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Caffeine reduces organophosphate-induced respiratory failure: Effect of caffeine on dichlorvos-induced central respiratory failure in a rat model

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ABSTRACT

Organophosphate (OP) poisoning is a serious global health concern responsible for more than three million poisonings worldwide each year. OP-induced respiratory failure is associated with an unacceptably high mortality rate. Caffeine has been successfully used in other cases of central respiratory failure. We hypothesized that caffeine administration would mitigate respiratory failure in an animal model of OP poisoning. We used a previously validated animal model of OP-induced central apnea with detailed physiologic respiratory recordings. The model consisted of Wistar rats that were anesthetized but spontaneously breathing 100% oxygen. Airflow, respiratory rate, tidal volume, mean arterial pressure and pulse rate were digitally recorded for 120 min following OP exposure or until death. The two study groups included dichlorvos and saline (n = 8) or dichlorvos and caffeine (n = 8). In all groups, dichlorvos was given as a single subcutaneous dose of 100 mg/kg (3x rat LD50). In each group caffeine (20 mg/kg) or saline (same volume) was given as a single intravenous (IV) injection 1 min prior to exposure to dichlorvos. Primary outcome was time to apnea. Data are presented as mean (stdev). Comparison between groups was performed using Student's t Test and analysis of variation (ANOVA). There was no difference between group respiratory parameters during the baseline period. There was no difference in mortality between the groups (100% both) but the time to apnea differed significantly ($p < 0.001$). Animals pre-treated with caffeine demonstrated apnea 30.6 (8.7) min post dichlorvos exposure. Animals pre-treated with saline demonstrated apnea 12.2 (3.9) min post exposure. The rate of decline in respiratory rate and tidal volume was less in animals pre-treated with caffeine. In conclusion, in a rat model of dichlorvos poisoning with central apnea, caffeine exposure prior to dichlorvos did not prevent OP-induced central apnea, but it did prolong the time to apnea after dichlorvos exposure.

KEYWORDS: organophosphate, insecticide, apnea, caffeine, central respiratory depression

1. INTRODUCTION

Organophosphate (OP) poisoning is a serious global health concern responsible for more than three million poisonings worldwide each year, and results in an estimated 300,000 deaths annually [1]. OP compounds are used globally in industry and agriculture where exposure and intentional ingestion has become a significant public health concern. Furthermore, these same compounds represent terrorist agents of opportunity. The consequences of such an incident were demonstrated in the 1995 Sarin gas attacks on the Tokyo City subway [2] and more recently the use of nerve agents in the chemical attacks in Syria.

Although treatment strategies exist, the mortality rate from acute OP poisoning remains high. Mortality

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occurs primarily from either acute respiratory failure due to a build up of excess acetylcholine at neuromuscular junctions, central respiratory depression from effects on the central nervous system (CNS), or the intermediate syndrome (IMS) where a delay in respiratory failure is attributed to increasing muscle weakness. Current therapies focus on anticholinergics such as atropine and other adjunctive treatments including oximes to reestablish cholinesterase function and reduce neurotoxic effects. Mechanical ventilation may be necessary following initial resuscitation and current therapies are frequently insufficient for severe exposures [3]. Alternatives to post exposure treatments are clinically important not only for hospital-based use, but for their potential use as a military aid in the field for exposures.

Caffeine and other methylxanthine compounds have been used in neonatal models of central respiratory depression for over four decades [4, 5]. The mechanism of action is thought to be secondary to a stimulation of central respiratory drive due to adenosine antagonism [6]. Studies have also shown that global stimulants of the brainstem mitigate the depressant respiratory effects of opiates [7, 8]. We hypothesized that caffeine administration would mitigate respiratory failure in an animal model of OP exposure.

2. MATERIALS AND METHODS

2.1. Study design

All animals were housed and cared for in accordance with the guidelines of the National Institute of Health. The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School. We used a previously validated animal model of OP poisoning with detailed physiologic respiratory recordings [9], which are described in the following sections. Male Wistar rats obtained from Charles River Laboratories (Wilmington, MA) were pair housed and maintained on a 12 hr light/dark cycle. Food and water was available *ad libitum* prior to sedation at the start of the study.

