChE in this study. GF-inhibited ChE, in general, is more difficult to reactivate with oximes than are GB- and VX-inhibited ChE [22]. Some very potent oximes, relative to MINA, such as 2-PAM, obidoxime, and TMB-4 (trimedoxime) are relatively poor reactivators of GF-inhibited ChE [22]. In this study 2-PAM was not able to re-activate GF-inhibited ChE in the RBC, brain regions, and three peripheral tissues, and, therefore, provided little or no protection against lethal effects of GF. On the contrary, MINA was capable of reactivating ChE inhibited by GF in blood, brain regions, diaphragm, and heart and also provided marked protection when given at doses of 60.0 or 100.0 mg/kg at 1 min after varying LD50 doses of GF. In this instance, the addition of atropine sulfate and atropine + 2-PAM to MINA treatment did not provide additional protection, thus, suggesting the importance of elevated brain ChE activity as a result of reactivation by MINA, the sole contributor to the therapeutic efficacy. MINA markedly reactivated ChE activity in blood, peripheral tissues, and multiple brain regions following GB intoxication, even at the lowest dose (35.0 mg/kg) of MINA tested. Consequently, its treatment alone without atropine sulfate was more efficacious than atropine sulfate alone, 2-PAM alone, or even the combination of atropine sulfate + 2-PAM (i.e., compared with 60.0 mg/kg of MINA treatment alone). Additionally, MINA alone was just as effective as MINA + atropine sulfate treatment. This implies that the antitodal requirements of CNS ChE activity are critical and thus, places the blocking of CNS muscarinic receptors by anticholinergic drug in a secondary role. The most dramatic benefit from the use of MINA occurred when it was used in combination with atropine sulfate + 2-PAM treatment. In these combination treatment groups, approximately 10–20% reactivation of CNS ChE by MINA in concert with the peripheral ChE reactivation by MINA and 2-PAM and a small dose of atropine sulfate (0.5 mg/kg, i.m.) to block muscarinic receptors resulted in large increases in survival. This further suggests that ChE, especially brain ChE, reactivation should be the root of medical countermeasures in nerve agent poisoning. Thus, a centrally active oxime will be most beneficial if it is able to reactivate ChE inhibited by a broad spectrum of nerve agents.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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