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RESEARCH ARTICLE

# Time-dependent reduction in the anticonvulsant effectiveness of diazepam against soman-induced seizures in guinea pigs

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## Abstract

Near-lethal exposure to nerve agents produces prolonged epileptiform seizures requiring the administration of benzodiazepine anticonvulsant drugs, such as diazepam. Clinically, benzodiazepines are reported to lose anticonvulsant effectiveness the greater the delay between seizure onset and benzodiazepine treatment. This time-dependent diminished effectiveness of diazepam was tested in the present study. Seizures elicited by the nerve agent, soman, were produced in guinea pigs instrumented to record brain electrocorticographic (ECoG) activity. Different groups of animals were administered 10 mg/kg, intramuscularly, of diazepam at 5, 40, 60, 80, or 160 minutes after the onset of seizure activity. There was a progressive loss in the anticonvulsant efficacy of diazepam as the treatment was delayed after seizure onset, but no differences in the time for diazepam to stop seizures. The results show a diminished ability of diazepam to stop nerve-agent-induced seizures the longer treatment is delayed.

**Keywords:** Nerve agent; soman; seizure; diazepam; benzodiazepine; anticonvulsant

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## Introduction

Nerve agents elicit prolonged seizures that have all the behavioral and electrographic characteristics of the clinical condition known as *status epilepticus* (Carpentier et al., 1990; Koplovitz and Skvorak, 1998). Benzodiazepines, such as diazepam, lorazepam, or midazolam, are the first-line therapy used to treat such ongoing seizures in humans (Shorvon, 1994), and diazepam is the current anticonvulsant used by the U.S. military to treat nerve-agent-induced seizures. Other military forces use a prodrug form of diazepam, known as avizafone, that is bioconverted into diazepam following injection (Lallement et al., 2000; Taysse et al., 2003, 2006). Both clinically and in various animal models of *status epilepticus*, it has been shown that the longer a seizure progresses before benzodiazepine treatment, the less responsive the seizure is

to the anticonvulsant effect of the drug (Towne et al., 1994; Lowenstein and Alldredge, 1998; Walton and Treiman, 1988; Rice and DeLorenzo, 1999; Jones et al., 2002). Previous work in our laboratory has shown that diazepam (20 mg/kg, intraperitoneally, i.p.) was not effective at all in stopping soman-induced seizures in rats when treatment was delayed for 40 minutes after seizure onset (Shih et al., 1999). Yet, in another study in guinea pigs, diazepam was still able to exert some control of soman-induced seizures when treatment was delayed for 40 minutes after seizure onset (McDonough et al., 1999, 2000). The present study was designed to more systematically examine the anticonvulsant effectiveness of diazepam in guinea pigs when given at varying delays following the onset of soman-induced seizures.

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## Methods

Male Hartley guinea pigs (CrI:(HA) BR COBS; Charles River Labs, Kingston, New York, USA), weighing 250–300 g before surgery, served as subjects. They were individually housed in temperature- ( $21 \pm 2^\circ\text{C}$ ) and humidity- ( $50 \pm 10\%$ ) controlled quarters that were maintained on a 12-hour light-dark cycle (lights on at 6:00 a.m.) and received food and water *ad libitum*, except during the experimental period.

Approximately 1 week before experimentation, the animals were implanted with stainless-steel cortical screw electrodes to record electrocorticographic (ECoG) signals. The animals were anesthetized with isoflurane (3% induction, 1.5–2% maintenance; with oxygen) and set in a stereotaxic frame. Three cortical stainless-steel screw electrodes were implanted in the skull: two were placed bilaterally  $\sim 3.0$  mm lateral from the midline and equidistant between the bregma and lambda; the third was placed on the posterior calvaria as the reference electrode. Stainless-steel wires attached the screws to a miniature connector plug. The electrodes, wires, and plug were encased in cranioplastic cement. The incision was sutured; the animal was removed from the frame, given buprenorphine HCl (0.03 mg/kg, subcutaneously; s.c.) for postoperative analgesia, and placed on a warming pad for at least 30 minutes before being returned to the animal quarters.

On the day of study, the animals were placed in a recording chamber, and at least 15 minutes of baseline ECoG was recorded. ECoGs were recorded by using CDE 1902 amplifiers and displayed on a computer running Spike2 software (Cambridge Electronic Design, Ltd., Cambridge, UK). The animals were then administered 0.026 mg/kg of pyridostigmine (i.m.), a dose determined to produce  $\sim 30\%$  whole-blood cholinesterase inhibition (Lennox et al., 1985). Thirty minutes later, the animals were challenged with 2X LD<sub>50</sub> soman (56  $\mu\text{g/kg}$ , s.c.) and, 1 minute later, were treated i.m. with 2 mg/kg of atropine mixed with 25 mg/kg of 2-PAM Cl. They were then monitored for seizure onset, which was defined as the appearance of  $\geq 10$  seconds of rhythmic, high-amplitude spikes or sharp waves.

