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Short Communication

Brief isoflurane administration as a post-exposure treatment for organophosphate poisoning

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ABSTRACT

Organophosphate chemical threat agents (OP-CTA) exert toxic effects through cholinergic over-activation. However, after the initial cholinergic phase, the pathophysiology shifts to a non-cholinergic phase which leads to prolonged status epilepticus (SE), irreversible neuronal degeneration and long-term damage to the central nervous system. The efficacy of delayed treatments against OP-CTA is generally low due to the fact that most drugs fail to inhibit the later phase of non-cholinergic activation. Recently, we reported that intranasal brain delivery of obidoxime (OBD) provides complete neuroprotection against a lethal dose of paraoxon when administered 5 min after intoxication. In follow-up studies, we examined the window of effectiveness and found that OBD lost effectiveness around 15 min post-exposure, which corresponds to the onset of the non-cholinergic phase of intoxication. However, we observed that a brief isoflurane administration, the inhalation anesthetic used to facilitate intranasal drug administration, was effective against paraoxon-induced neurotoxicity. Thus, the present study aimed to investigate the time-course and dose-response efficacy of a brief 4 min isoflurane administration as a treatment for neurotoxicity induced by OP-CTA. We found that isoflurane is a potent anti-seizure agent and neuroprotectant when administered between 20 and 30 min after paraoxon exposure, stopping SE within 10 min of administration and preventing acute neurodegeneration seen 24 h later. We also found that the seizure blocking and neuroprotectant properties of isoflurane, when administered 30 min after paraoxon, are dose-dependent. The effectiveness and current clinical use of isoflurane support its use as an innovative approach for post exposure treatment of organophosphate poisoning.

1. Introduction

Organophosphate poisoning is a significant world health problem, claiming thousands of lives per year through intentional and unintentional pesticide exposure (Jett, 2007, 2011). The prospect for industrial accidents or military/terrorism based organophosphate release further increases the need for treatments. Organophosphate based chemical threat agents (OP-CTA), including certain common pesticides, exert their toxic effects through cholinergic over-activation during the initial phase of intoxication. However, the pathophysiology shifts to a non-cholinergic phase which leads to prolonged seizures and a pathophysiological cascade culminating in irreversible neuronal degeneration and long term CNS damage (Deshpande et al., 2014; McDonough and Shih, 1997). The efficacy of delayed treatments against OP-CTA toxicity is limited due to the fact that most drugs fail to inhibit the later phase of non-cholinergic activation. For example, the current Food and Drug Administration approved anticonvulsant treatment for OP-CTA poisoning, diazepam, loses effectiveness if treatment is delayed (Apland et al., 2014; McDonough et al., 2010). Recently we reported that intranasal delivery of obidoxime (OBD) provides complete neuroprotection against a lethal dose of paraoxon when administered from 30 min before to 5 min after organophosphate intoxication (Krishnan et al., 2016). In follow-up studies we examined the post exposure window of effectiveness and found that intranasally administered

Abbreviations: AChE, acetylcholinesterase; BBB, blood brain barrier; FJC, Fluoro-Jade C; OBD, Obidoxime; OP-CTA, organophosphate chemical threat agents.

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OBD lost effectiveness when given more than 15 min after paraoxon exposure which corresponds to the onset of the non-cholinergic phase of intoxication. During the course of these studies we observed that isoflurane, the inhalation anesthetic used during intranasal drug delivery, was an effective post-exposure anticonvulsant and neuroprotectant even with a brief 4 min administration.

The potential use of anesthetics as neuroprotectants in nerve agent poisoning has been shown in several earlier studies. In one study, sub-anesthetic doses of ketamine were found to stop ongoing seizures when administered 30 min after soman challenge (Dorandeu et al., 2005). An extended delay before treatment of up to 2 h required an increase in ketamine dose that reached the anesthetic dose level. In another study, anesthesia using isoflurane maintained for 1.5 h during diffusion-weighted magnetic resonance imaging interrupted soman-induced seizures and attenuated edema in selected brain areas in mice (Testylier et al., 2007). In a later study the protective effects of several anesthetics on sarin poisoning were investigated in domestic swine (Sawyer et al., 2012). In this study 2% isoflurane was delivered in 100% oxygen continuously for 6 h and resulted in profound protection against sarin poisoning. In another recent study, isoflurane was found to have anticonvulsant and neuroprotective properties in a paraoxon model of acquired epilepsy in the rat (Bar-Klein et al., 2016). Again, a longer duration of 1 h isoflurane administration was used in these studies, raising the possibility for long term toxicity by isoflurane. None of the investigations examined shorter duration isoflurane administration.

