Comparative effects of phencynonate hydrochloride and scopolamine hydrobromide against organophosphorus nerve agent-induced seizure activity and neuropathology

Tsung-Ming Shih
Jeffrey A. Koenig
Cindy Acon-Chen

September 2018

Approved for public release; distribution unlimited

US Army Medical Research Institute of Chemical Defense
8350 Ricketts Point Road
Aberdeen Proving Ground, MD  21010-5400

an element of the
US Army Medical Research and Materiel Command
DISPOSITION INSTRUCTIONS:

Destroy this report when no longer needed. Do not return to the originator.

DISCLAIMERS:

The views expressed in this technical report are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.
Comparative effects of phencyononate hydrochloride and scopolamine hydrobromide against organophosphorus nerve agent-induced seizure activity and neuropathology

14. ABSTRACT

This study utilized the guinea pig NA-seizure model to assess and compare the effectiveness of phencyononate hydrochloride (PCH), a compound that possesses both anticholinergic and anti-N-methyl-D-aspartate activities, and of scopolamine hydrobromide (SCP), a purely anti-muscarinic compound, when given at the early (at time of seizure onset) or late (40 min after seizure onset) phase of seizure progression. Administered at seizure onset, PCH and SCP were both effective at terminating seizure activity against all NAs, with ED50 values for SCP generally lower. At the 40 min treatment time, ED50 values were obtained following GA, GD, GF, and VR challenges for SCP, but for PCH values could only be obtained following GD, indicating a superior efficacy of SCP. When seizure activity was controlled, a significant improvement in weight loss, neuropathology, and survival was observed, regardless of treatment or NA challenge. These results demonstrate the differing efficacies of these two similarly structured anticholinergic compounds with delayed administration and warrant further investigation into the timing and mechanisms of the seizure maintenance phase in different animal model.

15. SUBJECT TERMS

phencyononate; scopolamine; seizure activity; neuropathology; lethality; nerve agents; organophosphorus compounds; sarin; soman; cyclosarin; tabun; VR; VX

16. SECURITY CLASSIFICATION OF:

a. REPORT UNCLASSIFIED
b. ABSTRACT UNCLASSIFIED c. THIS PAGE UNCLASSIFIED

17. LIMITATION OF ABSTRACT UNLIMITED

18. NUMBER OF PAGES 33

19. NAME OF RESPONSIBLE PERSON Tsung-Ming Shih

410-436-3414
ACKNOWLEDGMENTS

The excellent technical team work of Jessica Chandler and Amy Wegener is acknowledged.
ABSTRACT

The efficacy of anticonvulsant therapies to curtail and stop seizure activities following acute exposure to organophosphorus nerve agents (NAs) has been well documented as being time-dependent: the effectiveness of anticholinergics and benzodiazepines decreases as time progresses from seizure onset, while the N-methyl-D-aspartate (NMDA) receptor antagonists still possess anticonvulsant activity when administered 40 min after seizure onset. Novel treatments exploiting more than one therapeutic target may increase this therapeutic window. This study utilized the guinea pig NA-seizure model to assess and compare the effectiveness of phencynonate hydrochloride (PCH), a compound that possesses both anticholinergic and anti-NMDA activities, and of scopolamine hydrobromide (SCP), a purely anti-muscarinic compound, when given at the early (at time of seizure onset) or late (40 min after seizure onset) phase of seizure progression. Guinea pigs implanted one week earlier with cortical electrodes for electroencephalographic (EEG) recordings were pretreated with pyridostigmine bromide (0.026 mg/kg, im) 30 min prior to exposure to a 2.0 x LD50 subcutaneous dose of a NA (GA, GB, GD, GF, VR or VX), followed one min later with atropine sulfate (0.1 mg/kg, im) and 2-PAM (25 mg/kg, im). At seizure onset (early phase) or at 40 min after seizure onset (late phase), animals were treated with one of several doses of PCH or SCP. The anticonvulsant ED$_{50}$ doses of these two treatments individually were determined. When administered at seizure onset, PCH and SCP were both effective at terminating seizure activity against all NAs, with ED$_{50}$ values for SCP generally being lower. At the 40 min treatment time, ED$_{50}$ values were obtained following GA, GD, GF, and VR challenges for SCP, but for PCH values could only be obtained following GD, indicating a superior efficacy of SCP. When seizure activity was controlled, a significant improvement in weight loss, neuropathology, and survival was observed, regardless of treatment or NA challenge. Overall, these results demonstrate the differing efficacies of these two similarly structured anticholinergic compounds with delayed administration and warrant further investigation into the timing and mechanisms of the seizure maintenance phase in different animal models.
INTRODUCTION

Organophosphorus (OP) nerve agents (NA) are the most toxic chemical threats to military personnel and civilian populations. NAs irreversibly bind the enzyme acetylcholinesterase (AChE), rendering it incapable of hydrolyzing the cholinergic neurotransmitter acetylcholine (ACh). This inhibition results in the accumulation of ACh and the hyperstimulation of the cholinergic system at central and peripheral sites throughout the body (Taylor, 2011). The consequences of this cholinergic crisis include tremors, muscle fasciculation, hypersecretions, respiratory distress, convulsions, seizures, and if a severe exposure is left untreated, death (Moore et al., 1995; Taylor, 2011). Various drug classes have been adopted to treat NA poisoning. Carbamate AChE inhibitors, such as pyridostigmine bromide (PB), temporarily bind and shield AChE in the periphery from NA inhibition (Berry and Davies, 1970; Dirnhuber et al., 1979). Anticholinergic drugs (e.g., atropine sulfate) antagonize muscarinic receptor sites, blocking ACh binding. Oximes (e.g., pyridine-2-aldoxime methylchloride [2-PAM]) reactivate unaged NA-inhibited AChE, and finally, benzodiazepines (e.g., diazepam or midazolam) decrease seizure activity (Philippens et al., 1992; McDonough et al., 2010; Reddy and Reddy, 2015). These treatments offer the best efficacy at preventing lethality when administered immediately following NA exposure. However, these medical countermeasures do not afford total protection against the neuropathological consequences of NA exposure (Hayward et al., 1990; McDonough and Shih, 1995) because seizure activity does not completely terminate (Harris et al., 1994; McDonough and Shih, 1993).

