REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (*Leave blank*)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	2000	Open Literature publication

4. TITLE AND SUBTITLE
Anticonvulsant Treatment of Nerve Agent Seizures: Anticholinergics versus Diazepam in Soman-intoxicated Guinea Pigs

5. FUNDING NUMBERS
30162384ATC2
62384A

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

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10. SPONSORING/MONITORING AGENCY REPORT NUMBER
USAMRICD-P98-041

11. SUPPLEMENTARY NOTES
Published in Epilepsy Research, 38, 1-14, 2000.

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (*Maximum 200 words*)
See reprint

14. SUBJECT TERMS
seizure, status epilepticus, nerve agent, soman, anticonvulsant, anticholinergic, diazepam, EEG, neuropathology, guinea pig

15. NUMBER OF PAGES
14

16. PRICE CODE

S17. ECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED

ELSEVIER

Epilepsy Research 38 (2000) 1–14

Epilepsy Research

www.elsevier.com/locate/epilepsyres

Anticonvulsant treatment of nerve agent seizures: anticholinergics versus diazepam in soman-intoxicated guinea pigs☆

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Received 12 January 1999; received in revised form 20 April 1999; accepted 8 June 1999

Abstract

A total of eight anticholinergic drugs (aprophen, atropine, azaprophen, benactyzine, biperiden, procyclidine, scopolamine, trihexyphenidyl) were tested in parallel with diazepam for the ability to terminate seizure activity induced by the nerve agent soman. Guinea pigs, implanted with electrodes to record cortical electroencephalographic (EEG) activity, were pretreated with pyridostigmine Br (0.026 mg/kg, i.m.) and 30 min later challenged with $2 \times LD_{50}$ soman (56 μg/kg, s.c.) followed 1 min later by treatment with atropine SO_4 (2 mg/kg, i.m.) and pralidoxime chloride (2-PAM Cl; 25 mg/kg, i.m.). All guinea pigs developed sustained seizure activity following this treatment. Dose-effect curves were determined for the ability of each drug to terminate seizure activity when anticonvulsant treatment was given either 5 or 40 min after seizure onset. Body weight gain and recovery of behavioral performance of a previously trained one-way avoidance task were measured after exposure. With the exception of atropine, all anticholinergic drugs were effective at lower doses than diazepam in terminating seizures when given 5 min after seizure onset; benactyzine, procyclidine and aprophen terminated seizures most rapidly while scopolamine, trihexyphenidyl, biperiden, and diazepam were significantly slower. When given 40 min after seizure onset, diazepam was the most potent compound tested, followed by scopolamine, benactyzine and biperiden; atropine was not effective when tested 40 min after seizure onset. For diazepam, the time to terminate the seizure was the same whether it was given at the 5- or 40-min delay. In contrast, most anticholinergics were significantly slower in terminating seizure activity when

☆ The animals used in this study were handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, proposed by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHHA, National Institute of Health Publication 85-23, 1985, and the Animal Welfare Act of 1966, as amended. The opinions or assertions contained therein are the private views of the authors, and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

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0920-1211/00/$ - see front matter Published by Elsevier Science B.V.

PII: S0920-1211(99)00060-1

given at the 40-min delay relative to when they were given at the 5-min delay. Successful control of seizure activity, regardless of the drug, was predictive of survival of the lethal effects of nerve agent exposure, a more rapid behavioral recovery (body weight, avoidance performance) and greater protection from neuropathology. In contrast, failure of a drug treatment to terminate seizure activity was closely associated with an increased probability of acute (< 24 h) and delayed (10-day survival) lethality, a slower behavioral recovery in survivors, and an increased incidence and degree of neuropathology. Published by Elsevier Science B.V.

Keywords: Seizure; Status epilepticus; Nerve agent; Soman; Anticonvulsant; Anticholinergic; Diazepam; EEG; Neuropathology; Guinea pig

1. Introduction

The nerve agents, such as soman, sarin, tabun and VX, are organophosphorus cholinesterase inhibitors. Exposure to these agents causes a progression of toxic signs, including hypersecretions, fasciculations, tremor, convulsions and respiratory distress. These toxic effects are due to hyperactivity of the cholinergic system as a result of inhibition of cholinesterase and the subsequent increase of the neurotransmitter acetylcholine at central and peripheral sites. A combined treatment regimen of prophylaxis and therapy is now generally agreed upon as the most effective medical countermeasure for dealing with the threat of nerve agent poisoning (Dunn and Sidell, 1989; Sidell, 1992). Pretreatment with carbamate cholinesterase inhibitors, such as pyridostigmine, shield a fraction of cholinesterase in the periphery from irreversible inhibition by the nerve agent. In the event of poisoning, an anticholinergic drug such as atropine sulfate is used to antagonize the effects of excess acetylcholine at muscarinic receptor sites, and an oxime such as pralidoxime chloride (2-PAM Cl) is used to reactivate any unaged inhibited enzyme (Sidell, 1992).

