Intramuscular Ophthalmic Homatropine vs. Atropine to Prevent Lethality in Rates with Dichlorvos Poisoning

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ABSTRACT

Introduction: Most hospitals lack a sufficient supply of atropine to treat, simultaneously, patients poisoned with multiple organophosphorous compound (OC) or nerve agent. The presence of a ubiquitous alternate antidote would prove useful if mass poisoning occurred. Our objective was to evaluate the effect of ophthalmic homatropine (Isopto Homatropine 5%) on survivability in a rat model of significant, acute OC poisoning.

Methods: Sprague-Dawley rats were randomized to one of five pre-treatment groups (N = 10 per group). Prior to experimentation, animals were pre-treated with intramuscular (IM) injections of either atropine 5 mg/kg, atropine 10 mg/kg, homatropine 10 mg/kg, or homatropine 20 mg/kg. The control group received 0.3 mL normal saline IM. Five minutes later, 25 mg/kg of dichlorvos was subcutaneously administered. Mortality rates were compared using Fisher's Exact test. Kaplan-Meier survival curves with Logrank analysis was also performed. If alive at 120 minutes, survival was assumed, and the study was terminated.

Results: All rats pre-treated with normal saline, atropine 5 mg/kg, and homatropine 10 mg/kg died. Survival in the homatropine (20 mg/kg) and atropine (10 mg/kg) groups was 30% and 40% respectively. Times to death ranged between 4 and 12 minutes. Overall comparison of time to death revealed a statistically significant improvement for groups pre-treated with homatropine (20 mg/kg) and atropine (10 mg/kg).

Conclusions: Pre-treatment with homatropine (20 mg/kg) was comparable with atropine (10 mg/kg) in preventing lethality in this rat model of acute OC poisoning.

Keywords: antidote, terrorism, homatropine, atropine, dichlorvos

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INTRODUCTION

Atropine is the first-line agent of choice to treat muscarinic overdrive in patients acutely poisoned by organophosphorous compounds (OCs) or nerve agents. Over stimulation of cholinergic receptors peripherally and centrally account for significant morbidity and mortality. In the wake of 9/11, anthrax dispersion, and the ongoing war on terror, mass poisoning by chemical agents exists and remains a real concern.

Patients presenting to a hospital from an exposure to OCs or carbamates may lead to depletion of a hospital's depot of atropine [1,2,3]. Doses as large as 11,000 mg have been used to treat a single patient [4]. Multiple patients presenting to a hospital after a chemical agent exposure poses a significant burden to healthcare facilities. In addition to dermal decontamination, triage, and appropriate delegation of care, specific antidotal therapy is critical to prevent mass casualties. The cholinesterase inhibiting action of OCs and nerve agents results in a constellation of signs such as bronchospasm, bronchorrhea, and neuromuscular weakness and potentially culminating in death. Antimuscarinic agents reverse muscarinic poisoning and decrease airway secretion.

Having the appropriate antidotal reserve to treat victims of these attacks is paramount. Consideration of ophthalmic antimuscarinic agents as an antidotal option in a mass casualty incident has been discussed; however, no study has experimentally examined the efficacy of this potential antidote [5].

The aim of this study is to determine whether pre-treatment with Isopto® homatropine hydrobromide 5% will increase survival in a rat model of acute, significant OC poisoning.

MATERIALS AND METHODS

Institutional Animal Care and Use Committee approval was obtained. Animals were housed in standard cages with 12-hour day/night cycles, and animals were given free access to food and water. Fifty adult male Sprague-Dawley rats (approximately 300 grams each) were utilized in a pre-treatment model as described previously [6,7]. Atropine (Sigma-Aldrich, St. Louis, MO) or ophthalmic homatropine (Alcon laboratories, Inc., Fort Worth, TX) was mixed in sterile normal saline to a final concentration of 3 mg/mL and administered IM.

Prior to experimentation, animals were pre-treated with IM injections of either atropine 5 mg/kg, atropine 10 mg/kg, homatropine 10 mg/kg, or homatropine 20 mg/kg. The control group received 0.3 mL normal saline IM. Dichlorvos (Pestanal, Sigma-Aldrich, St. Louis, MO) was suspended in sterile normal saline to a final concentration of 10 mg/mL. Five minutes following pretreatment, 25 mg/kg of dichlorvos was subcutaneously administered in all rats. A dichlorvos dose of 25 mg/kg represents two times the LD50, and it produces classic muscarinic and nicotinic toxicity followed by death [6,7,8].