At 5, 40, 60, 80, or 160 minutes after seizure onset, different groups of animals were treated with a fixed dose (10.0 mg/kg, i.m.) of diazepam. An initial pilot study ( $N=8$ ) showed that this dose of diazepam successfully controlled soman-induced seizures in all animals when given 5 minutes after seizure onset. The animals from the pilot study were included in the 5-minute treatment group reported here. The ECoG was continuously monitored for at least 5 hours after diazepam treatment and for 30 minutes at 24 hours

after exposure. Each animal was rated as having the seizure terminated or not terminated, based on the overall appearance of the ECoG record at the end of the experimental day and during the 24-hour recording. To be rated as having the seizure terminated, all spiking and/or rhythmic waves had to stop and the ECoG remain normal at all subsequent observation times. For each animal in which the seizure was terminated, the latency to seizure termination was measured as the time from when the animal received diazepam treatment to the last observable epileptiform event in the ECoG.

The proportion of animals in which the seizures were terminated was compared between groups by using Fisher's exact test. Likewise, the proportion of animals dying before the 24-hour observation time was also compared between groups by using Fisher's exact test. The latencies for seizure termination (i.e., time from diazepam injection to termination of seizure activity) were compared between groups by using a one-way analysis of variance. In all cases,  $P \leq 0.05$  was considered significant.

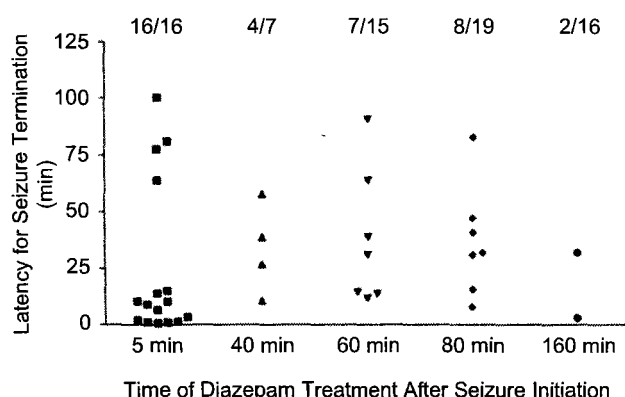
## Results

As was seen in the pilot study, diazepam, at 10 mg/kg i.m., was 100% effective in stopping soman-induced seizures when administered 5 minutes after seizure onset (Table 1; Figure 1). This dose of diazepam was progressively less effective in controlling soman-induced seizures at the later treatment delay times: 57% at 40 minutes, 47% at 60 minutes, 42% at 80 minutes, and 13% at 160 minutes. There were significant differences in anticonvulsant effectiveness (i.e., proportion of animals responding) between the 5-minute treatment delay and all other treatment delay times (Table 2). Diazepam was least effective in controlling seizures when given at the longest (160-minute) delay time; the proportion of animals responding at this treatment delay was significantly less than of those treated at the 5- or 40-minute treatment delays, while the 60- and 80-minute treatment delays showed nonsignificant, but clear, continuations of this trend (Table 2). Diazepam

**Table 1.** Summary of seizure control and lethality as a function of treatment delay after seizure onset.

|                   | 5 minutes | 40 minutes | 60 minutes | 80 minutes | 160 minutes |
|-------------------|-----------|------------|------------|------------|-------------|
| N tested          | 16        | 7          | 15         | 19         | 16          |
| Seizure off       | 16 (100%) | 4 (57%)    | 7 (47%)    | 8 (42%)    | 2 (13%)     |
| 24-hour lethality | 0 (0%)    | 0 (0%)     | 1 (7%)     | 2 (11%)    | 9 (56%)     |
| Died before Rx    | 0 (0%)    | 0 (0%)     | 0 (0%)     | 0 (0%)     | 3 (19%)     |

was highly effective in preventing the lethal consequences of soman exposure, and this, too, was related to treatment delay. All animals treated at the 5- or 40-minute delays survived 24 hours, and even those treated at the 60- or 80-minute delays experienced only an ~10% mortality rate (Tables 1 and 3). In contrast, the animals in the 160-minute treatment delay group experienced significantly higher mortality than those in the 5-, 60-, or 80-minute treatment groups (Table 3). Analysis of the seizure termination latencies showed treatment delay did not affect this factor; seizures took about the same amount of time to stop following successful diazepam treatment, regardless of the treatment delay.



**Figure 1.** Scatter plots of the latency for seizure control (times from drug injection until termination of seizure) for individual animals treated with 10.0 mg/kg of diazepam when treatment was given at 5-, 40-, 60-, 80-, or 160-minute delay times after seizure onset. The numbers at the top of each scatter plot show the number of animals in which seizures were successfully terminated/total number of animals tested in that group.