Years of research have shown that NA-induced neuropathology is a time-dependent process in which damage worsens with prolonged seizure activity (McDonough and Shih, 1995). Through systematic studies in rats, a three-phased progression of neuropharmacological events following NA-induced seizures has been proposed (Shih et al., 1991; McDonough and Shih, 1993, 1995, 1997; Shih and McDonough, 1997). First, an early cholinergic phase occurs, spanning from the time of NA exposure to about 5 min after seizure onset. This is followed by a transitional phase of progressively mixed cholinergic/non-cholinergic modulation that begins 5 min after seizure onset and lasts until 40 min after seizure onset. Finally, a non-cholinergic phase begins at about 40 min after seizure onset in which there is a high release of excitatory amino acids (e.g., aspartate and glutamate). These amino acid neurotransmitters activate the N-methyl-D-aspartate (NMDA) system and maintain on-going seizure activity thereafter (Lallement et al., 1991a, 1991b, 1991c, 1992). If seizures are not controlled, status epilepticus results, inducing severe neuropathology and lethality (Petras, 1981; Lemercier et al., 1983; McLeod, 1985; Raffaele et al., 1987; McDonough and Shih, 1995; Shih et al., 2003).

Anticholinergic drugs or benzodiazepines can effectively terminate seizures and prevent brain damage if they are administered shortly after seizure onset (within 5 to 10 min after seizure onset). However, if treatment administration is delayed 10 to 40 min after seizure onset, it is not as efficacious and neuropathology develops (McDonough et al., 2010; McDonough and Shih, 1995). In contrast, NMDA receptor antagonists have been shown to block seizures when given during the later stages of seizure activity (40 min after seizure onset), affording more neuroprotection (Braitman and Sparenborg, 1989; Figueiredo et al., 2011; Sparenborg et al., 1992); however, some of these drugs (e.g., MK-801) cause altered sensory perception, paranoia,
hallucinations (Muir and Lees, 1995), and lethal respiratory depression (McDonough and Shih 1993, 1995). Better drugs are necessary to adequately control NA-induced seizure, and a drug possessing both anticholinergic and anti-NMDA properties may be a more favorable choice.

In the past several decades, the effects of numerous anticholinergic compounds alone or in conjunction with other treatments after NA poisoning have been evaluated (Wills, 1963; Coleman et al., 1962, 1968; Brimblecombe et al., 1970). Using a mouse model, Jovic and Milosevic (1970) examined the efficacy of twelve anticholinergic drugs to determine if atropine was the most effective cholinolytic following exposure to different OP compounds including the NAs soman (GD), tabun (GA), and sarin (GB). They determined that caramiphene and benactyzine had activities comparable or greater than that of atropine after NA exposure. Furthermore, they concluded that the use of atropine as a treatment for OP poisoning could be enhanced with the use of other anticholinergics. In 1973, Ketchum et al. evaluated the relative potency and effects of belladonnoid compounds (e.g., atropine and scopolamine) in 158 US Army enlisted men and found that compared to scopolamine (SCP), atropine displays 8-9 times less central potency. Atropine also displayed a slower onset of action than SCP or other synthetic anticholinergics (Ketchum et al., 1973). These studies exposed the limitations of using atropine alone and revealed the need to research additional anticholinergics. In subsequent decades, researchers assessed the effects of purely anticholinergic SCP against GD intoxication using rat or guinea pig models, demonstrating this drug’s efficacy to improve behavioral symptoms and terminate seizure activity (Capacio and Shih, 1991; Anderson et al., 1994; Harris et al., 1994; Wetherell, 1994; McDonough et al., 2000; Philippens et al., 2000; Meshulam et al., 2001; Wetherell et al., 2002). This efficacy in the rat model, however, was shown to be ablated if SCP treatment was withheld until 40 min after seizure onset (McDonough and Shih, 1993). This is the time point associated with a shift of seizure maintenance from the cholinergic to excitatory amino acid neurotransmitter system. SCP was chosen as a testing candidate for our study, not only because it has previously shown efficacy against GD, but also because it has attained FDA approval as a transdermal extended release film for use as a motion sickness medication, smooth muscle spasmodic, and surgical preanesthetic (Clissold and Heel, 1985; Nachum et al., 2006).

Recent literature has also reported a novel anticholinergic compound, phencynonate hydrochloride (PCH), which was developed by the Beijing Institute of Pharmacology and Toxicology, China (Xu et al., 1993, Kou et al., 2008). This drug, structurally similar to SCP, possesses both muscarinic and nicotinic antagonistic properties as well as anti-NMDA properties (Ge et al., 2015, Wang et al., 2005a; 2005b). While it has previously been developed as a safe and effective drug for the prevention of motion sickness in tablet form (Xu et al., 1993; Dai et al., 1997), it also demonstrates clear anticonvulsant effectiveness after GD poisoning in a rat model (Wang et al., 2005a). In this rat model, animals received HI-6 (125 mg/kg, ip) 30 min prior to challenge with a convulsant dose of GD (180 µg/kg, sc). PCH, when administered intraperitoneally, has shown excellent anticonvulsant properties at 5 min, 20 min, and 40 min after seizure onset in this rat model, with few adverse effects (Wang et al., 2005a). Thus, this drug possesses the characteristics, as described earlier, to be a possible new type of medical countermeasure treatment for NAs.

Here we evaluated and compared the efficacy of PCH and SCP (structures shown in Fig. 1) when given intramuscularly during the early (at time of seizure onset) or late (at 40 min after seizure
onset) phases of seizure progression using our standardized guinea pig model (Shih et al., 2003, 2007). This guinea pig model simulates the field pretreatment (pyridostigmine bromide) and therapy (atropine/oxime) regimen in NA intoxication. Although rat and mouse models have been employed for NA studies, the use of a guinea pig model may be more appropriate (Inns and Leadbeater, 1983; O'Donnell et al., 2011) since guinea pigs do not possess a high level of carboxylesterase. Carboxylesterase nonspecifically binds NAs such as GB or GD and reduces the availability of NAs in the bloodstream (Maxwell et al., 1987). Guinea pigs have also been used previously to study acute toxicity, treatment, and pathology of NA intoxication (Shih et al., 1996; 2003; 2007; 2009; McDonough and Shih, 1997; Shih and McDonough, 1999; Atchison et al., 2004), and their sensitivity to NA poisoning and response to treatment are similar to that of non-human primates (Inns and Leadbeater, 1983). While previous studies have focused solely on treatments for GD exposures, we expanded our present study to evaluate against six traditional NAs: GA, GB, GD, cyclosarin (GF), VX, and a Russian V agent, VR.