However, this treatment regimen does not control the development of nerve agent-induced seizures (Dunn and Sidell, 1989; McDonough and Shih, 1997). Prolonged generalized seizures (status epilepticus) can begin rapidly after nerve agent exposure in humans (Morita et al., 1995; Nozaki et al., 1995; Okumura et al., 1996; Ohbu et al., 1997). Animal studies show these seizures can result in prolonged physical incapacitation and neuropathology (Lipp, 1968; Lemercier et al., 1983; McDonough et al., 1989, 1995; Carpentier et al., 1990; Hayward et al., 1990; Castro et al., 1991; Shih et al., 1991; Philippens et al., 1992; Baze, 1993; Lallement et al., 1994). Although diazepam is used as an immediate anticonvulsant treatment for seizures induced by nerve agents or other organophosphorus anticholinesterase compounds (Sidell, 1992, 1994; Moore et al., 1995), research has demonstrated that diazepam is not always completely effective in protecting animals against nerve agent-induced neuropathology (Martin et al., 1985; McDonough et al., 1989, 1995; Hayward et al., 1990; Philippens et al., 1992; Clement and Broxup, 1993; Lallement et al., 1997). Because of this, diazepam may not be the optimal drug to serve as the sole immediate treatment of nerve agent-induced seizures.

Muscarinic anticholinergic drugs with strong central activity are also very effective in antagonizing nerve agent-induced seizures and in protecting against the subsequent neuropathology (Green et al., 1977; McDonough et al., 1989; Capacio and Shih, 1991; McDonough and Shih, 1993; Sparenborg et al., 1993; Shih et al., 1997). Anticholinergics possess several features that potentially make them as attractive as diazepam for the treatment of nerve agent seizures. Specifically, many anticholinergics are more potent than diazepam in stopping seizures when they are given shortly after seizure onset (Capacio and Shih, 1991; McDonough and Shih, 1993); anticholinergics reportedly reverse the immediate physical incapacitation of agent intoxication more rapidly than diazepam (Anderson et al., 1994a,b, 1997); and seizures tend not to recur as frequently following anticholinergic treatment when compared to diazepam (McDonough and Shih, 1993; Anderson et al., 1997; Shih et al., 1997).

However, the previous studies of anticholinergic drugs to moderate nerve agent-induced seizures used a variety of procedures that make a true assessment of their anticonvulsant potential, in comparison with diazepam, difficult. For example, the studies of Capacio and Shih (1991), McDonough and Shih (1993) and Shih et al. (1997) used rats that were pretreated with high doses of the oxime HI-6. In some instances, the anticholinergics were given as pretreatments and assessed for their ability to block the development of seizure activity, or the drug treatments were given by varying routes of administration and/or at differing times after agent exposure. Thus, none of the previous experiments modeled the conventional medical treatment protocols for treatment of severe nerve agent exposure.

The present study compared the anticonvulsant activity of several anticholinergic drugs with diazepam when given as part of a standard treatment protocol following nerve agent exposure. The treatment regimen included carbamate (i.e. pyridostigmine) pretreatment followed by atropine + oxime (2-PAM Cl) therapy after exposure to the nerve agent soman in guinea pigs. The potential anticonvulsant drugs were given at either a short (5 min) or delayed (40 min) time after seizure onset. This was done to test the effectiveness of the drugs against the two phases of the seizure that are controlled by different neuropharmacological mechanisms (McDonough and Shih, 1993, 1997). All animals were monitored electrophysiologically to determine whether the test drug terminated seizure activity. The animals were also assessed for immediate and long-term survival, recovery of body weight and recovery of previously trained behavioral performance. Finally, animals were evaluated histologically for neuropathological changes that may have resulted from nerve agent exposure.

2. Methods

2.1. Subjects

A total of 521 male Hartley guinea pigs (Charles River Laboratories, Wilmington, MA) weighing 250–300 g served as subjects. Animals were individually housed in temperature- (21 ± 2°C) and humidity-controlled (50 ± 10%) quarters under a 12-h light-dark cycle with lights on at 06:00 h. Animals had ad libitum access to food and water except during experimental periods.

2.2. Surgery

The guinea pigs were implanted with cortical screw electrodes to monitor EEG activity using standard small animal surgical procedures. The screws were placed approximately equidistant between bregma and lamda and ~ 3.0 mm lateral to the midline. After surgery the animal was given buprenorphine HCl (0.03 mg/kg, s.c.) for relief of pain. Nerve agent exposure occurred at least 1 week after surgery.

2.3. Nerve agent exposure procedure

On the day of the experiment, the guinea pigs were placed in recording chambers (30 cm L; 24 cm W; 35 cm H), and EEG was monitored for at least a 15-min baseline period. The animals were then pretreated with a dose of pyridostigmine Br (0.026 mg/kg, i.m.; 0.5 ml/kg) calculated to achieve 20–40% inhibition of blood cholinesterase (Lennox et al., 1985). Then 30 min after the pyridostigmine injection, the animals were challenged with $2 \times LD_{50}$ of soman (56 μg/kg, s.c.; 0.5 ml/kg) and 1 min after soman exposure the animals were injected i.m. with atropine SO_4 (2 mg/kg) admixed with 2-PAM Cl (25 mg/kg) (total volume = 0.5 ml/kg). For each animal, the onset of seizure activity was identified on the EEG tracing (repetitive high amplitude spike/sharp wave activity, > 10-s duration). Either 5 or 40 min following seizure onset, the anticonvulsant treatment drug was given, i.m., at a concentration to deliver 0.5 ml/kg. The EEG of each animal was recorded continuously for at least 4 h after the anticonvulsant treatment, and another 30-min EEG sample was obtained the next day (24-h observation). Each animal was rated as having the seizure terminated (OFF) or not terminated (NOT OFF) based on the overall appearance of the EEG at the end of the day and during the 24-h