We determined that a sample size of ten rats in each group provided 90% power to detect a statistically significant difference using an alpha error of 0.05. Two-hour mortality rates were compared using Fisher's Exact test. Kaplan-Meier survival curves with Logrank analysis were also performed. Survival was assumed if rats remained alive at 120 minutes. Data were analyzed using SPSS statistical software (Version 13.0; SPSS Inc., Chicago, IL). At the end of study, animals were euthanized with the use of pentobarbital (intraperitoneal) and potassium chloride (intracardiac).

RESULTS

All rats pre-treated with normal saline, atropine 5 mg/kg and homatropine 10 mg/kg, died. Survival in the homatropine (20 mg/kg) and atropine (10 mg/kg) groups was 30% ($p = 0.105$; 95% CI 0.02, 0.58) and 40% ($p = 0.043$: 95% CI 0.10, 0.70) respectively compared to controls (95% CI 0.00, 0.28). Time of death ranged between 4 and 12 minutes. Overall comparison via Kaplan-Meier with Logrank analysis revealed a statistically significant improvement in length of survival for groups pre-treated with homatropine (20 mg/kg) and atropine (10 mg/kg) ($p < 0.001$) (*Figure 1*).

DISCUSSION

Our study revealed a significant survival benefit for rats treated with 10 mg/kg of atropine. Improvements in time to death were observed in the 10 mg/kg atropine and 20 mg/kg homatropine groups. All subjects consistently exhibited fasciculations within 2–3 minutes of dichlorvos administration. Additionally, all rats that died did so within 12 minutes; however, most died much earlier (4–5 minutes). The endpoint of our study was set at 2 hours. Other studies, using 24 hours as an endpoint, discovered that rats living beyond 30 minutes survived for 24 hours [6,7].

Other antimuscarinic antidotes have been studied. Investigators have considered the efficacy of expired atropine, reformulation of high-concentration atropine from bulk powder, and alternate antimuscarinic agents. One study found minimal degradation in atropine concentrations from outdated injectable atropine samples [9]. Even a WWII era atropine autoinjector proved to retain a significant concentration of atropine. Another study validated the use of reformulation of bulk atropine powder, and the study concluded that the process was inexpensive, accurate, and took approximately one hour to complete [10]. Nebulized ipratropium was successfully used in a case of OC poisoning presenting with significant pulmonary secretions [11]. Other investigated antimuscarinic agents included glycopyrrolate bromide, scopolamine, diphenhydramine, and jimson weed extract [12,6,7]. Despite demonstrating some efficacy, these agents all share limitations in availability and/or ease of administration. In the midst of a terrorist event, overwhelmed hospital staff, substantial restrictions, and burdens exist in taking time to reformulate powdered atropine, locate and procure atropine stocks (new or outdated), or administer scopolamine, glycopyrrolate bromide, or ipratropium to a mass of victims.

Our emergency department (ED) has a total of 150 mg of atropine readily available, while our Eye/ENT room within the ED maintains 30 bottles of Isopto® Homatropine 5%. Ophthalmic products are highly concentrated in order to supply sufficient