![Figure 1. Chemical structures of phenecynonate hydrochloride and scopolamine hydrobromide.](image-url)
MATERIALS AND METHODS

Subjects
Male Hartley guinea pigs (Crl: (HA) BR COBS; Charles River Labs, Kingston, NY), weighing 250-350g, served as subjects. Upon arrival, the animals were quarantined for a week and tested for evidence of disease. They were individually housed in polycarbonate cages in temperature (20-26 °C) and humidity (30-70%) controlled animal quarters maintained on a 12-hour light-dark lighting cycle with lights on at 0600 hr. Guinea pig diet #7006 (Harlan Teklad, Madison, WI) and filtered tap water were freely available whenever the animals were in home cages. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Materials
Sterile saline (0.9% NaCl) injection, USP, was purchased from Cutter Labs, Inc. (Berkeley, CA). Atropine sulfate, scopolamine hydrobromide, and sodium pentobarbital were purchased from Sigma-Aldrich (St. Louis, MO). Phencyonate hydrochloride (99.6%) was custom-synthesized by ChemPacific Corporation (Baltimore, MD). Buffered formalin (10%) was purchased from Fisher Scientific (Hampton, NH). PB (pyridostigmine bromide) was obtained from Hoffmann-La Roche Inc. (Nutley, NJ), and 2-PAM was purchased from Ayerst Labs, Inc. (New York, NY). Attane™ (Isoflurane, USP) was purchased from Minrad, Inc. (Bethlehem, PA). The six OP NAs studied were tabun (GA; ethyl N,N-dimethyl phosphoramidocyanidate), sarin (GB; isopropyl methylphosphonofluoridate), soman (GD; pinacolyl methylphosphonofluoridate), cyclosarin (GF; cyclohexylmethyl phosphonofluoridate), VX (0-ethyl S-(2-(diisopropylamino)ethyl) methylphosphonothioate), and VR (2-isobutyl S-(2-(diethylamino)ethyl)methylphosphonothioate). These NAs were obtained from the US Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD) and diluted in ice-cold sterile normal saline prior to subcutaneous injection. PB, atropine sulfate, scopolamine hydrobromide, 2-PAM, and phencyonate HCl were prepared in sterile normal saline and administered intramuscularly. Atropine sulfate and 2-PAM were admixed in a solution just before injection; all other solutions were prepared and injected separately. Injection volume was 0.5 ml/kg for saline, NAs, and treatment drugs.

Surgery
All animals were anesthetized with isoflurane then implanted with electroencephalographic (EEG) screws and a miniature connector plug according to previously described procedures (Shih and McDonough, 1999; Shih et al., 2003, 2007). The guinea pigs received 0.07 ml of buprenorphine HCl subcutaneously (sc) (0.15 mg/ml) after surgery for pain control. A 7- to 10-day recovery period was allowed prior to experimentation.
Experimental Procedure
On the day of the experiment, unanesthetized guinea pigs were placed in individual recording chambers (23 cm deep x 31 cm wide x 45 cm high) and continuously monitored for EEG activity. EEGs were recorded using CDE 1902 amplifiers and displayed on a computer running Spike2 software (Cambridge Electronic Design, Ltd., Cambridge, UK). After a 15-minute recording of baseline EEG activity, animals received a dose of PB (0.026 mg/kg, im) to produce 20-30% whole blood AChE inhibition (Lennox, 1985). Thirty min later, animals were challenged sc with 2 x LD50 of GA (240 µg/kg), GB (84 µg/kg), GD (56 µg/kg), GF (114 µg/kg), VR (22.6 µg/kg), or VX (16 µg/kg). One min after NA challenge, animals were treated intramuscularly (im) with atropine sulfate (0.1 mg/kg) plus 2-PAM (25 mg/kg). This dose of 2-PAM approximates the total dose of 2-PAM in 3 autoinjectors (600 mg per injector) given to a 70-75 kg human. The 0.1 mg/kg dose of atropine sulfate approximates the total dose of atropine sulfate in the 3 autoinjectors (2.0 mg per injector) given to a 70-75 kg human. Previous work with this model (Shih et al., 2003, 2007) showed that a 2 x LD50 challenge level of these NAs will induce seizures in all animals. Immediately (usually within 15 sec) after the onset of EEG seizure activity or at 40 min after seizure onset, PCH or SCP at various doses was given im. The dosage ranges used for PCH and SCP are summarized in Table 1.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Phencynonate Seizure onset</th>
<th>Phencynonate 40 min after onset</th>
<th>Scopolamine Seizure onset</th>
<th>Scopolamine 40 min after onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>0.18-1.0</td>
<td>0.56-18.0</td>
<td>0.018-0.56</td>
<td>0.56-5.6</td>
</tr>
<tr>
<td>GB</td>
<td>0.032-0.56</td>
<td>1.0-56.0</td>
<td>0.032-0.18</td>
<td>3.2-100.0</td>
</tr>
<tr>
<td>GF</td>
<td>0.1-1.8</td>
<td>1.0-18.0</td>
<td>0.056-0.32</td>
<td>0.056-32.0</td>
</tr>
<tr>
<td>VX</td>
<td>0.032-0.18</td>
<td>1.0-18.0</td>
<td>0.018-0.18</td>
<td>10.0-100.0</td>
</tr>
<tr>
<td>VR</td>
<td>0.056-1.0</td>
<td>0.1-5.6</td>
<td>0.056-0.18</td>
<td>1.0-10.0</td>
</tr>
</tbody>
</table>

Dosage ranges in mg/kg (im) utilized to investigate and compare the efficacy of phencynonate (PCH) and scopolamine (SCP) against various OP nerve agents at either seizure onset or 40 min after seizure onset in guinea pigs.

Animals were observed continuously for seizure activity for the first hour following NA exposure and drug treatment and periodically thereafter for at least 5 more hours. EEG activity was recorded continuously throughout this time and for another 30 min at 24 hr after exposure. Seizure onset was operationally defined as the appearance of ≥ 10 sec of rhythmic high amplitude spikes or sharp wave activity in the EEG tracing. Each animal was rated as having the seizure terminated (OFF) or not terminated (NOT OFF) based on the overall appearance of the EEG record at the end of the experimental day and during the 24-hour observation. (Note: An animal was rated as OFF if the seizure was terminated and the EEG remained normal at all subsequent observation times.) Lethality was recorded 24 hr after NA exposure. Animals that survived 24 hr were weighed, deeply anesthetized with sodium pentobarbital intraperitoneally (75.0 mg/kg), and euthanized by exsanguination via transcardial perfusion with normal saline, followed by 10% phosphate buffered formalin.
The brain was blocked, embedded in paraffin, cut 5-10 µm thick, stained with hematoxylin and eosin, and evaluated by a board-certified pathologist who was unaware of the experimental history of a given subject. The procedures and criteria used for pathological evaluation have been published elsewhere (McDonough and Shih, 1995; Shih et al., 2003). Briefly, six brain areas (cerebral cortex, pyriform cortex, amygdala, hippocampus, thalamus, caudate/putamen) were evaluated in each animal, and each area was rated for neuropathological damage on a scale from 0-4: 0 = no damage, 1 = minimal damage (1-10% necrotic neurons), 2 = mild (11-25%), 3 = moderate (26-45%), and 4 = severe (>45%). The magnitude of total brain damage was assessed by summing the neuropathology scores of the six areas.