observation. (Note: An animal was rated as OFF if the seizure was terminated and the EEG remained normal at all subsequent observation times.) Animals were weighed before and daily following exposure and were tested daily for recovery of one-way avoidance performance. Then 10 days after exposure, surviving animals were deeply anesthetized with pentobarbital (75 mg/kg, i.p.), and perfused through the aorta with saline followed by 10% neutral buffered formalin. The brain was blocked, embedded in paraffin, cut 6–10 μm thick, stained with hematoxylin and eosin and then evaluated by a board certified pathologist (C.D.S.) who was unaware of the experimental history of a given subject. The procedures and criteria used for pathological evaluation have been published (McDonough et al., 1989, 1995). Briefly, six brain areas (cerebral cortex, pyriform cortex, amygdala, hippocampus, caudate nucleus, thalamus) were evaluated in each animal; each area was given a score that described brain lesion severity based on the approximate percentage of tissue involvement: 0 = none; 1 = minimal, 1–10%; 2 = mild, 11–25%; 3 = moderate, 26–45%; 4 = severe, > 45%. For each animal, a total neuropathology score was obtained by summing the scores of the six brain areas. The criteria used to characterize the lesion/pathology was neuronal necrosis, e.g. shrunken eosinophilic neurons with dark, round, pyknotic nuclei.

2.4. One-way avoidance procedures

The apparatus was a jump-up shelf avoidance device. It consisted of an enclosed box (22 cm L; 21 cm W; 20 cm H) with a grid floor and a movable door that could slide back to reveal a 'safe' shelf 8 cm above the floor. Training consisted of 30 trials per day with a 20-s intertrial interval (ITI). Each trial began with a 4-s presentation of the conditioned stimulus (CS), which consisted of the illumination of a light and the sliding back of the movable door exposing the 'safe' shelf. If the animal did not jump up to the shelf within the 4-s CS period an 0.8-mA scrambled shock was applied to the grid bars until the animal responded or for a maximum duration of 4 s. If the animal jumped up, it was allowed to remain on the shelf for 10-s before the sliding door closed, pushing it back down to the grid floor for the start of the ITI. The animal's jumping up during the 4-s CS period was an avoidance response. Criterion performance was defined as an avoidance response on $\geq 80\%$ (24 trials) of the daily trials. Animals were trained to criterion before exposure and then daily after exposure until criterion was again met or for a maximum of ten sessions.

2.5. Experimental design

The drugs tested were aprophen HCl, atropine SO_4, azaprophen HCl, benactyzine HCl, biperiden HCl, diazepam, procyclidine HCl, scopolamine HBr, and trihexyphenidyl HCl. Preliminary range-finding studies were conducted using the up-down procedure of Dixon and Massey (1981) to estimate an ED_{50} for each drug at each treatment time (5- or 40-min). Based on these estimated ED_{50}s, a probit experiment was performed that utilized at least five groups of six animals each with the middle dose group receiving the ED_{50} estimated from the range-finding study.

2.6. Drugs

Atropine SO_4, benactyzine HCl, procyclidine HCl, scopolamine HBr, and trihexyphenidyl HCl were purchased from Sigma (St. Louis, MO). Biperiden HCl was purchased from Knoll Pharmaceuticals (Whippany, NJ). Diazepam and pyridostigmine Br were purchased from Hoffman-La Roche (Nutley, NJ). Aprophen HCl and azaprophen HCl were prepared by Starks Associates (Buffalo, NY) under contract to the Walter Reed Army Institute of Research. Pralidoxime chloride was purchased from Ayerst Labs (New York, NY). Soman (pinacolyl methylphosphonofluoridate) was obtained from the U.S. Army Edgewood Research, Development and Engineering Center (Aberdeen Proving Ground, MD). Biperiden HCl, diazepam, and trihexyphenidyl HCl were diluted in a vehicle containing 40% propylene glycol, 10% ethanol, 1.5% benzyl alcohol, and 48.5% distilled water. All other drugs were diluted in saline.

2.7. Data analysis

The anticonvulsant ED_{50} for each compound was determined using probit analysis (Bliss, 1952). The proportion of animals surviving as a function of successful control of the seizure, as well as the incidence of neuropathology as a function of seizure control, was evaluated using the chi-square procedure with Yates correction (Winer, 1971). The latency to seizure termination was evaluated between drugs using the Kruskal–Wallis one-way analysis of ranks (Winer, 1971). The number of sessions to reacquire the one-way avoidance task, the number of days to regain baseline body weight, and total neuropathology scores were categorized by whether seizure was turned OFF versus NOT OFF and by drug, and then each was evaluated by a two-way analysis of variance (Winer, 1971).

3. Results

3.1. Toxic signs

All the animals developed continuous seizures when the pyridostigmine pretreatment, soman exposure and immediate atropine and 2-PAM Cl post-exposure treatment protocol described above was followed. The earliest sign of soman intoxication was repetitive chewing followed by mild to moderate, episodic, whole body tremors. At the onset of EEG spikes, in many cases there was no obvious accompanying motor sign; the animal would stare, and some chewed, while maintaining an immobile posture. Onset of seizure activity was $X = 523$ s (8 min, 43 s; S.D. = 182 s; $n = 521$). After EEG seizure activity began, the physical signs of intoxication continued to evolve: the tremor recurred and episodes of forepaw and hindpaw clonus developed, and eventually the righting reflex was lost. Under the conditions of this study, the 24-h lethality, regardless of drug treatment, was 34% (175 of 521). However, there was significant continuing mortality in survivors; at 10 days after exposure the cumulative lethality was 48% (249 of 521). Of the 346 survivors at 24 h, 21% (74 animals; 27 of the OFF category, 47 of the NOT OFF category) had died by 10 days after the exposure.