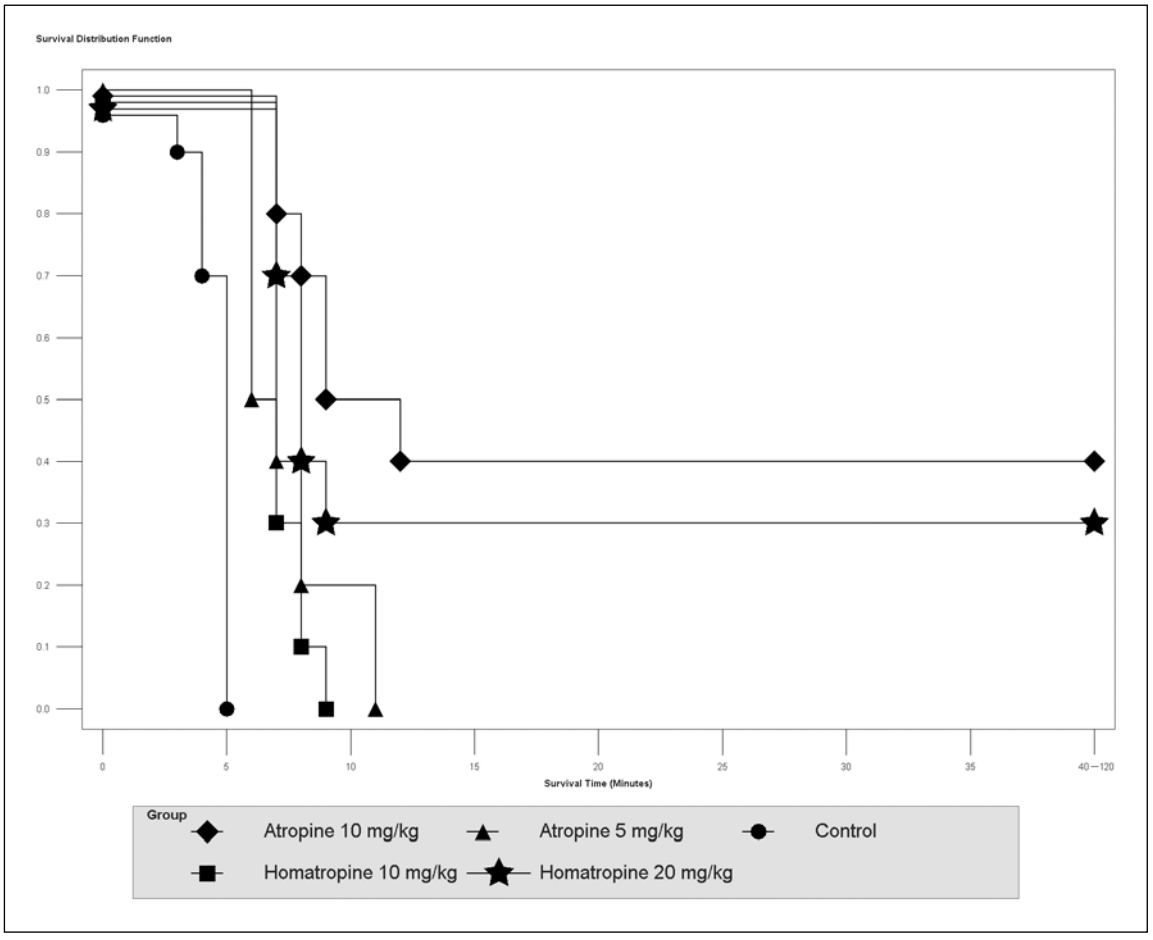

Figure 1. Kaplan-Meier curve comparing time to death between all groups. Proportion alive is plotted versus survival minutes.

doses in small volumes. Each dropper bottle contains 15 mL of sterile homatropine hydrobromide ophthalmic solution. Our ED holds 750 mg of homatropine per bottle and 22.5 g of homatropine. This massive amount of antimuscarinic agent could therefore be a consideration when supplies of atropine are diminishing during a mass casualty event.

Homatropine, closely related to atropine chemically, only differs by possessing one less methyl group. Ophthalmic homatropine also contains inert additives (sodium chloride, sodium hydroxide and/or hydrochloric acid to adjust for pH, and water) and the preservative benzethonium chloride 0.005%. While these substances could potentially result in toxicity with massive doses, their concentrations are so low that one would not anticipate harm. Kinetic data on "parenteral" homatropine is limited; however, homatropine is reported to have less systemic toxicity than atropine [13]. The lesser systematic toxicity could explain the need to increase dosing of homatropine. In order to obtain similar results in our study, the homatropine dosing requirement was twice that of atropine. Classic antimuscarinic poisoning has been reported with systemic poisoning of homatropine after ophthalmic administration for the treatment of anterior uveitis [14].

Several limitations of our study exist. Extrapolating animal data to human utilization is challenging. A pre-exposure regimen was used in this model; however, this does not simulate real life poisoning and treatment scenarios. Intramuscular administration of an antidote has been previously utilized [6]. We found this difficult due to the limited amount of muscle available in our rats. In our laboratory environment, dosing for dichlorvos and atropine was not consistent with the previous studies. Future work may consider intraperitoneal injection instead and the consideration of a dose response phase prior to experimentation in order to validate published LD50 values in our laboratory setting [7].

We believe it is premature to administer ophthalmic homatropine (as a first line antidote) parenterally to humans poisoned by OCs or nerve agents. However, our study does suggest that ophthalmic homatropine may be a viable and realistic alternative antidote in a mass casualty setting. Further studies should be directed at evaluating antimuscarinic ophthalmic products as alternative antidotes for OC and/or nerve agent poisoning.

The authors have no potential conflicts of interest to report.

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