Data Analysis
Dose-effect curves and the median effective dose (ED$_{50}$) for anticonvulsant activity of each individual drug were determined using 4-7 doses with 5-6 animals per group. A probit regression analysis (SPSS for Windows, Version 17.0, Chicago, IL) was used to estimate the ED$_{50}$ values along with the 95% confidence intervals for each drug treatment and NA combination (Bliss, 1952). An SAS program was used to compare the ED$_{50}$ values of SCP and PCH within a NA group and treatment time using the regression output. This allowed for the calculation of a ratio and a confidence interval (CI) around the ratio. The ED$_{50}$ values were determined to be significantly different if the CI of the ratio did not include the value of 1. Average latencies for seizure onset between the different NAs were not normally distributed and were evaluated utilizing the Kruskal-Wallis analysis of variance (ANOVA) on ranks followed by Dunn’s multiple comparison test. Average latencies for seizure terminations were evaluated using both one- and two-way ANOVA procedures followed by Tukey multiple comparison tests. Differences in overall neuropathology scores between seizure control groups or treatments were analyzed using a Mann Whitney test. The differences between proportions of animals surviving challenge with each NA was analyzed using a two-tailed Fisher’s exact test. In all statistical analysis, $p<0.05$ was considered significant.

RESULTS

Seizure Occurrence
The percent of seizure occurrence was 100% in GA-, GB-, GD-, GF-, and VR-exposed guinea pigs, while it was 95.4% in VX-exposed animals.

Seizure Onset
Seizure onset times after 2 x LD$_{50}$ of each NA are shown in Figure 2. The average seizure onset times (mean ± 95% CI min) for GA, GB, GD, GF, VX, and VR were 8.9 ± 0.64 (N=109), 6.3 ± 0.25 (N=129), 7.5 ± 0.26 (N=114), 5.5 ± 0.49 (N=135), 21.1 ± 1.06 (N=102), and 11.0 ± 0.47 (N=126), respectively. The average times to seizure onset among all NA groups are significantly different from each other ($p<0.05$), except for the times between GA and GD groups. Thus, the rank order for average latency to seizure onset from short to long for these six NAs is GF>GB>GD=GA>VR>VX.
Figure 2. Average seizure onset times for guinea pigs pretreated with pyridostigmine bromide (0.026 mg/kg, im) 30 min prior to a nerve agent (2 x LD50, sc) and treated 1 min later with 2-PAM (25 mg/kg, im) and atropine sulfate (0.1 mg/kg, im). p<0.05 between all nerve agent groups except GA vs. GD groups.

Seizure Termination

Treatment at seizure onset

The seizure termination times of all animals within a particular NA challenge following either PCH or SCP treatment were averaged and expressed regardless of the doses used. The average seizure termination times for animals exposed to NA and treated with PCH or SCP at seizure onset are shown in Figure 3A. The average seizure termination time (mean ± 95% CI min) in ascending order for PCH-treated animals was GD (8.9 ± 2.22 min), GF (12.2 ± 6.65 min), VR (14.4 ± 5.72 min), GA (31.9 ± 43.21 min), GB (34.1 ± 18.54 min), and VX (43.4 ± 24.26 min). For SCP-treated animals, the average seizure termination time (mean ± 95% CI min) in ascending order was VR (12.7 ± 3.51 min), GB (16.8 ± 7.45 min), GA (18.0 ± 11.15 min), GF (21.0 ± 14.82 min), VX (22.6 ± 14.16 min), and GD (23.0 ± 18.25 min). There was no significant difference in seizure termination between the PCH and the SCP treatment groups with each respective NA.
A. Seizure Onset

Figure 3. Time to seizure termination (in min) by phenecynonate (PCH) and scopolamine (SCP) treatment at seizure onset (A) or 40 min after onset (B). Average time (displayed as mean ± 95% CI min) to termination of EEG seizure in all guinea pigs that were pretreated with pyridostigmine bromide (0.026 mg/kg, im) 30 min prior to a nerve agent (2 x LD50, sc) and treated 1 min later with 2-PAM (25 mg/kg, im) and atropine sulfate (0.1 mg/kg, im). PCH or SCP was given im.

* p<0.05 PCH vs. SCP within the individual NA group.
& p<0.05 vs. treatment at seizure onset within NA and treatment group.

B. 40 min after Onset
Treatment at 40 min after seizure onset
The average seizure termination time for PCH- and SCP-treated guinea pigs at 40 min after seizure onset is shown in Figure 3B. The seizure termination times (mean ± 95% CI min) in ascending order for animals receiving PCH were VR (14.4 ± 5.72 min), GF (20.2 ± 26.41 min), VX (36.5 ± 14.88 min), GD (43.9 ± 28.57 min), GA (69.1 ± 68.01 min), and GB (no termination). For treatment with SCP, seizure termination times in ascending order were GD (52.7 ± 13.89 min), GF (65.4 ± 14.97 min), GA (65.5 ± 21.68 min), GB (110.0 ± 4.12 min), VR (129.2 ± 18.16 min), and VX (no termination). There were no significant differences in seizure termination between the PCH and the SCP treatment groups exposed to GA, GB, GD, or VX. However, animals receiving GF or VR and treated with PCH experienced seizure termination significantly faster than those treated with SCP. Additionally, when compared to treatment at seizure onset within each treatment group seizures in animals treated at 40 min after seizure onset took significantly longer to terminate following treatment with PCH after exposure to GD and following treatment with SCP after exposure to GA, GB, GD, GF, and VR.

Anticonvulsant Efficacy
Table 2 and Figure 4 show the anticonvulsant ED$_{50}$ results for both PCH and SCP treatment at seizure onset or 40 min after seizure onset. The anticonvulsant ED$_{50}$ doses (mg/kg with 95% confidence limits in parenthesis) of PCH for animals treated at onset of seizure were GA 0.46 (0.26-0.86), GB 0.10 (0.07-0.15), GD 0.59 (0.38-1.20), GF 0.25 (0.15-0.37), VX 0.07 (0.05-0.09), and VR 0.19 (0.07-0.33). Following treatment with SCP at time of seizure onset, the ED$_{50}$ values were GA 0.12 (0.08-0.18), GB 0.06 (0.04-0.09), GD 0.11 (0.07-0.17), GF 0.13 (0.09-0.20), VX 0.06 (0.04-0.09), and VR 0.09 (0.07-0.11). With the exception of treatment after VX, all ED$_{50}$ values were significantly lower with SCP treatment compared to PCH. For treatment given at 40 min after seizure onset, the anticonvulsant ED$_{50}$ values were significantly higher for both treatments when compared to treatment at onset. Only one ED$_{50}$ dose could be calculated for PCH-treated animals: GD 10.66 (7.67-17.00). For SCP-treated guinea pigs, the following anticonvulsant ED$_{50}$ doses were obtained: GA 1.88 (0.75-11.66), GD 1.41 (0.46-2.27), GF 0.28 (0.12-0.48), and VR 1.81 (0.00-4.28); the ED$_{50}$ dose for GB and VX could not be determined.
Table 2. Anticonvulsant ED$_{50}$ doses for phencynonate and scopolamine following exposure to various OP nerve agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Phencynonate</th>
<th>Scopolamine</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seizure Onset</td>
<td>40 min after onset</td>
<td>Seizure Onset</td>
</tr>
<tr>
<td>GA</td>
<td>0.46 (0.26-0.86)</td>
<td>ND</td>
<td>0.12 (0.08-0.18)</td>
</tr>
<tr>
<td>GB</td>
<td>0.10 (0.07-0.15)</td>
<td>ND</td>
<td>0.06 (0.04-0.09)</td>
</tr>
<tr>
<td>GD</td>
<td>0.59 (0.38-1.20)</td>
<td>10.66 (7.67-17.01)$^a$</td>
<td>0.11 (0.07-0.17)</td>
</tr>
<tr>
<td>GF</td>
<td>0.25 (0.15-0.37)</td>
<td>ND</td>
<td>0.13 (0.09-0.20)</td>
</tr>
<tr>
<td>VX</td>
<td>0.07 (0.05-0.09)</td>
<td>ND</td>
<td>0.06 (0.04-0.09)</td>
</tr>
<tr>
<td>VR</td>
<td>0.19 (0.07-0.33)</td>
<td>ND</td>
<td>0.09 (0.07-0.11)</td>
</tr>
</tbody>
</table>