3.2. Anticonvulsant effects

All the drugs were effective anticonvulsants when given 5 min after seizure onset, and all, with the exception of atropine, were effective when given at the 40-min treatment time. The ED_{50} and 95% fiducial limits for the drugs are given in Table 1; Fig. 1 displays the dose-effect curves. At the 5-min treatment time all anticholinergics, with the exception of atropine, had lower ED_{50}s than diazepam. Scopolamine, biperiden, trihexyphenidyl, procyclidine and, possibly, benactyzine, were clearly more potent than diazepam, while aprophen and azaprophen overlapped the lower effective dose range of diazepam.

Diazepam was the most potent drug for producing an anticonvulsant effect at the 40-min treatment time, and the effective dose range for diazepam was virtually identical to its effective dose range at the 5-min treatment time. Scopolamine, benactyzine and biperiden were the most potent anticholinergics at the 40-min treatment time. However, the 40-min treatment time anticonvulsant ED_{50}s of all anticholinergics were 10–100 times greater than the ED_{50}s of these drugs at the 5-min treatment time. In addition, the therapeutic effectiveness was variable enough in many cases (benactyzine, biperiden, procyclidine, scopo-

Table 1
ED_{50}s (mg/kg) for an anticonvulsant effect at the 5-min and 40-min treatment times[a]

Drug	5-min Treatment	40-min Treatment
Aprophen	2.93 (1.96–5.26)	33.84 (27.59–49.11)
Atropine	12.15 (8.45–16.65)	ND
Azaprophen	1.87 (1.40–4.12)	22.48 (15.68–1984.0)
Benactyzine	1.09[b]	13.98[b]
Biperiden	0.57 (0.38–0.84)	19.73[b]
Diazepam	4.81 (3.01–23.20)	7.05 (5.05–9.53)
Procyclidine	1.57 (0.97–2.38)	32.93[b]
Scopolamine	0.12 (0.09–0.16)	11.75[b]
Trihexyphenidyl	0.82 (0.45–1.13)	37.05[b]

[a] 95% Fiducial limits in parentheses. ND, ED_{50} could not be determined.

[b] 95% Fiducial limits could not be determined.

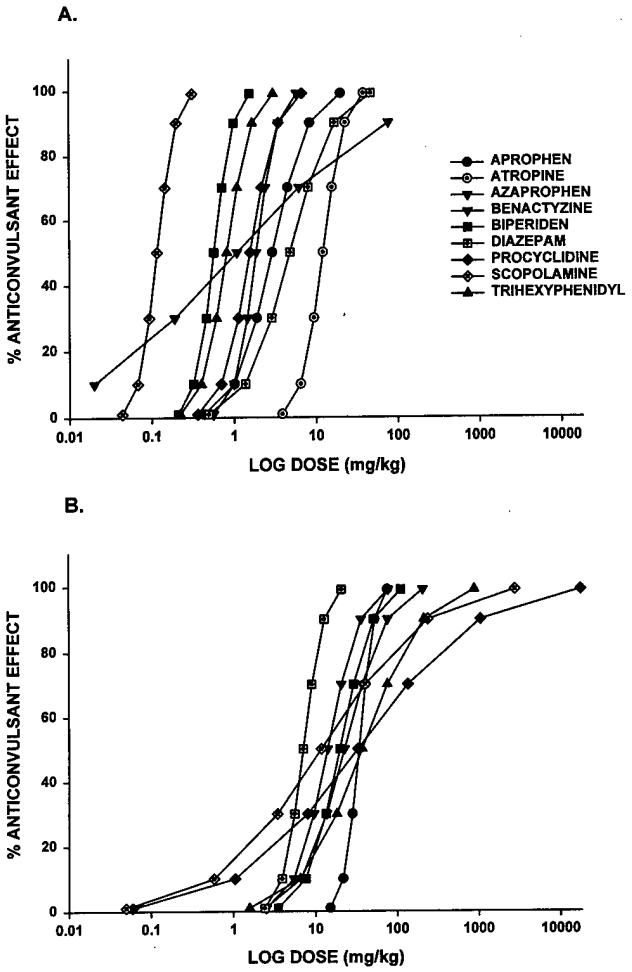

Fig. 1. Log dose-effect curves for an anticonvulsant effect of the eight anticholinergic drugs and diazepam in guinea pigs pretreated with pyridostigmine (0.026 mg/kg, i.m.), challenged with $2 \times LD_{50}$ soman (56 μg/kg, s.c.), and treated 1 min later with atropine (2 mg/kg, i.m.) and 2-PAM (25 mg/kg, i.m.). Panel A represents data for when the treatment drugs were given 5 min after seizure onset; panel B represents data for when treatment drugs were given 40 min after seizure onset.

lamine and trihexyphenidyl) to obviate the calculation of valid confidence limits for the probit lines. In the case of atropine, anticonvulsant effects were only sporadically obtained at the 40-min treatment delay, and these occurred at doses so high (>40 mg/kg) as to produce lethal toxic effects in all animals within 1–4 h after treatment.

3.3. Latency of seizure control

Latencies for seizure termination were tabulated by drug for all subjects rated as having the seizure turned OFF regardless of the dose. These data are presented in Fig. 2. Kruskal–Wallis tests of both the 5- and 40-min treatment data revealed statistically significant effects (5-min: $H = 51.50$, $df = 8$, $P < 0.001$; 40-min: $H = 29.13$, $df = 7$, $P < 0.001$). At the 5-min treatment time benactyzine, procyclidine and aprophen terminated seizures significantly faster than biperiden, trihexyphenidyl and diazepam; benactyzine and procyclidine also terminated the seizures more rapidly than scopolamine. There were no differences between aza-prophen, atropine, trihexyphenidyl, biperiden,

scopolamine and diazepam in the time to terminate seizures. At the 40-min treatment time, procyclidine and biperiden terminated seizures more rapidly than azaprophen; procyclidine was also more rapid than scopolamine in terminating seizures. All other drugs were equivalent in the speed to terminate seizures at the 40-min treatment time. Diazepam terminated seizures with equivalent speed when given at either the 5- or 40-min treatment time. In contrast, seizure termination latencies for all the anticholinergic drugs at the 40-min treatment time were markedly slower when compared with their 5-min treatment time latencies.