**Table 2.** Differences in seizure control between treatment delay groups (Fisher's exact test).

|            | 40 minutes | 60 minutes  | 80 minutes  | 160 minutes       |
|------------|------------|-------------|-------------|-------------------|
| 5 minutes  | $P < 0.02$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$       |
| 40 minutes |            | NS          | NS          | $P < 0.05$        |
| 60 minutes |            |             | NS          | NS ( $P = 0.06$ ) |
| 80 minutes |            |             |             | NS ( $P = 0.07$ ) |

NS, not significant.

**Table 3.** Differences in lethality between treatment delay groups (Fisher's exact test).

|            | 40 minutes | 60 minutes | 80 minutes | 160 minutes       |
|------------|------------|------------|------------|-------------------|
| 5 minutes  | NS         | NS         | NS         | $P < 0.007$       |
| 40 minutes |            | NS         | NS         | NS ( $P = 0.06$ ) |
| 60 minutes |            |            | NS         | $P < 0.04$        |
| 80 minutes |            |            |            | $P < 0.001$       |

NS, not significant.

## Discussion

The results show that the benzodiazepine diazepam loses anticonvulsant effectiveness the longer treatment is delayed following the start of *status epilepticus* seizure activity induced by the nerve agent, soman. In this respect, soman-induced seizures respond to diazepam like other models of *status epilepticus*, indicating that the neuropharmacological processes underlying this drug resistance are common to prolonged seizure activity, regardless of the eliciting stimulus. The results also show, not surprisingly, that the lethal effects of soman exposure are also linked to treatment delay, and that prompt administration of diazepam is effective in preventing lethality.

Clinically, it has been shown that the longer a seizure progresses before benzodiazepine treatment, the less responsive the seizure is to the anticonvulsant effect of the drug (Towne et al., 1994; Lowenstein and Alldredge, 1998). Likewise, in a variety of different animal models of *status epilepticus*, seizure duration was shown to also influence the anticonvulsant effectiveness of benzodiazepines, such as diazepam (Walton and Treiman, 1988; Rice and DeLorenzo, 1999; Jones et al., 2002) as well as of the barbiturate, phenobarbital, but not that of phenytoin (Jones et al., 2002). Benzodiazepines and barbiturates exert their anticonvulsant effects by enhancing gamma-aminobutyric-acid<sub>A</sub> (GABA<sub>A</sub>) receptor-mediated inhibitory neural activity. Thus, prolonged soman-induced seizures in either rats (Shih et al., 1999) or guinea pigs (this study) elicit the same seizure-dependent neuropharmacological changes in GABA<sub>A</sub> responsiveness to diazepam as reported in other models of *status epilepticus*. Drugs that act on receptor systems other than the GABA<sub>A</sub> sites may be necessary to effectively stop these prolonged nerve-agent-induced seizures when treatment is substantially delayed.

The results also confirm previous observations that diazepam, or, for that matter, any benzodiazepine treatment, is effective in antagonizing the lethal effects of nerve agents. We have noted in several studies that diazepam or other benzodiazepines are effective in enhancing survival following lethal nerve-agent challenge, regardless of whether seizures were controlled or not (McDonough et al., 1999, 2000; Shih et al., 2003, 2007). The data in Tables 1 and 3 show that diazepam treatment prevented the lethal effects of soman exposure to a greater extent than it controlled seizure activity in the 40-, 60-, 80-, or 160-minute treatment delay groups. Diazepam/benzodiazepine treatment thus provides additional therapeutic benefits in nerve-agent intoxication over and above the control of seizures. This is potentially an area for additional research.

The present results also show that guinea pigs respond to delayed diazepam treatment under these circumstances with a different time profile, compared to that of rats. In our previous work, we found that even 20 mg/kg, i.p., of diazepam failed to stop soman-induced seizures in a rat model when diazepam treatment was delayed 40 minutes after seizure onset (Shih et al., 1999). These results are similar to those reported in other models of *status epilepticus* that have used rats. Walton and Treiman (1988) and Jones et al. (2002) reported that rats were totally resistant to the anticonvulsant effects of 20 mg/kg, i.p., of diazepam after 30 minutes of seizure in the lithium-potentiated pilocarpine model of *status epilepticus*. However, in the present study, 10 mg/kg, i.m., of diazepam was clearly effective in controlling seizure activity in a substantial percentage (57–42%) of the guinea pigs at the 40- through 80-minute treatment delays, respectively. Rats also more rapidly become resistant to the anticonvulsant effects of anticholinergic drugs to terminate nerve-agent-induced seizures than do guinea pigs (McDonough and Shih, 1993; McDonough et al., 2000; Shih et al., 2007). This suggests that the duration of seizure activity needed to develop pharmacoresistance may be progressively longer in more developed species.