Anticonvulsant ED$_{50}$ doses in mg/kg (with 95% confidence limits in parentheses) of phencynonate or scopolamine treatment at time of seizure onset or at 40 min after seizure onset in guinea pigs that were pretreated with pyridostigmine bromide (0.026 mg/kg, im) 30 min prior to a nerve agent (2 x LD$_{50}$, sc) and treated 1 min later with 2-PAM (25 mg/kg, im) and atropine sulfate (0.1 mg/kg, im).

ND = ED$_{50}$ could not be determined.

Values in **bold** are significant (p<0.05) vs phencynonate at the same treatment time.

$^a$p<0.05 vs. seizure onset treatment within same treatment group.
Figure 4. Dose-response curves for the anticonvulsant effects of PCH (black square) and SCP (grey triangle) in guinea pigs that were pretreated with pyridostigmine bromide (0.026 mg/kg, im) 30 min prior to a nerve agent (2 x LD$_{50}$, sc) and treated 1 min later with 2-PAM (25 mg/kg, im) and atropine sulfate (0.1 mg/kg, im). PCH or SCP was given im at time of seizure onset (solid line) or at 40 min after seizure onset (dotted line).
Table 3 shows the variable anticonvulsant effects of PCH (3a) and SCP (3b) administered at 40 min after onset for which an ED50 dose could not be determined. For PCH treatment, ED50 values could not be determined following exposure to GA, GB, GF, VX, or VR. There were few seizure terminations following GA or GB exposure, with 3/39 (8%) and 0/26 animals, respectively, experiencing seizure termination. PCH efficacy following GF, VX, or VR exposure was more varied, with 12/36 (33%), 11/31 (35%), and 24/43 (56%) animals, respectively, having seizures terminate. The efficacious doses, however, were too variable to properly calculate ED50 values. SCP treatment demonstrated very little efficacy following exposure to GB and VX, with 2/38 (5%) and 0/15 animals, respectively, experiencing seizure termination across the assessed doses.

**Table 3.** Anticonvulsant effects of phencynonate and scopolamine administered 40 minutes after seizure onset for which an ED50 and 95% confidence limits could not be determined.

### A. Phencynonate at 40 min after seizure onset

<table>
<thead>
<tr>
<th>dose (mg/kg)</th>
<th>GA</th>
<th>GB</th>
<th>GF</th>
<th>VX</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0/3</td>
</tr>
<tr>
<td>0.18</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>6/8</td>
</tr>
<tr>
<td>0.32</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>4/7</td>
</tr>
<tr>
<td>0.56</td>
<td>0/1</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>1/7</td>
</tr>
<tr>
<td>1.0</td>
<td>0/2</td>
<td>0/1</td>
<td>0/6</td>
<td>0/2</td>
<td>5/7</td>
</tr>
<tr>
<td>1.8</td>
<td>0/3</td>
<td>--</td>
<td>3/6</td>
<td>1/6</td>
<td>4/6</td>
</tr>
<tr>
<td>3.2</td>
<td>1/7</td>
<td>0/1</td>
<td>1/6</td>
<td>2/6</td>
<td>2/3</td>
</tr>
<tr>
<td>4.2</td>
<td>---</td>
<td>0/1</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>5.6</td>
<td>1/9</td>
<td>0/3</td>
<td>4/6</td>
<td>4/6</td>
<td>2/2</td>
</tr>
<tr>
<td>7.5</td>
<td>---</td>
<td>0/3</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>10.0</td>
<td>1/11</td>
<td>0/3</td>
<td>4/6</td>
<td>2/6</td>
<td>---</td>
</tr>
<tr>
<td>13.0</td>
<td>---</td>
<td>0/4</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>18.0</td>
<td>0/6</td>
<td>0/4</td>
<td>0/6</td>
<td>2/5</td>
<td>---</td>
</tr>
<tr>
<td>24.0</td>
<td>---</td>
<td>0/6</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

### B. Scopolamine at 40 min after seizure onset

<table>
<thead>
<tr>
<th>dose (mg/kg)</th>
<th>GB</th>
<th>VX</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>0/1</td>
<td>---</td>
</tr>
<tr>
<td>5.6</td>
<td>0/2</td>
<td>---</td>
</tr>
<tr>
<td>10.0</td>
<td>0/6</td>
<td>0/1</td>
</tr>
<tr>
<td>18.0</td>
<td>1/7</td>
<td>0/2</td>
</tr>
<tr>
<td>32.0</td>
<td>0/7</td>
<td>0/2</td>
</tr>
<tr>
<td>56.0</td>
<td>1/8</td>
<td>0/5</td>
</tr>
<tr>
<td>100.0</td>
<td>0/7</td>
<td>0/5</td>
</tr>
</tbody>
</table>

*Number of animals whose seizure terminated over the total number of animals treated.
Weight Loss at 24 hours

Treatment at seizure onset

Figure 5A displays the 24 hr weight change for animals whose seizure terminated after being treated at the time of seizure onset. On average, animals treated with PCH at onset and exposed to GA, GD, or GF experienced more weight loss than those exposed to GB, VX or VR. The average 24 hr weight losses (mean ± 95% CI grams) were GA (27.78 ± 8.24), GB (17.94 ± 2.97), GD (33.38 ± 6.57), GF (38.63 ± 3.18), VX (14.00 ± 2.82), and VR (14.19 ± 2.83). These weight losses were comparable to the weight losses that SCP-treated animals experienced, with the exception of the GA-exposed group where SCP-treated animals lost significantly more weight. The average 24 hr weight losses (mean ± 95% CI grams) for animals treated with SCP at seizure onset were GA (38.53 ± 3.71), GB (19.95 ± 4.32), GD (40.09 ± 4.27), GF (38.10 ± 5.73), VX (13.64 ± 5.04), and VR (18.87 ± 4.75).

