Pretreating Rats with Parenteral Ophthalmic Antimuscarinic Agents Decreases Mortality from Lethal Organophosphate Poisoning

Sean M. Bryant, MD, James W. Rhee, MD, Trevonne M. Thompson, MD, Steven E. Aks, DO

Abstract

Background: In the event of a large scale organophosphate (OP) or nerve agent exposure that depletes a hospital's atropine stores, alternative antidotes should be considered.

Objectives: To test the effects of parenteral administration of ophthalmic antimuscarinic agents on survivability in a rat model of acute, lethal OP poisoning.

Methods: After determining appropriate dosing for comparison, rodents were randomized to receive one of four intraperitoneal antidotes (n = 10 per group): 1) normal saline (0.3 mL), 2) atropine sulfate (10 mg/kg), 3) ophthalmic atropine sulfate (1%; 10 mg/kg), or 4) ophthalmic homatropine (5%; 20 mg/kg). Five minutes after pretreatment, dichlorvos (10 mg/kg) was administered subcutaneously. Mortality rates and time to death were compared by using Fisher's exact test and the Kaplan-Meier method with log rank test, respectively. If the animal was alive at 120 minutes, survival was assumed.

Results: Survival in rats pretreated with standard atropine was 100%. Survival in rats pretreated with ophthalmic homatropine and atropine sulfate were 100% ($p < 0.001$; 95% CI = 0.98 to 1.02) and 90% ($p < 0.01$; 95% CI = 0.71 to 1.09), respectively, compared with controls (20% survival; 95% CI = 0.04 to 0.45). Time of death ranged between 7 and 19 minutes. Comparison of survival times revealed a statistically significant improvement in experimental groups compared with controls ($p < 0.0001$).

Conclusions: Parenteral pretreatment with ophthalmic preparations of homatropine or atropine sulfate was equal to standard atropine in preventing lethality in this rat model of acute, lethal OP poisoning.

ACADEMIC EMERGENCY MEDICINE 2007; 14:370–372 © 2007 by the Society for Academic Emergency Medicine

Keywords: organophosphate poisoning, ophthalmic antimuscarinic agents, homatropine

Proper emergency department preparedness includes having adequate antidote supplies when treating any patient(s) poisoned by an organophosphate (OP) or nerve agent (e.g., sarin and soman). Significant morbidity and mortality result when excessive stimulation of peripheral and central cholinergic receptors takes place. The cholinesterase inhibiting action of OPs and nerve agents results in a constellation of signs that culminate in death secondary to hypoxia from bronchospasm, bronchorrhea, and neuromuscular weakness.

Depletion of a hospital's supply of atropine has been reported after patients have presented from an exposure to OPs or carbamates.[1–3] Doses as large as 11,000 mg have been documented to treat a single patient.[4] Multiple patients, presenting after a chemical agent exposure, pose a significant burden to health care facilities. The goal of reversing muscarinic poisoning and decreasing airway secretions is normally accomplished with an antimuscarinic agent (namely, atropine).

Having the appropriate antidotal reserve to treat victims of these exposures is paramount. Because atropine supplies could quickly become depleted in mass casualty incidents, consideration of using concentrated topical

From the Department of Emergency Medicine, Cook County-Stroger Hospital, Rush Medical College (SMB, SEA); Illinois Poison Center (SMB); Toxikon Consortium (SMB, JWR, TMT, SEA); Section of Emergency Medicine, University of Chicago (JWR); Department of Emergency Medicine, University of Illinois at Chicago Medical Center (TMT), Chicago, IL.

Received August 4, 2006; revision received October 13, 2006; accepted October 14, 2006.

Presented as an abstract and poster at the North American Congress of Clinical Toxicology, San Francisco, CA, October 2006.

Partially supported by the 2004 American College of Medical Toxicology (ACMT) and Agency for Toxic Substances and Disease Registry (ATSDR) Grant for Chemical Terrorism or Mass Chemical Exposure Incident Preparedness.

