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A rodent model of human organophosphate exposure producing status epilepticus and neuropathology

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ABSTRACT

Exposure to organophosphates (OPs) often results in seizures and/or status epilepticus (SE) that produce neural damage within the central nervous system (CNS). Early control of SE is imperative for minimizing seizure-related CNS neuropathology. Although standard therapies exist, more effective agents are needed to reduce OP-induced SE and neuronal loss, particularly therapies with efficacy when administered 10's of minutes after the onset of SE. To evaluate novel antiseizure compounds, animal models should simulate the CNS effects of OP exposure observed in humans. We characterized in rats the effects of the OP, diisopropyl flourophosphate (DFP) as a function of dose and route of administration of supporting agents (pyridostigmine, 2-PAM, atropine); outcome measures were mortality, electrographic seizure activity during SE, and subsequent CNS neuropathology. Doses of DFP between 3 and 7 mg/kg consistently caused SE, and the latency to behavioral tremors and to subsequent initiation of SE were dose related. In distinction, all doses of DFP that resulted in electrographic SE (3–7 mg/kg) produced seizures of similar intensity and duration, and similar CNS neuropathology (i.e., the effects were all-or-none). Although SE was similar across doses, mortality progressively increased with higher doses of DFP. Mortality was significantly lower when the route of administration of therapeutic agents was intramuscular compared to intraperitoneal. This rodent model of OP poisoning demonstrates pathological characteristics similar to those observed in humans, and thus begins to validate this model for investigating potential new therapeutic approaches.

1. Introduction

Organophosphates (OPs) comprise the active components of many insecticides including parathion and malathion, as well as the nerve agents (NA) soman, tabun and sarin. Accidental or deliberate exposure to these OP-containing compounds is a major, worldwide health problem. OPs are potent inhibitors of cholinesterase (Lotti, 2001); therefore, OP exposure induces the accumulation of acetylcholine in peripheral synapses, which results in muscle tremors, cardiovascular dysfunction, hypotension, and bronchial spasms (Hulse et al., 2014; Lotti, 2001). OP-related morbidity most frequently results from respiratory failure due to brochospasm, bronchorrhea, and paralysis of respiratory muscles (Karalliedde and Senanayake, 1989; Lotti, 2001).

In addition to peripheral effects, OPs, such as diisopropyl flourophosphate (DFP) (Deshpande et al., 2010; Todorovic et al., 2012), parathion (Garcia et al., 2003; Hoffmann and Papendorf, 2006), dimethoate (Hoffmann and Papendorf, 2006), paraoxon (Deshpande et al., 2014; Shrot et al., 2014), dichlorvos (Gaspari and Paydarfar, 2007), and NAs (McDonough and Shih, 1997; Nozaki et al., 1995) all can act in the central nervous system (CNS) to induce seizures and/or status epilepticus (SE). SE is characterized by continuous or recurrent seizure activity that often results in neuronal injury in the CNS and mortality (Fujikawa, 2005; Fujikawa et al., 2000; Wasterlain et al., 1993). This central neuropathology produced by SE can result in long-term neurological and behavioral disorders (Apland et al., 2010; Prager et al., 2014). The CNS neuronal damage can occur after only 20 min of continuous seizure activity, and worsens as the duration of the SE increases (McDonough et al., 1995). Consequently, early control of SE is critical for survival and CNS neuroprotection following OP exposure (Shih et al., 2003).

Benzodiazepines are the first-line standard-of-care pharmacotherapy for OP-induced SE (Alldredge et al., 2001; Newmark, 2007;

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Rotenberg and Newmark, 2003; Treiman, 2007; Walton and Treiman, 1988). Although these agents can be effective if an appropriate dose is administered shortly after initiation of SE, these drugs fail to terminate SE in many cases of OP poisoning, and become less effective when administered >30 min after exposure, as SE becomes increasingly resistant to benzodiazepines as the duration of the SE increases (Todorovic et al., 2012). Furthermore, studies in animal models found that these agents do not prevent brain damage or behavioral deficits resulting from SE if administration is delayed (Apland et al., 2014; Myhrer et al., 2005; Todorovic et al., 2012). In the case of a mass civilian exposure to NA or other OP, it is unlikely that first responders would be on site and administer these standard therapeutic agents within the relatively short post-exposure time period needed for maximum efficacy. Consequently, development of novel therapies that can effectively control OP-induced SE and reduce CNS neuropathology, when administered following a significant time period after exposure, is essential.