2.1.1. Anesthetic circuit

All animals were spontaneously breathing and received oxygen and isoflurane via a 3/32 inch kynar barbed 'Y' fitting (Small Parts, Inc., Miami Lakes, FL). The gas mixture was delivered through one arm, while the other was connected to a scavenger system (Surgivet, Waukesha, WI) connected to a ventilated hood system. An oxygen concentrator (SeQual Technologies, San Diego, CA) was used in line with the titrated isoflurane, producing an oxygen concentration of 30-40%. An end tidal CO_2 detector (Columbus Instruments, Columbus, OH) was connected in series to the tracheal tube and pneumotachometer (HSE, March-Hugstetten, Germany). The dead space of the entire system totaled 0.33 cm^3 or approximately 10% of the tidal volume of the animal.

Animals were administered a continuous dose of isoflurane (Webster Veterinary, Sterling, MA) titrated to between 2-4%. Anesthetic sufficiency was determined by lack of response to painful stimulus on the foot or tail. Titration was aimed at sustaining a respiratory rate between 45 and 60 breaths per minute, and no changes occurred 10 minutes prior to the initiation of the experimental protocol.

2.1.2. Surgical setup

A tracheostomy was performed by cannulating the trachea using polyethylene tubing (0.24 mm). A cannula (0.05 mm) was then visually inserted into the femoral artery to monitor blood pressure throughout the study. A second cannula (0.05 mm) was placed in the femoral vein for intravenous applications. Catheterization of the bladder was unnecessary, as the study did not exceed 2.5 hours.

2.1.3. Respiratory and hemodynamic recordings

Respiratory parameters that were collected included end-tidal P_{CO_2}. Respiratory rate was calculated from this airflow signal using peak of inspiration as the marker of an individual breath. Volume of expired gas was also calculated through integration of the airflow tracing. Minute ventilation was further determined by multiplying the respiratory rate by the volume of expiration. A non-invasive pulse oximeter (MouseOx, Starr Life Sciences Corp., Oakmont, PA) attached to the right back paw measured arterial oxygen saturation and pulse rate. One animal in the experimental group did not have an arterial pressure recording. All tracings were recorded and displayed for the duration of the study.

2.1.4. Data acquisition

Signals were digitally recorded using a data acquisition system (PowerLab, ADInstruments, Inc., Colorado

Springs, CO) and a PC computer (Dell, Round Rock, TX). Recordings were filtered and amplified using the CyberAmp (AutoMate Scientific, Berkeley, CA) and sampled at 400 Hz. Arterial pressure was filtered by a separate amplifier (ADInstruments, Colorado Springs, CO). Data was then stored on a personal computer for later analysis.

2.2. Protocol

Animals were randomized into two study groups (n = 8 per group); caffeine and dichlorvos (Group 1) and dichlorvos only (Group 2). In each group, either caffeine (Sigma-Aldrich, St. Louis, MO) or saline was given intravenously one minute prior to OP exposure. Caffeine was dissolved in warm saline and animals were given 20 mg/kg over 40 seconds (dose based on previous studies). In all animals, dichlorvos (Sigma-Aldrich, St. Louis, MO) was given as a single subcutaneous dose of 100 mg/kg. Dichlorvos is an oxon-OP that has been used previously for studies of respiratory function in OP poisoning. 100 mg/kg was chosen based on previous studies that demonstrate reliable respiratory failure at that dose [10]. Animals were monitored for 120 minutes post exposure, or until death. Animals that survive 120 minutes were euthanized with bilateral pneumothoraxes and intravenous pentobarbital (100 mg/kg).

2.3. Data analysis

Data is presented as mean +/- standard deviation unless otherwise noted. Comparison of time to apnea between the exposed and controls groups was performed using an unpaired student's t test for independent means. Rate of decline of continuous physiologic variables was compared between the groups using repeated measures ANOVA. The average point-to-point slope of decline was calculated for both groups to compare the rate of change in respiratory variables.

A power calculation was performed *a priori*. Assuming an alpha of 0.05 and a beta of 0.8 the study was powered to detect a difference of 50% between the groups using time to apnea as the primary comparison. Statistics was performed using online statistical tools (www.statpages.org).

3. RESULTS

The baseline parameters of both groups are shown in table 1 and were not found to be significantly different between the groups with the exception of the mean arterial pressure and animal weight. There was no difference in respiratory parameters during the baseline period.

Changes in respiratory parameters over time are detailed in figures 1-3. Data represents the changes in respiratory rate, tidal volume and minute ventilation over one hour from exposure to OP for each group. All animals demonstrated agradual decrease in respiratory parameters over time following exposure to OP. In addition, minute ventilation and tidal volume demonstrated a slight increase (roughly 5%) immediately following OP exposure before declining. Animals in the control group demonstrated a significantly increased rate of decline in

Table 1. Baseline parameters of study groups.