Contact for correspondence and reprints: Sean M. Bryant, MD; e-mail: sbryant@ccbh.org.

ISSN 1069-6563
PII ISSN 1069-6563583

doi: 10.1197/j.aem.2006.10.099

ophthalmic antimuscarinic agents (15 mL of homatropine hydrobromide 5% = 750 mg; 15 mL of ophthalmic atropine sulfate 1% = 150 mg) parenterally as an antidotal option has been discussed; however, experimental research in this field is lacking.[5]

The aim of this study was to determine whether pretreatment with Isopto homatropine hydrobromide 5% (Alcon Laboratories, Inc., Fort Worth, TX) or with ophthalmic atropine sulfate 1% will increase survival (compared with controls) in a rat model of acute, lethal OP poisoning.

METHODS

Study Design

This was a laboratory study that used a rodent or rat model. Approval of the institutional animal care and use committee was obtained.

Animal Subjects and Preparation

Animals were housed in standard cages with 12-hour day–night cycles and were given free access to food and water. A total of 70 adult male Sprague-Dawley rats (mean weight = 255 g) were used in a pretreatment model, as described elsewhere.[6,7] Atropine sulfate (Sigma-Aldrich, St. Louis, MO), ophthalmic atropine sulfate (Alcon Laboratories, Inc., Fort Worth, TX), or ophthalmic homatropine (Alcon Laboratories, Inc.) was mixed in sterile normal saline and administered via the intraperitoneal (IP) route.

Study Protocol

Our previous work determined that reported LD50 values for dichlorvos and atropine dosing that were used in published studies were not adequate in our laboratory and thus necessitated a dose response phase before experimentation.[8] Before ophthalmic antidote testing, 30 animals were used to obtain an appropriate dose response. It was discovered that administration of dichlorvos (Pestanal; Sigma-Aldrich) subcutaneously (SC; 10 mg/kg) resulted in death within 7 minutes in 10 rats. However, when another group of rats was pretreated with IP atropine sulfate (10 mg/kg) 5 minutes before administration of dichlorvos (10 mg/kg), all 10 rats survived. In light of this information, a dose of 10 mg/kg of dichlorvos was chosen for study, and standard atropine sulfate (10 mg/kg) would be compared against the experimental ophthalmic antidotes.

The experimental phase of the study consisted of randomly assigning rats to one of four groups to test the alternate antidotes against a control group and the standard atropine sulfate group. Each group received one of the following IP antidotes 5 minutes before poisoning with SC dichlorvos: 1) control, normal saline (0.3 mL); 2) atropine sulfate (10 mg/kg); 3) ophthalmic atropine sulfate (10 mg/kg); or 4) ophthalmic homatropine (20 mg/kg). Total volume administered did not exceed 0.4 mL. Animals were killed at the end of study with IP pentobarbital and intracardiac potassium chloride.

Data Analysis

Two-hour mortality rates were compared by using Fisher's exact test. Kaplan-Meier survival curves with log rank analysis were also performed. A priori, survival was assumed if the rats remained alive at 120 minutes. We determined that a sample size of 10 rats in each group provided 90% power to detect a difference in survival between experimental groups and controls by using an alpha error of 0.05. Data were analyzed by using SAS statistical software (version 8.1; SAS Institute, Cary, NC).

RESULTS

Survival in rats pretreated with standard atropine sulfate was 100%. Survival in rats pretreated with ophthalmic homatropine and atropine sulfate was 100% ($p < 0.001$; 95% confidence interval [CI] = 0.98 to 1.02) and 90% ($p < 0.01$; 95% CI = 0.71 to 1.09), respectively, compared with controls (20% survival; 95% CI = 0.04 to 0.45). Time of death ranged between 7 and 19 minutes. Overall comparison of survival time revealed a statistically significant improvement in experimental groups compared with controls ($p < 0.0001$; Figure 1).