3.4. EEG response to drug treatment and acute behavioral effects

Differences in the EEG characteristics of seizure termination and immediate behavioral re-

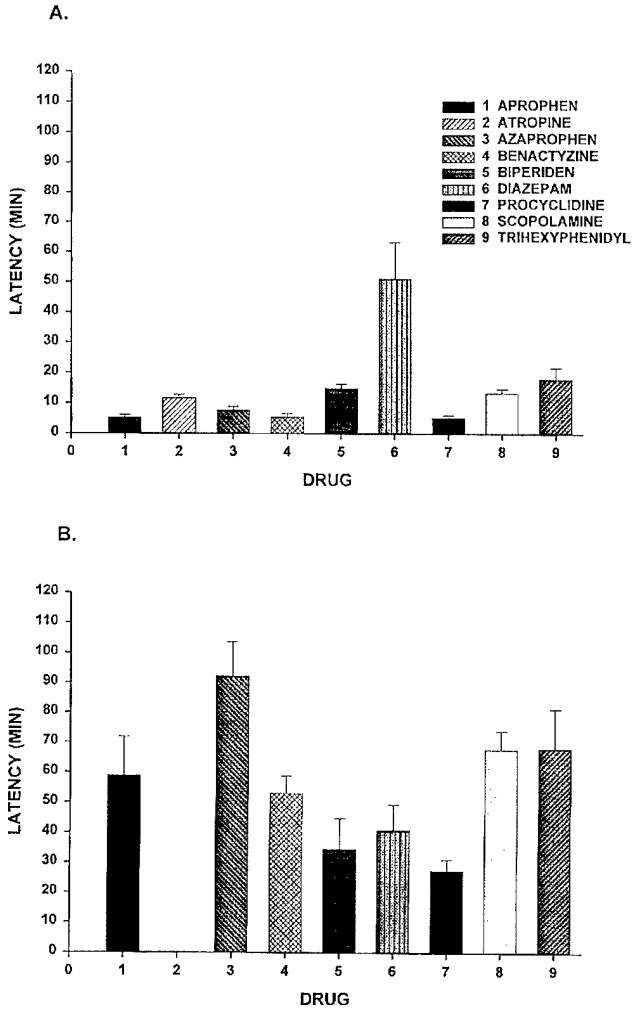

Fig. 2. Mean latencies (min) and S.E.M. for termination of seizure activity following drug treatment. Panel A represents data for when the treatment drugs were given 5 min after seizure onset; panel B represents data for when treatment drugs were given 40 min after seizure onset. Note that atropine was not tested at the 40 min-treatment time: the corresponding slot was left blank for easier comparisons of the drugs at the different treatment times.

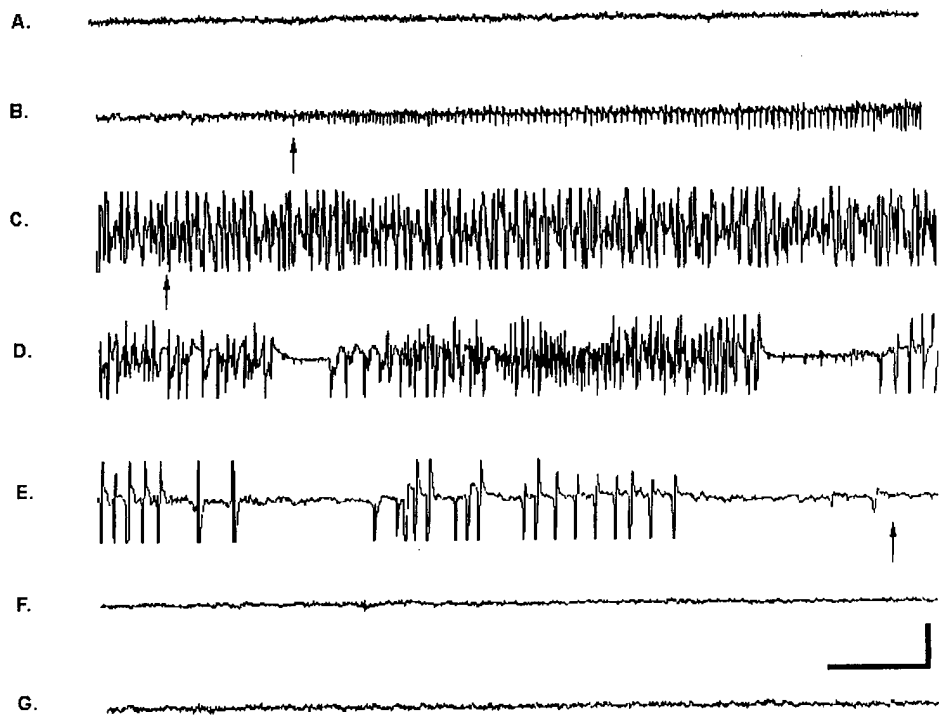

Fig. 3. An example of the control of a soman-induced seizure by an anticholinergic drug. Each discontinuous strip represents approximately 30 s of EEG. (A) Baseline; (B) seizure onset (arrow); (C) injection of scopolamine (0.09 mg/kg, i.m.) (arrow) 5 min after seizure onset; (D) approximately 10.5 min after scopolamine injection: note brief breaks in spike discharges; (E) continuation of record shown in (D): note the discharge now consists of isolated high amplitude spikes followed by longer pauses; seizure was judged off at the arrow, 670 s from the time scopolamine was injected; (F–G) EEG remains free of epileptiform activity 5 min and 4 h, respectively, after the termination of the seizure displayed in E. Calibration: 5 s; 1000 μV.