## Conclusions

The results of this and other studies show that there is a rapid change in the pharmacoresponsiveness of GABA<sub>A</sub> receptors to the anticonvulsant effects of benzodiazepines following the onset of prolonged seizures. When this change begins is still poorly understood, but all the data show pronounced changes after 5 minutes of seizure and before 30–40 minutes of seizure. This reinforces the fact that early, aggressive treatment of nerve-agent seizures is essential to the total medical management of nerve-agent casualties.

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accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The opinions or assertions contained herein 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. This work has not been published elsewhere, nor has it been submitted simultaneously for publication elsewhere.

## Declaration of interest

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## References

- Carpentier, P., Delamanche, I. S., Le Bert, M., Blanchet, G., Bouchaud, C. (1990). Seizure-related opening of the blood-brain barrier induced by soman: possible correlation with the acute neuropathology observed in poisoned rats. *Neurotoxicology* 11:493–508.
- Jones, D. M., Esmaeil, N., Maren, S., Macdonald, R. L. (2002). Characterization of pharmacoresistance to benzodiazepines in the rat Li-pilocarpine model of status epilepticus. *Epileps Res* 50:301–312.
- Koplovitz, I., Skvorak, J. P. (1998). Electroconvulsive changes during generalized convulsive status epilepticus in soman intoxicated rats. *Epileps Res* 30:159–164.
- Lallement, G., Renault, F., Baubichon, D., Peoc'h, M., Burckhart, M. F., Galonnier, M., et al. (2000). Compared efficacy of diazepam or avizafone to prevent soman-induced electroencephalographic disturbances and neuropathology in primates: relationship to plasmatic benzodiazepine pharmacokinetics. *Arch Toxicol* 74:480–486.
- Lennox, W. J., Harris, L. W., Talbot, B. G., Anderson, D. R. (1985). Relationship between reversible acetylcholinesterase inhibition and efficacy against soman lethality. *Life Sci* 37:793–798.
- Lowenstein, D. H., Alldredge, B. K. (1998). Status epilepticus. *NEJM* 338:970–976.
- McDonough, J. H., McMonagle, J., Copeland, T., Zoeffel, D., Shih, T.-M. (1999). A comparative evaluation of benzodiazepines for control of soman-induced seizures. *Arch Toxicol* 73:473–478.
- McDonough, J. H., Shih, T.-M. (1993). Pharmacological modulation of soman-induced seizures. *Neurosci Biobehav Rev* 17:203–215.
- McDonough, J. H., Zoeffel, L. D., McMonagle, J., Copeland, T. L., Smith, C. D., Shih, T.-M. (2000). Anticonvulsant treatment of nerve agent seizures: anticholinergics vs. diazepam in soman-intoxicated guinea pigs. *Epileps Res* 38:1–14.
- Rice, A. C., DeLorenzo, R. J. (1999). N-methyl-D-aspartate receptor activation regulates refractoriness of status epilepticus to diazepam. *Neuroscience* 93:117–123.

- Shih, T.-M., Duniho, S. M., McDonough, J. H. (2003). Control of nerve agent-induced seizures is critical for neuroprotection and survival. *J Appl Toxicol* 188:69-80.
- Shih, T.-M., McDonough, J. H., Koplovitz, I. (1999). Anticonvulsants for soman-induced seizure activity. *J Biomed Sci* 6:86-96.
- Shih, T.-M., Rowland, T. C., McDonough, J. H. (2007). Anticonvulsants for nerve agent-induced seizures: the influence of the therapeutic dose of atropine. *J Pharmacol Exp Ther* 320:154-161.
- Shorvon, S. (1994). Status epilepticus: its clinical features and treatment in children and adults (pp 244-254).Cambridge, UK: Cambridge University Press.
- Taysse, L., Calvet, J. H., Buee, J., Christin, D., Delamanche, S., Breton, P. (2003). Comparative efficacy of diazepam and avizafone against sarin-induced neuropathology and respiratory failure in guinea pigs: influence of atropine dose. *Toxicology* 188:197-209.
- Taysse, L., Daulon, S., Delamanche, S., Bellier, B., Breton, P. (2006). Protection against soman-induced neuropathology and respiratory failure: a comparison of the efficacy of diazepam and avizafone in guinea pig. *Toxicology* 225:25-35.
- Towne, A. R, Pellock, J. H., Ko, D., DeLorenzo, R. J. (1994). Determinants of mortality in status epilepticus: a retrospective study of 292 adult patients. *Epilepsia* 35:27-34.
- Walton, N. Y., Treiman, D. M. (1988). Response of status epilepticus induced by lithium and pilocarpine to treatment with diazepam. *Exp Neurol* 101:267-275.