DISCUSSION

Other reports have investigated the efficacy of various antimuscarinic agents in treating muscarinic poisoning. Nebulized ipratropium was used successfully in a case of OP poisoning presenting with significant pulmonary secretions.[9] In addition, glycopyrrolate bromide, scopolamine, diphenhydramine, and jimson weed extract have been studied.[6,7,10] Despite demonstrating some efficacy, these agents all share limitations in availability, in ease of administration, or in both. We believe that the consideration of ophthalmic antimuscarinic products may provide a more natural, convenient, and logical approach to a mass casualty event.

The advantage of ophthalmic antimuscarinic products is not only because of their availability. The highly concentrated nature of these products could allow treatment of several patients with just one dropper bottle. Ophthalmic products are highly concentrated to supply sufficient doses in small volumes. Each dropper bottle of 5% homatropine contains 15 mL of sterile homatropine

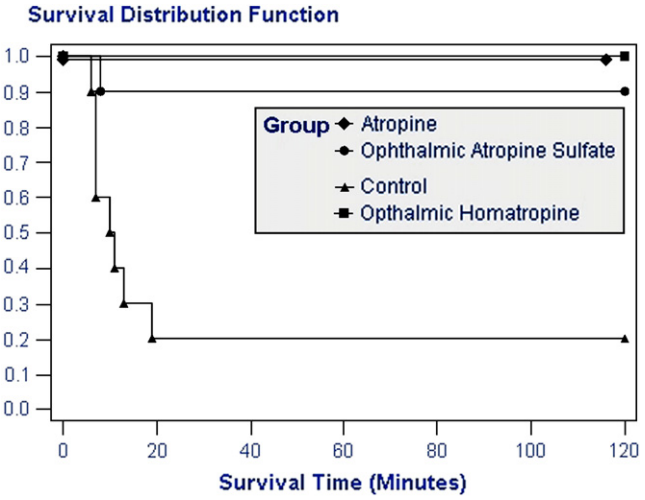

Figure 1. Kaplan-Meier survival curves comparing proportion survived vs. time. (Color version of this figure available online at www.aemj.org.)

hydrobromide ophthalmic solution. This represents 750 mg of homatropine per bottle. Ophthalmic homatropine also contains inert additives (sodium chloride, sodium hydroxide, or hydrochloric acid to adjust for pH, and water) and the preservative benzethonium chloride 0.005%. Although these substances could potentially result in toxicity in massive doses, their concentrations are so low that one would not anticipate harm in this setting. In addition, one dropper bottle of 1% ophthalmic atropine sulfate contains a total of 150 mg of available antidote. To put things in perspective, our emergency department has 125 mg of standard atropine sulfate available. However, several bottles of ophthalmic homatropine and atropine sulfate are readily accessible, which could greatly increase our antimuscarinic antidotal supply.

Kinetic data on "parenteral" homatropine are limited. Homatropine is reported to have less systemic toxicity than atropine.[11] One case report describes classic systemic antimuscarinic poisoning after ophthalmic administration of homatropine for the treatment of anterior uveitis.[12] In choosing a dose of homatropine for study, we found that doubling the dose of homatropine (20 mg/kg vs. 10 mg/kg of atropine sulfate) was equivalent to standard atropine sulfate in preventing death in our previous work in the laboratory as well as in this current experiment.[8]

Our study revealed a significant survival benefit for rats treated with standard atropine sulfate (10 mg/kg), ophthalmic homatropine (20 mg/kg), and ophthalmic atropine sulfate (10 mg/kg). All subjects consistently exhibited fasciculations within 2–3 minutes of dichlorvos administration. In addition, all rats that died did so within 19 minutes; however, most died much earlier (7–13 minutes). The endpoint of our study was set at two hours. Other studies using 24 hours as an endpoint discovered that if rats lived beyond 30 minutes, they survived for 24 hours.[6,7] In light of this, a two hour endpoint was sufficient to decrease any unnecessary suffering by the animals. In addition, those animals that survived the duration of the two hour observation after experimentation appeared healthy and vibrant after a short period of fasciculations and lethargy.