covery were also apparent. At the 5-min treatment delay time, anticholinergic drugs tended to abruptly terminate seizure activity (Fig. 3). Brief breaks in the almost continuous spiking of the seizure would first become apparent and then there would be a sudden termination. Occasionally there would be some residual spiking at a lower amplitude and frequency for 1–5 min, but after this period the EEG would revert to a relatively normal pattern. Behaviorally there was also a reversal of the physical signs of incapacitation. The animal could right itself and within 15–30 min appeared to become oriented to its surroundings. At the 40-min treatment time this abrupt termination of the high amplitude spiking would also occur with anticholinergic drugs, but the period of residual isolated spikes, sharp waves and/or slow wave activity was longer. When anticholinergic drugs terminated seizure activity, a tremor and a general unsteadiness was still evident upon handling and could persist >4 h following treatment. In contrast, the EEG response of animals that received diazepam at either the 5- or 40-min treatment time, and in which the seizure turned off, would generally show a progressive diminution of spike amplitude and frequency that resolved gradually over time (Fig. 4). Some diazepam-treated animals ($\sim 25\%$ at both treatment times) showed a more rapid termination of seizure activity, but the prolonged pattern was by far the more typical response. Behaviorally, diazepam-treated subjects displayed a marked physical incapacitation after treatment and rarely regained a righting reflex within 2 h.

The EEG and behavioral response of animals in which drug treatment failed to stop the seizure

was markedly different. Anticholinergic-treated animals showed the brief breaks in the spiking, but these were typically short and failed to increase in duration. Gradually the duration of these breaks grew less and the spiking would merge back to the continuous pattern seen before treatment. Diazepam-treated subjects showed an initial decrease in seizure spike amplitude and frequency, but these changes did not progress beyond a point and there would be a gradual return of more intense epileptiform activity. Behaviorally, all the animals would display continuing evidence of limbic seizure, primarily facial, forelimb and head clonus, but these became less notable the longer the seizure continued, and the animal became more debilitated. By ~1 h after exposure and treatment most of the animals that had continuing strong seizures had lost righting reflex; long-term survival of animals displaying this condition was not likely. A smaller percentage of animals (~15%) in which the seizure failed to stop displayed an attenuated, but continuing, EEG seizure pattern in the absence of notable motor clonus. They were able to maintain righting reflex and were less physically compromised.

3.5. Seizure control and lethality

There was a strong relationship between the control of seizures and protection against the lethal effects of soman exposure. Animals were categorized by seizure outcome (OFF, NOT OFF)

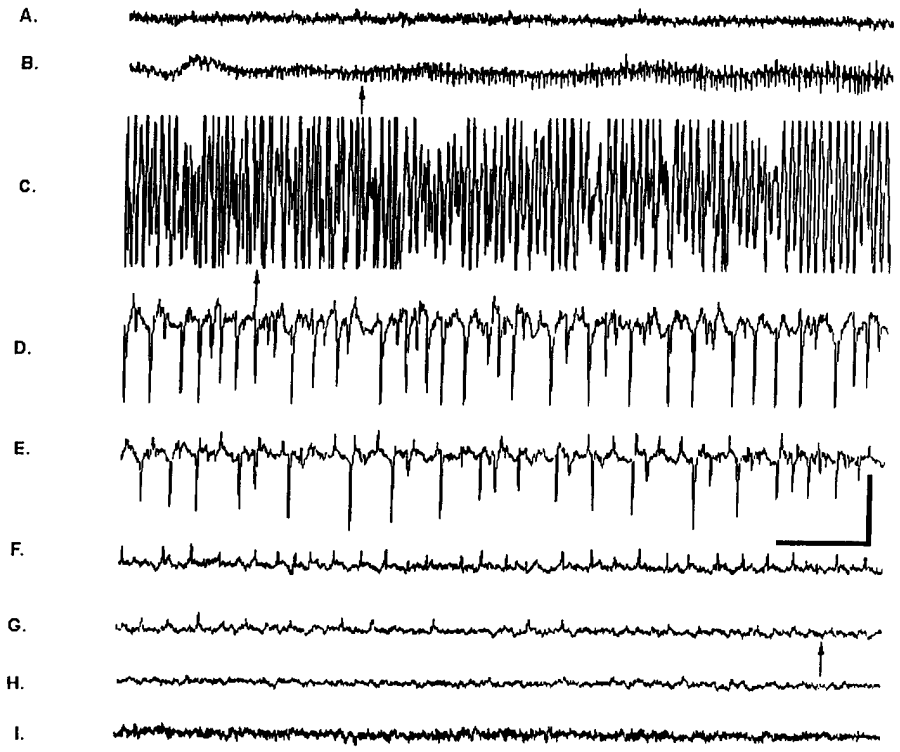

Fig. 4. An example of the control of a soman-induced seizure by diazepam. Each discontinuous strip represents approximately 30 s of EEG. (A) Baseline; (B) seizure onset (arrow); (C) injection of diazepam (6.89 mg/kg, i.m.) 5 min after seizure onset (arrow); (D) 5 min after diazepam injection: note the attenuation of the frequency and amplitude of the discharge; (E) 10 min and (F) 15 min, after diazepam injection: note continuing diminution of both the frequency and amplitude of the discharge; (G) epileptiform activity fades so as to be indistinguishable from background EEG: seizure was judged off at the arrow, 18 min 15 s after diazepam injection; (H–I) EEG remains free of epileptiform activity 5 min and 4 h, respectively, after the termination of the seizure displayed in (G). Calibration: 5 s; 1000 μV.