Treatment 40 min after seizure onset

Figure 5B displays the 24 hr weight change for animals whose seizure terminated after being treated at 40 min after seizure onset. The average 24 hr weight losses for PCH-treated animals at 40 min after seizure onset were GA (19.50 ± 12.74), GD (16.75 ± 4.57), GF (11.67 ± 6.10), VX (21.38 ± 11.35), and VR (10.91± 4.81). These weight losses were also comparable to those for SCP-treated guinea pigs in all NA-exposed groups with the exception of GD- and GF-exposed groups, where SCP-treated animals displayed significantly more weight loss. The average 24 hr weight losses for SCP-treated animals at this time point were GA (34.00 ± 6.38), GD (35.77 ± 5.02), GF (42.25 ± 2.16), and VR (15.11 ± 3.70). There was limited survival for GB-exposed animals treated at 40 min (PCH N=0; SCP N=1). Weight loss could not be observed for VX-exposed subjects treated with SCP at 40 min as there were no survivors.
A. Seizure Onset

![Graph showing weight change (g) for PCH and SCP at seizure onset.]

**PCH or SCP at Onset**

B. 40 min after Onset

![Graph showing weight change (g) for PCH and SCP at 40 min after seizure onset.]

**PCH or SCP at 40 min.**

**Figure 5.** Weight loss (in grams) at 24 hr in guinea pigs that were pretreated with pyridostigmine bromide (0.026 mg/kg, im) 30 min prior to a nerve agent (2 x LD$_{50}$, sc) and treated 1 min later with 2-PAM (25 mg/kg, im) and atropine sulfate (0.1 mg/kg, im). PCH or SCP was given im at either seizure onset (A) or 40 min after seizure onset (B).

* p<0.05 PCH vs. SCP within the individual nerve agent group.
**Lethality**

The effects of anticonvulsant treatment on 24 hr lethality after 2 x LD$_{50}$ of a NA are shown in Table 4. As reported in previous research studies (Shih et al., 2003, 2007), there was an overall strong relationship between the control of seizure activity and protection against NA-induced lethality. For guinea pigs that exhibited seizure termination after treatment with PCH at onset, no lethality was observed at 24 hr. In contrast, 55.6% (50/90) of animals expired within 24 hr if PCH treatment at onset failed to stop seizure activities. This difference in lethality between “seizure on” and “seizure off” groups was significant for all NAs with the exception of GB, which produced the lowest lethality (23%) among the active seizure groups. For guinea pigs treated with SCP at onset a similar pattern was observed: only 3.7% (3/81) of animals died within 24 hr if seizure termination occurred. If seizure activity was continuous after SCP treatment, 61.8% (42/68) of the animals expired. This difference in lethality between “seizure on” and “seizure off” groups was significant across all NA groups.

Table 4. Lethality at 24 hours as a function of OP nerve agent challenge, anticholinergic treatment, treatment time, and seizure control.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Phenocyloate</th>
<th>Scopolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seizure onset</td>
<td>40 min after onset</td>
</tr>
<tr>
<td></td>
<td>Seizure on</td>
<td>Seizure off</td>
</tr>
<tr>
<td>GA</td>
<td>6/10</td>
<td>0/9</td>
</tr>
<tr>
<td>GB</td>
<td>5/22</td>
<td>0/16</td>
</tr>
<tr>
<td>GD</td>
<td>15/21</td>
<td>0/14</td>
</tr>
<tr>
<td>GF</td>
<td>10/12</td>
<td>0/19</td>
</tr>
<tr>
<td>VX</td>
<td>8/14</td>
<td>0/16</td>
</tr>
<tr>
<td>VR</td>
<td>6/11</td>
<td>0/16</td>
</tr>
</tbody>
</table>

**Bold** values indicate a significant difference (p<0.05) from ‘Seizure on’ within treatment and treatment time.

Delayed treatment with PCH resulted in some lethality within 24 hr even after seizure activity was controlled. Of the animals treated with PCH at 40 min whose seizure terminated, 14.3% (9/63) expired within 24 hr. Nevertheless it was an improvement in survival compared to that of animals whose seizures did not terminate as 53.4% (78/146) of the animals with continuous seizure died within 24 hr. Among the individual NA groups, only GD and VR reached significant improvement levels when seizure was terminated. Similarly to PCH, there was 13.9% (11/79) lethality for animals treated with SCP at 40 min after seizure onset among animals whose seizure terminated. Lethality was greater at 24 hr (39.4%, 37/94) for animals that displayed continuous seizure activity throughout the experimentation period. GA and GF were the only NA groups to reach a significant improvement with less lethality when seizure was terminated.
Neuropathology
Table 5 shows the neuropathology obtained 24 hr after NA exposure. Similarly to the previously reported studies (Shih et al., 2003), there was a strong relationship between the control of seizure activity and the prevalence of neuropathology. All of the NAs were capable of producing neuropathology when the treatment did not terminate seizures. Animals treated with either SCP or PCH at time of seizure onset displayed little to no brain damage (total pathology scores of 0-1) when seizures were controlled, with a significant reduction seen across all NAs. The GF group with PCH treatment and GA, GD, and GF groups with SCP treatment had an insufficient number of subjects in the continuous seizure (i.e., “Seizure on”) group to make a statistical comparison.

For treatment at 40 min after seizure onset, there was a similar improvement in the animals that experienced seizure termination compared with those that seized continuously. There was a significant improvement with PCH treatment following GD or VR exposure. For SCP-treated animals, a significant improvement was noted following GA or GF exposure. Additionally, treatment with PCH showed significantly lower neuropathology scores following GF exposure when compared to SCP treatment at this time point.
<table>
<thead>
<tr>
<th>Agent</th>
<th>Seizure Onset</th>
<th>Seizure Off</th>
<th>40 min after seizure onset</th>
<th>Seizure Onset</th>
<th>Seizure Off</th>
<th>40 min after seizure onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>20 (18.5-20.5)</td>
<td>0 (0-1.25)</td>
<td>11 (5-17)</td>
<td>5 (4.5-5.5)</td>
<td>0 (0-0)</td>
<td>15 (7-15)</td>
</tr>
<tr>
<td>GB</td>
<td>11 (9.25-13.75)</td>
<td>0 (0-0)</td>
<td>9 (6-10)</td>
<td>--</td>
<td>11 (8-15)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>GD</td>
<td>4 (3-5)</td>
<td>0 (0-0)</td>
<td>9 (7-11)</td>
<td>3 (2-4.5)</td>
<td>1 (0.5-1.5)</td>
<td>1 (0-1)</td>
</tr>
<tr>
<td>GF</td>
<td>1.5 (0.75-2.25)</td>
<td>0 (0-0)</td>
<td>0 (0-0.5)</td>
<td>0 (0-0)</td>
<td>11 (5-14)</td>
<td>8 (5-6)</td>
</tr>
<tr>
<td>VX</td>
<td>9 (8.25-11.25)</td>
<td>0 (0-0)</td>
<td>3.5 (0-5.25)</td>
<td>0 (0-0.5)</td>
<td>16 (13.5-17)</td>
<td>0 (0-1)</td>
</tr>
<tr>
<td>VR</td>
<td>6 (4-16)</td>
<td>0 (0-1)</td>
<td>16 (4.5-18)</td>
<td>3 (1-4)</td>
<td>9 (4.5-14.5)</td>
<td>0 (0-0)</td>
</tr>
</tbody>
</table>