LIMITATIONS

Extrapolating animal data to human utilization is challenging. Without parenteral kinetic data on these products, it could be risky administering them parenterally. A preexposure regimen was used in this model; this does not simulate real life poisoning and treatment scenarios. A two hour endpoint was sufficient for these pilot data, as mentioned in the previous paragraph. Morbidity and mortality secondary to OPs and nerve agents may warrant further treatment beyond the acute toxicity phase with oximes that have not been considered in this laboratory experiment. Finally, practically speaking, if a clinician was to entertain using these agents in the emergency department, strict guidelines would need to be implemented in relation to dilution, preparation, and administration.

CONCLUSIONS

Parenteral pretreatment with ophthalmic preparations of homatropine or atropine sulfate was equal to standard atropine in preventing lethality in this rat model of acute, lethal OP poisoning. We believe that it may be premature to administer ophthalmic homatropine or atropine sulfate (as a first line antidote) parenterally to human beings poisoned by OPs or nerve agents. However, even though the goal of this study was not to test these agent's capabilities in a mass casualty setting, further studies should be directed at evaluating antimuscarinic ophthalmic products as alternative antidotes for OP poisoning, nerve agent poisoning, or both.

The authors thank Manuel Elazegui for his invaluable assistance in the laboratory. In addition, they thank Linda M. Kampe and Ibrar Ahmad for their statistical expertise.

References

1. Tsao TCY, Juang YC, Lan RS, Shieh WB, Lee CH. Respiratory failure of acute organophosphate and carbamate poisoning. Chest. 1990; 98:631–6.
2. Skofield S, Lambert D, Tomassoni A, Wallace K. Inadequate regional antidote and medication supplies for poisoning emergencies [abstract]. J Toxicol Clin Toxicol. 1997; 35:490.
3. Dart RC, Stark Y, Fulton B, Koziol-McLain J, Lowenstein SR. Insufficient stocking of poisoning antidotes in hospital pharmacies. JAMA. 1996; 276:1508–10.
4. Hopmann G, Wanke H. Maximum dose atropine treatment in severe organophosphate poisoning. Dtsch Med Wochenschr. 1974; 99:2106–8.
5. Holstege CP, Kirk M, Sidell FR. Chemical warfare. Nerve agent poisoning. Crit Care Clin. 1997; 13: 923–42.
6. Bird SB, Gaspari RJ, Lee WJ, Dickson EW. Diphenhydramine as a protective agent in a rat model of acute, lethal organophosphate poisoning. Acad Emerg Med. 2002; 9:1369–72.
7. Bania TC, Chu J, Bailes D, O'Neill M. Jimson weed extract as a protective agent in severe organophosphate toxicity. Acad Emerg Med. 2004; 11:335–8.
8. Bryant SM, Wills BK, Rhee JW, et al. Intramuscular ophthalmic homatropine vs. atropine to prevent lethality in rats with dichlorvos poisoning. J Med Toxicol. 2006; 2:156–9.
9. Perrone J, Henretig, Sims M, Beers M, Grippi MA. A role for ipratropium in chemical terrorism preparedness [letter]. Acad Emerg Med. 2003; 10:290.
10. Stein CM, Neill P. Atropine or hyoscine in treatment of acute organophosphate poisoning? Lancet. 1985; 8432:823–4.
11. Franz DN. Antimuscarinic and antispasmodic drugs. In: Gennaro AR, ed. Remington, The Science and Practice of Pharmacy. 19th ed. Easton, PA: Mack Publishing Company, 1995. pp 1020–6.
12. Reilly KM, Chan L, Mehta NJ, Salluzzo RF. Systemic toxicity from ocular homatropine. Acad Emerg Med. 1996; 9:868–71.