Variable	Group 1: caffeine & dichlorvos (n = 8)	Group 2: dichlorvos only (n = 8)	
Weight	295 (7.6)	272 (22.7)	p = 0.0167
Respiratory rate	54.0 (2.4)	49.1 (8.9)	p = 0.1549
Tidal volume	2.4 (0.45)	2.5 (0.9)	p = 0.7828
Minute ventilation	129.1 (22.4)	123.6 (57.0)	p = 0.8032
Pulse rate	385.1 (31.4)	388.3 (32.9)	p = 0.8451
Pulse oxygenation	99.5 (0.2)	99.6 (0.1)	p = 0.2266
Mean arterial pressure*	90.4 (9.6)	77.8 (7.3)	p = 0.0104

*One animal in the experimental group did not have an arterial pressure recording due to technical issues.

respiratory rate (RR), tidal volume (TV) and minute ventilation (MV) when compared to animals exposed to caffeine (Figures 1-3). The average decline in RR was 2.2 times less in those animals exposed to caffeine. The average decline in MV and TV over the course of the study was 1.8 and 2.6 times less, respectively, in those animals exposed to caffeine.

There was no significant change in blood pressure parameters or pulse rate before respiratory parameters

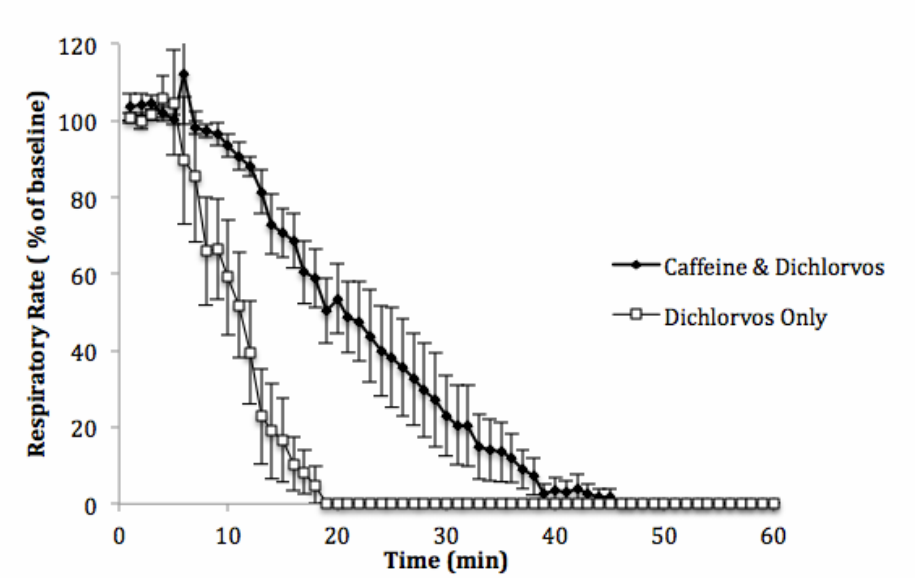

Figure 1. Respiratory rate decline after dichlorvos exposure.
Decline of respiratory rate between the groups is shown as a percentage of baseline respiratory rate. The data was binned into 1-minute intervals post dichlorvos administration. Data is presented as mean (dot) and standard deviation (error bar).