Table 2
Acute (24-h) and long-term (10-day) survival as a function of seizure control

	24-h Survival		10-day Survival	
	ALIVE	DEAD	ALIVE	DEAD
OFF	221	11	194	38
NOT OFF	125	164	78	211

and lethality (ALIVE, DEAD) for two survival times: 24 h (a measure of acute lethality) and 10 days (a measure of long-term survival). These data are presented in Table 2. Chi-square analysis showed a highly significant effect for either the 24-h survival time ($\chi^2 = 153.7$, df = 1, $P < 0.001$) or the 10-day survival time ($\chi^2 = 163.15$, df = 1, $P < 0.001$). At the 24-h survival time, < 5% of the animals that had their seizure successfully controlled died, whereas > 55% of animals that failed to have the seizure controlled had died by that time. Additionally, of the animals that did not have their seizure controlled by the drug treatment and survived 24 h, 25% had received diazepam as the anticonvulsant treatment. Thus, diazepam was more likely to protect against the acute lethal effects of soman exposure even though the seizure was not totally controlled. None of the eight anticholinergic drugs showed a similar trend. There was a continuing mortality over days, and the 10-day survival data show that 73% of the seizure NOT OFF animals had died, whereas only 16% of the seizure OFF animals had died. Again, if drug treatment did not stop the seizures, diazepam treated subjects were more likely to survive long-term (22 of 36, 61%) than anticholinergic-treated subjects (survival percentages ranged from 13 to 31%).

3.6. One-way avoidance behavior and body weight recovery

The number of training sessions to regain criterion avoidance performance or days to regain body weight on the days after exposure were tabulated by drug treatment and categorized by treatment outcome: seizure OFF versus NOT OFF. (Note: the data were collapsed across doses and treatment times within a drug because of the low number of survivors in some dose groups.) A two-way ANOVA with drug and seizure outcome as main variables showed the main effects for treatment outcome ($F = 12.07$, df = 1,7, $P < 0.001$) and drug ($F = 4.37$, df = 7,208, $P < 0.001$) were significant; the treatment outcome × drug interaction was not significant. Animals with seizure OFF recovered avoidance performance significantly faster ($X = 3.1$ sessions) compared with seizure NOT OFF animals ($X = 4.12$ sessions). In addition, regardless of treatment outcome, biperiden-treated animals required more sessions to regain criterion ($P < 0.05$) than animals treated with benactyzine, scopolamine, azaprophen or diazepam, and aprophen-treated animals required significantly more sessions ($P < 0.05$) to regain criterion than benactyzine-treated subjects. The lack of a significant treatment outcome × drug interaction indicated that no particular drug was superior to another in allowing more rapid recovery of behavioral performance as a function of treatment outcome. The results of the body weight data were somewhat similar; there was a significant effect of treatment outcome on body weight recovery ($F = 5.23$, df = 1,7, $P < 0.02$); the main effect for drug and the drug × treatment outcome interaction were not significant. Seizure OFF animals recovered body weight ($X = 6.44$ days) significantly sooner than animals rated NOT OFF ($X = 7.44$ days). No particular drug was superior to another in producing recovery.

3.7. Neuropathology

The brains of 280 animals were available for detailed neuropathology evaluation. No drug completely protected against neuropathology development, but control of the seizure clearly had an influence on both incidence and severity of neuropathology. Animals that were rated seizure OFF were significantly more likely to be free of any neuropathology (5- and 40-min treatments combined: $\chi^2 = 15.81$, df = 1, $P < 0.001$). This was also reflected in the analysis of the total neuropathology scores that used treatment outcome (OFF, NOT OFF) and drug as factors (NOTE:

dose of drug was not considered a factor due to no/small number of survivors in some dose groups.) (5-min treatment: $F = 25.78$, df = 1,127, $P < 0.001$; 40-min treatment: $F = 16.65$, df = 1,119, $P < 0.001$). Animals in which seizures were NOT OFF had significantly higher neuropathology scores (5-min treatment, $X = 2.28$; 40-min treatment, $X = 2.86$) than animals rated as OFF (5-treatment, $X = 0.50$; 40-min treatment, $X = 1.25$). In both analyses the drug factor was significant. In the 5-min treatment case this was due to a single exceptionally high score (one animal in the atropine, NOT OFF, category had a neuropathology score of 11). In the 40-min treatment case there was only one significant difference of 36 possible comparisons (diazepam-treated animals had significantly higher neuropathology scores than biperiden-treated animals). The drug × treatment outcome interactions were also significant. In all cases, subjects in the seizure NOT OFF category for certain drugs had significantly higher neuropathology scores than animals in the seizure OFF category for the same drug. There were no differences in neuropathology scores between drugs in the seizure OFF category in either analysis. Thus, no drug was superior or inferior to another in protecting against brain damage given that the seizure was controlled.

The prevalence of damage to the six neural areas that were evaluated showed distinct differences. There were 123 subjects rated as having neural damage; in these subjects the amygdala was most frequently damaged ($n = 83$, 67%), followed by the cerebral cortex ($n = 66$, 54%), piriform cortex ($n = 38$, 31%), thalamus ($n = 36$, 29%), hippocampus ($n = 31$, 25%), and the caudate nucleus ($n = 12$, 10%).