Although non-cholinergic intervention in nerve agent poisoning has been proposed as a potential neuroprotective strategy, efforts to bring this treatment to the field have not been forthcoming (Dorandeu et al., 2013). To our knowledge there are no studies that have investigated the anticonvulsant properties of isoflurane when used in a brief administration without maintenance of anesthesia for longer duration. Therefore in the present study we investigated the time course and dose response efficacy of a brief isoflurane administration as an anticonvulsant and neuroprotective treatment against a lethal dose of paraoxon in view of its several attractive features including 1) current widespread availability in hospitals, 2) lack of long term toxicity due to the brief administration time and 2) the simple, noninvasive nature of isoflurane administration. All of these factors make isoflurane a potentially useful treatment in both hospital settings and field applications following OP-CTA release events.

2. Experimental procedure

All animal experiments were conducted following the NIH Guidelines for the Care and Use of Laboratory Animals, and the animal protocol was approved by the animal care and use committee (IACUC) of the Uniformed Services University of the Health Sciences, Bethesda, MD. Adult male rats Sprague-Dawley rats (250 ± 40 g) were used for all studies (Taconic Biosciences, NY). Animals were housed individually in an environmentally controlled room (20–23 °C, ~44% humidity, 12 h light/dark cycle, 350–400 lx, lights on at 6:00 am), with food (Teklad Global; 18% protein #2018 rodent diet; Harlan Laboratories, IN) and water available continuously. Animal handling was minimized to reduce animal stress. All reagents not indicated otherwise, were from Sigma Aldrich (St. Louis, MO).

2.1. Paraoxon exposure and behavioral studies

Paraoxon solutions were prepared fresh by adding 10 μl of stock solution (1.27 g/ml) to 3 ml of ice cold phosphate buffered saline (PBS) in a glass vial and mixing thoroughly. A lethal dose of paraoxon (4 mg/kg) (Deshpande et al., 2014) was administered subcutaneously followed by intramuscular atropine sulphate (2 mg/kg) and intramuscular 2-PAM (25 mg/kg) irrespective of group (Fig. 1). Rats were observed independently by two trained researchers who were blinded to the treatment groups for signs of seizure onset, and continuously rated for seizure severity according to a modified Racine Scale: Stage 0, no behavioral response; Stage 1, behavioral arrest, orofacial movements, chewing; Stage 2, head nodding/myoclonus; Stage 3, unilateral/bilateral forelimb clonus without rearing, straub tail, extended body posture; Stage 4, bilateral forelimb clonus plus rearing; Stage 5, rearing and falling; Stage 6, full tonic seizures (Dorandeu et al., 2013; Krishnan et al., 2016; Thiermann et al., 2013). Overall mortality was assessed at 24 h.

2.2. Time-course study

Animals were exposed to isoflurane in an anesthesia chamber at a concentration of 2% isoflurane (Baxter; Deerfield, IL) for 3 min followed by 5% for 1 min in 100% oxygen at 10, 20, 30, 45, 60 or 120 min after paraoxon intoxication. This pattern of isoflurane administration is based on the protocol used for anesthesia in our laboratory. The control group received paraoxon, 2-PAM and atropine sulphate, but was not treated with isoflurane.

2.3. Dose-response study

We selected the 30 min paraoxon post-administration time point for the dose response study because it was the optimal time point determined in the time-course study. Thirty minutes after onset of paraoxon exposure, animals were randomly placed in 5 groups. Control animals received no treatment. The four treated groups all received a total of 4 min of isoflurane administration. Two groups received the same dose throughout the 4 min period, and two groups had the dose increased for the last 1 min of treatment. The 4 treatment groups included; 1) 1% isoflurane for 4 min, 2) 2% for 4 min, 3) 2% isoflurane for 3 min followed by 3.5%

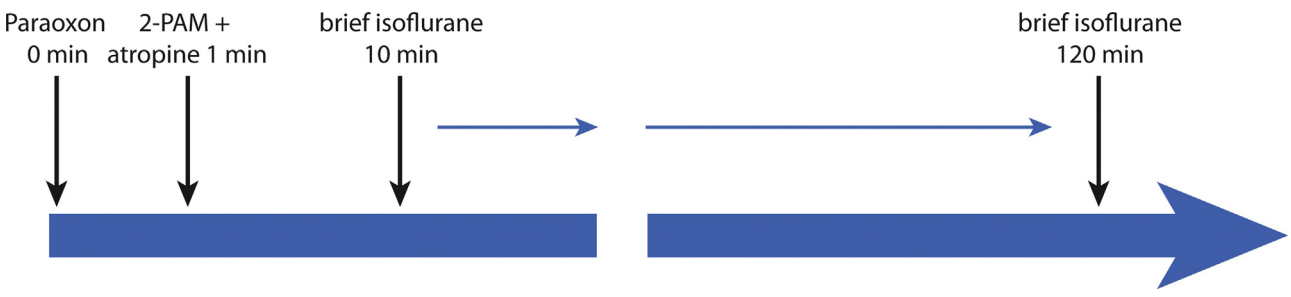

Fig. 1. Treatment schedule for paraoxon, 2-PAM, atropine sulfate and isoflurane.