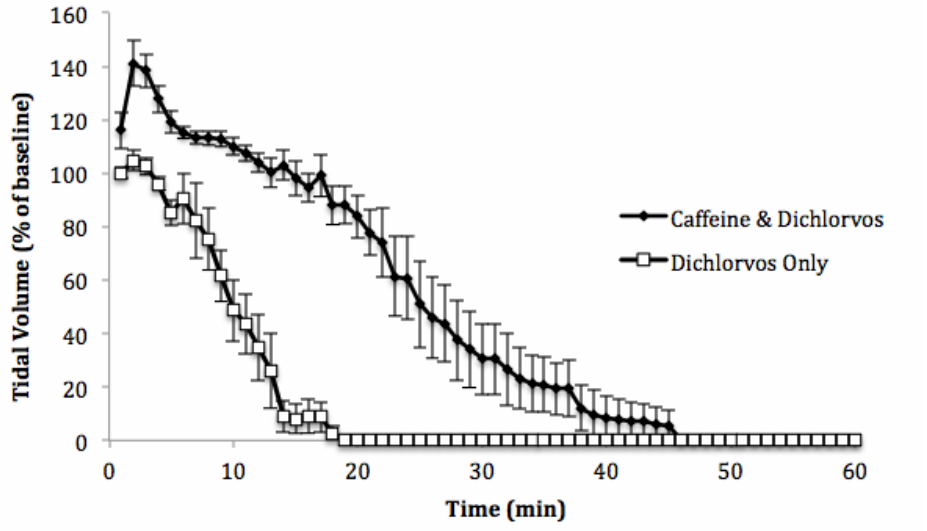

Figure 2. Tidal volume decline after dichlorvos exposure.
Decline of tidal volume between the groups is shown as a percentage of baseline. The data was binned into 1-minute intervals post dichlorvos administration. Error bars are representative of standard deviation for each point as an average of n = 8.

dropped at least 50%. No animal in either group survived to the study endpoint of one-hour post OP exposure (i.e. a 100% mortality in all animals). Animals exposed to caffeine (Group 1) experienced a longer time to apnea following OP exposure compared to those in the control group, $p < 0.0001$. (Figure 4) The mean time to apnea occurred at 30.6 (± 8.7) minutes in animals exposed to caffeine,

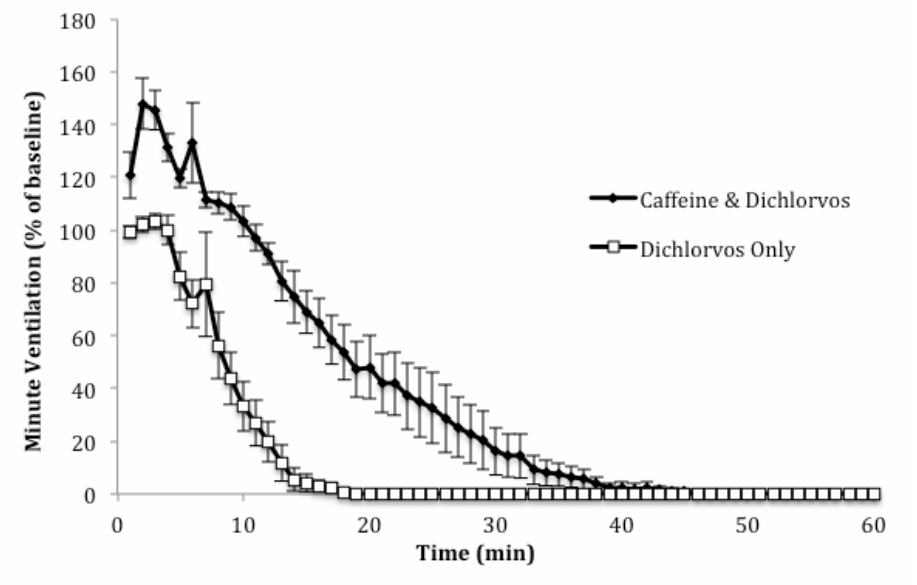

Figure 3. Minute ventilation decline after dichlorvos exposure.
Decline of minute ventilation between the groups is shown as a percentage of baseline. The data was binned into 1-minute intervals post dichlorvos administration. Error bars are representative of standard deviation for each point as an average of n = 8.

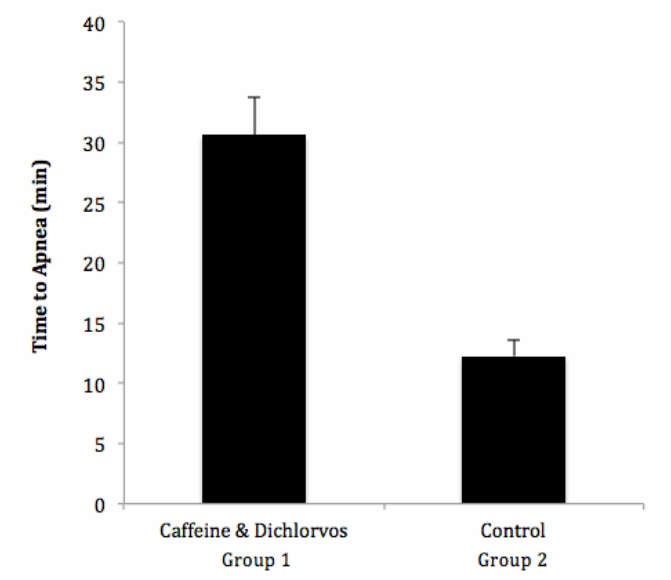

Figure 4. Time to apnea following dichlorvos exposure.
Average time in minutes to apnea shown between Group 1 and Group 2. Group 1 averaged approximately 30 minutes. Group 2 averaged 12 minutes. $p < 0.0001$.

compared to 12.2 (± 3.9) min in those exposed to OP alone.