4. Discussion

The results show that anticholinergic drugs can exert significant anticonvulsant effects against seizures induced by nerve agent. When given shortly after seizure onset, anticholinergics were more potent and more rapidly acting than diazepam, and they produced a more rapid reversal of the physical incapacitation produced by the intoxication. However, the data also indicate that anticholinergics work most effectively when given shortly after seizure onset. Because of this, they cannot serve simply as total replacements for diazepam.

The ability of low doses of anticholinergic drugs to rapidly stop nerve agent-induced seizures has been seen in previous studies (Capacio and Shih, 1991; Shih et al., 1991, 1997; McDonough and Shih, 1993; Sparenborg et al., 1993; Anderson et al., 1994a,b). Likewise, the need for larger amounts of anticholinergic drugs to terminate seizures when treatment is delayed also parallels previous findings in rats, where anticholinergics lose anticonvulsant effectiveness the longer treatment is delayed (McDonough and Shih, 1993; Shih et al., 1997). Although there are certain minor differences between all these studies, taken as a whole, the findings support the proposed hypothesis that the induction of nerve agent-induced seizures is due to cholinergic hyperstimulation, and, if not promptly controlled, the seizure activity per se then progressively activates other, non-cholinergic (e.g. excitatory amino acid), neurotransmitter systems that then come to exert control over the seizure process (McDonough and Shih, 1997; Lallement et al., 1998). The dose and latency of a drug's anticonvulsant effect is dependent primarily on the mechanism of action of the particular drug and its pharmacokinetic profile. Anticholinergics are highly effective when given early because they specifically block the cholinergic hyperactivity that initially drives the seizure. At the long treatment delay times, anticholinergic drugs may also exert anticonvulsant effects, but this may be through non-cholinergic mechanisms. Several studies have shown that high doses of anticholinergic drugs exert *N*-methyl-D-aspartate (NMDA) antagonist activity (Olney et al., 1987; McDonough and Shih, 1995). This would explain the need for such large increases in drug dosage and why latencies for seizure control increased at the 40-min as compared with the 5-min treatment times for the anticholinergic drugs. In contrast, diazepam exerts its anticonvulsant effect by enhancing inhibitory gamma amino butyric acid (GABA) activity. Because this action is not specific to the mechanisms of nerve agent seizure

induction or maintenance, diazepam is equally effective (ED_{50}, latency for seizure control) at either of the treatment delay times.

The results show that the doses of diazepam required for anticonvulsant effectiveness against soman-induced seizures are comparable with those (10–30 mg/kg) reported to be effective in other rodent models of status epilepticus (Morrisett et al., 1987; Walton and Treiman, 1988, 1991; Fujikawa, 1995). However, the doses of diazepam used in many previous studies of nerve agent seizures were most likely less than optimum for producing robust anticonvulsant effects (McDonough et al., 1989; Shih, 1990; Philippens et al., 1992; Clement and Broxup, 1993; Sket, 1993; Anderson et al., 1994a,b; Harris et al., 1994).

In a previous rat study, early successful treatment of soman-induced seizures totally protected animals from neuropathology development (McDonough et al., 1995). In contrast, successful treatment of seizure activity at the same delay time in guinea pigs did not produce comparable levels of protection, although it clearly reduced the incidence and severity of neuropathology. Also, in the previous rat study (McDonough et al., 1995) the development of neuropathology in animals that experienced uncontrolled seizures was >95%, whereas in this study 62% (54 of 87) of the guinea pigs developed neuropathology under similar seizure conditions. Another difference between the rat study and the present guinea pig experiment was the relative frequency and ranking of the different neural structures that developed neuropathology following soman. In the rat study (McDonough et al., 1995), the incidence of damage was as follows: piriform cortex = 89%, amygdala = 72%, hippocampus = 70%, thalamus = 55%, cerebral cortex = 49%, caudate/putamen = 41%. In the present study, the relative percentage of guinea pigs experiencing damage was lower, and the piriform cortex and hippocampus displayed lower frequencies of damage than the cerebral cortex, which had a much higher frequency in guinea pigs than in rats. These different results may be due to species differences, may be related to the way the pretreatment/treatment drugs affected the expression of the damage, or may be due to some interaction of these factors. This will require further study.

When this study was conceived, it was thought that one or several of the anticholinergic drugs might stand out as being superior to the others or to diazepam. While there are some clear distinctions between the anticholinergics and diazepam, the differences between anticholinergics are relatively negligible. At doses that terminate the seizure the largest differences between the individual anticholinergic drugs and diazepam are in the ED_{50}s and in the rapidity with which the seizure is controlled. The other dependent variables, body weight recovery, one-way avoidance performance recovery and neuropathology, did not show that any particular drug was 'better' given that the seizure was controlled.

In conclusion, the present data show that while anticholinergic drugs may be beneficial anticonvulsant adjuncts, diazepam, or other such benzodiazepines, will continue to be a mainstay for the treatment of nerve agent-induced seizures. To achieve maximum therapeutic effectiveness, anticholinergics must be given promptly after exposure. In a military setting, where personnel are equipped with antidote drugs in automatic injectors for immediate use, anticholinergics may be especially helpful in controlling seizures. However, in some poisoning situations (e.g. Tokyo subway incident) the ready availability or use of antidote drugs may not be possible. Under such delayed treatment conditions, diazepam would continue to act as effective anticonvulsant against nerve agent-induced seizures.

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