*Six brain areas (cerebral cortex, pyriform cortex, amygdala, hippocampus, thalamus, caudate/putamen) from each animal were evaluated and rated on a scale from 0-4 for neuropathological damage: 0 = no damage, 1 = minimal damage (1-10% necrotic neurons), 2 = mild (11-25%), 3 = moderate (26-45%), and 4 = severe (>45%). The severity of total brain damage was assessed by summing the neuropathology scores of the six areas (maximum = 24). Scores were expressed as median with interquartile range (IQR); Cells without IQR only have one subject. Values in **bold** are significant (p<0.05) vs. “Seizure on” group within treatment and treatment time.

ap<0.05 vs. scopolamine at same treatment time and seizure status.

bInsufficient number of survivors to determine statistical significance.
DISCUSSION

Our current study examined the efficacy of PCH and SCP following exposure to six traditional NAs (GA, GB, GD, GF, VX, and VR) in a guinea pig model that simulates the fielded human pretreatment and therapy regimen. Guinea pigs possess a lower level of carboxylesterase compared with rats (Inns and Leadbeater, 1983; O’Donnell et al., 2011) and are thus much more similar to humans. PCH or SCP treatment was given at either seizure onset or 40 min after seizure onset. These time points were chosen based on previous studies (McDonough and Shih, 1997; Shih and McDonough, 1997) that established the tri-phasic seizure model of NA intoxication. We chose to evaluate and compare the efficacy of PCH and SCP as they are both anticholinergic drugs and have been used as anti-motion sickness medications. PCH is an anticholinergic drug reportedly capable of antagonizing both muscarinic and nicotinic receptors and displays anti-NMDA properties following GD poisoning (Wang et al., 2005a), while SCP is a pure muscarinic antagonist compound that readily enters the brain (Ketchum et al., 1973). SCP has demonstrated efficacy as an adjunct to carbamate pyridostigmine, atropine, and oxime reactivator treatments following GD exposure to terminate seizures and reduce morbidity and mortality (Harris et al., 1994; Koplovitz et al., 2001; Koplovitz and Schulz, 2010). Previous results have also shown that SCP interacts with atropine sulfate to diminish seizure activity, improve survival rates, and reduce the amount of atropine needed for treatment of NA intoxication. Data further suggest that SCP may possess greater potency in the CNS compared with atropine sulfate (Koplovitz and Schulz, 2010).

Using a rat model, Wang et al. (2005a) examined the effectiveness of PCH and SCP following GD exposure. To study the drugs’ effects during the early cholinergic phase of NA-induced seizures, these researchers administered either PCH (4 mg/kg, ip) or SCP (2 mg/kg, ip) 5 min after seizure onset and found that both compounds terminated seizure at 8.9 ± 2.7 min and 4.6 ± 1.1 min, respectively. When these treatment drugs were administered im at seizure onset following GD intoxication in the guinea pigs, we observed similar seizure termination times for PCH-treated animals (8.9 ± 2.22 min) but obtained a different result for SCP administration (23.0 ± 18.25 min). This discrepancy may be a result of different routes of drug administration (ip vs. im), the differing animal models (rat vs. guinea pig), and the way the seizure termination times were utilized (based on a fixed dose by Wang et al. vs. varied doses in this study). However, the success of both of these treatments when given at seizure onset is expected given that the early stages of NA-induced seizure activity are modulated by cholinergic crisis, and both SCP and PCH exhibit excellent anticholinergic properties, with SCP showing higher overall efficacy (i.e., lower LD50 values). These results are also in agreement with previously published data using various cholinergic agonists at an earlier phase of NA exposure (Capacio and Shih, 1991; Anderson et al., 1994; McDonough et al., 2000; Shih et al., 2007).

When treatment was withheld until 40 min after seizure initiation, the efficacy of these two drugs began to diverge. In the rat study by Wang et al. (2005a), when PCH (8 mg/kg, ip) or SCP (8 - 60 mg/kg, ip) was administered 40 min after seizure onset following GD exposure, PCH continued to terminate seizure activity (9.3 ± 2.2 min), while SCP displayed no efficacy at all. Data on SCP were supported by a GD study conducted in rats by McDonough and Shih (1993) that showed that seizures were uncontrollable by SCP when administered 40 min after seizure.
onset. In our current guinea pig study, GD-induced seizures were terminated following the late administration of either drug, with PCH and SCP terminating seizure activity at a similar time delay. The inconsistency with regards to SCP’s effectiveness at the delayed treatment time could be explained by the different animal models employed. Possibly, in the guinea pig model, the transition from cholinergic to non-cholinergic seizure maintenance phase was much delayed and took a longer time to be established (i.e., prolonged transitional phase). This would increase the window of efficacy, even at 40 min after seizure onset, for purely anticholinergic compounds such as SCP. With regards to other NAs (i.e., GA, GB, GF, VX and VR) when compared with treatment at seizure onset, ongoing seizure in animals treated at 40 min after seizure onset took a significantly longer time to terminate following SCP treatment, while the time to terminate seizure following PCH treatment was similar, indicating the additional anti-NMDA action exerted by PCH during transitional phase.

To date, no publications have comprehensively investigated the efficacy of delayed anticonvulsant treatment against NA challenges other than GD. Although we were able to calculate the ED50 value for PCH administered 40 min after seizure onset for GD, we could not obtain the ED50 values for the other remaining NAs. We noted that little to no seizure termination was observed against GA and GB, while some degree of seizure termination was achieved following GF and VR exposures at sporadic doses of PCH. Nevertheless, seizures following GF and VR exposures terminated sooner for PCH-treated groups than for those receiving SCP. It was also interesting to note that in the case of VX challenge PCH was able to achieve 11/31 (35%) seizure terminations across various doses, compared to the 0/15 for SCP. In contrast, SCP demonstrated strong anticonvulsant properties against GA with an ED50 value of 1.88 mg/kg, whereas PCH could only achieve 3/39 (8%) terminations across various doses. These results demonstrate the different NA specificities of these two similarly structured anticholinergic compounds with delayed administration. The inconsistent drug efficacies against the different NA challenges could be attributable to varying transitional times to the non-cholinergic seizure maintenance phase. Also important to note is that the establishment of the tri-phasic model of NA-induced seizure maintenance exclusively evaluated GD exposure in a rat model (McDonough and Shih, 1997; Shih and McDonough, 1997). It is possible that NA compounds other than GD differ slightly in this timing scheme (e.g., NA-AChE binding time), shifting the efficacy window of anticholinergic compounds.