4. DISCUSSION

In this study caffeine mitigated the respiratory effects of organophosphate poisoning. Caffeine exposure prior to dichlorvos did not prevent OP-induced central apnea, but it did prolong the time to apnea after dichlorvos exposure. The clinical effects of caffeine treatment were roughly the same for all respiratory parameters. We hypothesize that the limited effect of caffeine on respiration was related to dosing. It is likely that the amount of caffeine required to fully reverse OP-induced apnea will be significantly higher. This is analogous to the dosing requirements for atropine following OP exposure, which can be 70 times higher than the dose used for other clinical conditions such as bradycardia [3]. We chose to test a single bolus dose of 20 mg/kg caffeine. This dose was based on previous rodent models which demonstrated this dose to be safe [11-13] and effective in stimulating respiration [14]. It is unclear what upper limit of caffeine may be required to fully combat the central respiratory depression from organophosphates. Prior studies of caffeine poisoning have described the rat LD50 for caffeine as 105 mg/kg [15].

The mechanism of action of caffeine in this study is likely centrally mediated. Previous studies from our group and others have demonstrated that the central respiratory effect of dichlorvos is related to an exposure to respiratory centers in the brainstem [16]. Animal models have shown that direct exposure of specific areas of the central respiratory centers in the brainstem with a variety of compounds can mitigate central respiratory depression. Caffeine is an unspecific antagonist of adenosine receptors active both centrally and at the carotid body. The mechanism of action of caffeine on respiration is poorly understood, but other animal models imply that the effect of caffeine on respiration is likely centrally mediated [17]. Our results do not imply a site of action or mechanism, but they do suggest a potential role in the future for caffeine in treating OP exposures.

Caffeine has been suggested as a therapeutic measure to combat other agents that produce respiratory depression and other conditions that produce central apnea. In a rat model of respiratory depression induced with morphine, IV caffeine (20 mg/kg) almost completely reversed the respiratory depression [7]. Kasaba and colleagues demonstrated a 57% decrease in respiratory rate with animals exposed to IV morphine, with 85% recovery following IV caffeine. Apnea of prematurity is thought to represent the immature maturation of the respiratory centers and represents a significant central apnea. A meta-analysis in 2012 found that caffeine treatment in these patients result in significantly fewer apneic spells [14].

The potential role for caffeine in the treatment of acute OP exposure remains speculative, but our results provide some evidence to support its role. It is noteworthy that in this model we did not employ other pharmacotherapies to treat the OP poisoning. Standard treatment of acute OP poisoning includes atropine or other anti-muscarinic agent, an oxime such as pralidoxime, and benzodiazepines to mitigate neurocognitive sequelae [3]. In this study, however, we sought to observe the isolated effects of caffeine on OP-induced respiratory depression. The majority of OP exposures occur in agrarian countries in the developing world with limited health care resources [1]. Patients presenting to hospitals in these countries require a high level of services that are not available in the majority of hospitals handling OP-poisoned patients. The ability of caffeine to potentially mitigate respiratory depression could have a large impact in a health care system where mechanical ventilation is limited. However, more research is needed to determine if caffeine has a role in preventing endotracheal intubation in OP-poisoned patients, or reducing the days needed for mechanical ventilator support.

4.1. Limitations

This manuscript suffers from the same limitations of all animal model work in that any attempt to extrapolate this to humans should be done with significant caution. Furthermore, in order to isolate the effect of caffeine we did not use comprehensive medical therapy, as survival is high in animal models of acute dichlorvos poisoning treated with atropine and pralidoxime. Additional limitations relate to the fact that caffeine was given as a pre-treatment, and not post exposure. Also, a single 20 mg/kg bolus of IV caffeine was used in this study. With a plasma half-life of approximately 1.25 hours [18] it is possible that the plasma caffeine

concentration in our animals fell to such a degree within 30 minutes that it no longer confers any respiratory benefit. Future studies with varying caffeine doses or treatment duration may be beneficial. Additional limitation include a small sample size, use of single dose of caffeine (and not multiple doses), single bolus dose of caffeine and not continuous infusion.

5. CONCLUSION

In a rat model of dichlorvos poisoning with central apnea, caffeine exposure prior to dichlorvos did not prevent OP-induced central apnea, but it did prolong the time to apnea after dichlorvos exposure.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

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