Delayed treatment with SCP maintained strong anticonvulsant properties overall for the remaining NA exposures, with the exception of GB or VX challenge, where few seizure terminations occurred. The loss of SCP efficacy in the latter case is not surprising given that this drug is reportedly a purely anti-muscarinic compound and would work best as an early therapeutic treatment during cholinergic excitation. The continued strong efficacy of delayed treatment following GA, GD, GF, and VR exposure was a bit unexpected but is supported by previously reported results in the guinea pig model following GD challenge (McDonough et al., 2000). Previous studies have demonstrated the anti-NMDA characteristics of certain anticholinergic compounds, including PCH, when administered at high concentrations. However, scopolamine was not among them, repeatedly failing to show any NMDA antagonistic properties (McDonough and Shih, 1995; Olney et al., 1987; Wang et al., 2005a). This lends support to the idea that at the 40 min time point after seizure initiation in the guinea pig model, at least a portion of the seizure maintenance is being supported by cholinergic processes (i.e., an extended
transitional phase of progressively mixed cholinergic/non-cholinergic). Further work investigating these transition time points in the guinea pig model will be necessary.

When investigating the various deleterious outcomes of NA-induced seizure activity such as weight loss, total neuropathology, and overall lethality, termination of seizure activity was the primary factor in obtaining a positive outcome. This is supported by several previous publications that demonstrated the control of seizure activity as being the primary determinant of prevention of neuropathology and lethality (Lallement et al., 1997; McDonough et al., 2000; Shih et al., 2003). No substantial differences in efficacy were observed across NA challenges or treatment if seizure activity was controlled effectively.

The pharmacokinetic (PK) properties of therapeutics under investigation are also of importance when evaluating their suitability as a novel treatment. However, few studies have investigated the PK properties of PCH, especially through intramuscular administration, as this compound is typically delivered orally for the treatment of motion sickness. And although much more PK data have been reported for SCP in both animal models and clinical settings, the available data have shown great inter-individual variability, making it difficult to draw strong conclusions (Renner et al., 2005). Nevertheless, the available data demonstrate that both PCH and SCP display rapid absorption and distribution, reaching $C_{\text{max}}$ values in approximately 4 - 20 min in both rodent studies and human trials following intramuscular administration, as summarized in Table 6 (Capacio et al., 2014; Ebert et al., 2001; Kou et al., 2008).

**Table 6.** Comparison of pharmacokinetic properties of scopolamine and phencynonate administered via intramuscular injection from published literature

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Capacio et al. 2014</th>
<th>Ebert et al. 2001</th>
<th>Kou et al. 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
<td>Guinea Pig</td>
<td>Human</td>
<td>Rat</td>
</tr>
<tr>
<td><strong>Detection</strong></td>
<td>LC-MS/MS; plasma</td>
<td>GC/ion trap tandem MS</td>
<td>LC-ESI/MS; blood</td>
</tr>
<tr>
<td><strong>Compound</strong></td>
<td>Scopolamine</td>
<td>Scopolamine</td>
<td>S-Phencynonate</td>
</tr>
<tr>
<td><strong>Dose (mg/kg)</strong></td>
<td>0.1</td>
<td>~0.007 (0.5 mg total)</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>$C_{\text{max}}$ (ng/mL)</strong></td>
<td>32.88</td>
<td>0.96</td>
<td>51.91</td>
</tr>
<tr>
<td><strong>$t_{\text{max}}$ (min)</strong></td>
<td>4.04</td>
<td>18.5</td>
<td>5.28</td>
</tr>
<tr>
<td><strong>$T_{1/2\beta}$ (hrs)</strong></td>
<td>N/D</td>
<td>1.15</td>
<td>4.68</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$ Maximum recorded plasma concentration.
$t_{\text{max}}$ Time to maximum plasma concentration.
$T_{1/2\beta}$ Elimination half-life; time for drug to reach half concentration after steady state.

Overall, this guinea pig study demonstrated the strong anticonvulsant properties of both PCH and SCP when administered at the time of seizure initiation among the 6 potential NAs investigated, with SCP displaying overall greater efficacy (lower ED$_{50}$ values). When treatment was withheld until the 40 min time point, there was a great deal more inter-NA variability, again with SCP displaying anticonvulsant efficacy in more NAs. Previous publications have almost exclusively utilized a GD challenge model for anticonvulsant studies. Our results demonstrate that PCH and SCP possess strong efficacy for both immediate and delayed treatment following GD exposure.
However, the efficacy of delayed treatment following the other investigated NAs was not as clear-cut; thus the data presented here on GA, GB, GF, VX, and VR should be of particular value when considering the efficacy of novel therapeutic compounds. Additionally, since SCP has been reported as a purely anti-muscarinic compound, its efficacy at the time point of seizure maintenance (late phase) thought to be controlled mainly by excitatory amino acids will require further investigation in animal models outside of what has been previously reported for the rat. Finally, although PCH displayed somewhat less consistent efficacy in this guinea pig study, its reported anti-NMDA properties warrant further evaluation as a delayed NA treatment in other animal models.
REFERENCES


Abbreviations:
ACh, acetylcholine
AChE, acetylcholinesterase
BBB, blood brain barrier
ChE, cholinesterase
CNS, central nervous system
DFP, diisopropylfluorophosphates or fluostigmine or dyflos
EEG, electroencephalogram
GA, tabun; GB, sarin
GD, soman
GF, cyclosarin
HI-6, 1-(4-carbamoylpyridino)methoxymethyl-2-(hydroxyiminomethyl) pyridinium dichloride
im, intramuscular
iv, intravenous
LD₅₀, median lethal dose
MMB-4, methoxime or 1,1’-methylene-bis[4-(hydroxyimino) methyl] pyridinium dichloride
NMDA, N-methyl-D-aspartate
OP, organophosphorus compound
2-PAM, pralidoxime or pyridine-2-aldoxime methylchloride
PCH, phencynonate hydrochloride
sc, subcutaneous
SCP, scopolamine hydrobromide
VR, o-isobutyl S-(2-(diethylamino)ethyl) methylphosphonothioate
VX, o-ethyl S-(2-(diisopropylamino)ethyl) methylphosphonothioate.