In order to experimentally evaluate potential therapies, the development of a new, delayed treatment, animal model of OP exposure is required. Although both kainic acid and pilocarpine induce SE and neuropathology, their mechanisms of action are different than OPs. Consequently, the optimal animal model should use an OP and/or NA to induce SE of adequate intensity and duration to induce neuropathology, but not result in rapid mortality that would preclude determination of the complete time course and duration of SE, or prevent appropriate processing of CNS tissue for histopathology. The initial phase of developing a new, delayed treatment model of OP poisoning, which would mimic a mass civilian exposure is to determine the dose of an OP that induces seizure activity and neuropathology with an experimentally acceptable rate of mortality. These studies were designed to determine the optimal dose of the prototypical OP DFP that meets these criteria.

We characterized behavioral and electrographic indicators of SE activity, CNS neuropathology, and mortality following administration of DFP (2–7 mg/kg), along with clinically appropriate supporting compounds (pyridostigmine, atropine, and 2-PAM) (Bajgar, 2010; Jokanovic and Stojiljkovic, 2006, Leadbeater et al., 1985; Voicu et al., 2010; Wetherell et al., 2002) in rats. Appropriate adjustment of OP dose and route of administration of supporting agents resulted in an animal model producing SE and CNS neuropathology similar to effects reported in patients (Holstege and Dobmeier, 2005; Shetty, 2014; Xiang et al., 2014), with an experimentally tolerable level of mortality.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (200–250 g) were purchased from Charles River, were housed in the temperature-controlled vivarium, and maintained on a 12hr:12hr light:dark cycle with *ad libitum* access to food and water. The University of Utah Institutional Animal Care and Use Committee approved all surgical and experimental procedures used in these studies.

2.2. Drug preparation

DFP was dissolved in 0.9% sterile saline to a concentration of 10 ul/ml that was stored frozen (−80°C) until the day of the experiment. DFP was kept on ice until injected. Pyridostigmine was prepared in 0.9% sterile saline (0.052 mg/ml) and refrigerated until used. Atropine methyl nitrate and 2-PAM were combined and dissolved in sterile 0.9% saline to final concentrations of 4 mg/ml and 50 mg/ml, respectively. Pyridostigmine, atropine, and 2-PAM were prepared on the day of testing.

2.3. Surgical procedures

Animals were implanted with electrodes for electroencephalographic (EEG) recording. The rats were anesthetized with 2% isoflourane and placed in a stereotaxic instrument. An incision was then made on the midline and the scalp was retracted laterally. Six holes were drilled in the skull, three for support screws and three for the electrode wires. Bipolar recording electrodes (MS333-3-B, Plastics One, Roanoake, VA) were placed right of midline, while the ground electrode was positioned on the left. All electrode wires were trimmed for epidural, differential recording of EEG activity. Dental cement was used to secure the support screws and electrode pedestal, and the wound was sutured shut. All animals were then returned to their home cages and allowed at least 7 days to recover from these procedures prior to testing.

2.4. Video and EEG recordings

Following recovery, the conscious, unrestrained rats were placed into Plexiglas recording chambers with swivel communicators for testing. The implanted electrodes were connected to spring-covered EEG cables (Plastics One, Roanoke, VA). EEG100 amplifiers (high-pass filter, 1 Hz, low-pass, 100 Hz, notch filter at 60 Hz, 5000× gain) amplified the signals, which were then digitized at 500 Hz with an MP150 digital-to-analog converter. The EEG signals were recorded using AcqKnowledge software (BioPac Systems, Inc. Santa Barbara, CA).

During testing, the rats were continuously monitored using an infrared surveillance system whose output was recorded by DVD player/recorders for subsequent evaluation (DMR-ES20, Panasonic).

2.5. Experimental protocols

2.5.1. Experiment 1

Fig. 1 is a schematic representation of the protocol used in these studies. To attenuate the peripheral lethal effects of OP and decrease mortality, animals were pretreated with pyridostigmine bromide (0.026 mg/kg, IM) 30 min prior to DFP administration, and atropine methyl nitrate (2 mg/kg, IM) plus 2-PAM (25 mg/kg, IM) 1 min after the injection of DFP. Pyridostigmine is a competitive cholinesterase inhibitor that reduces binding of the irreversible inhibitor DFP, and consequently is beneficial following DFP

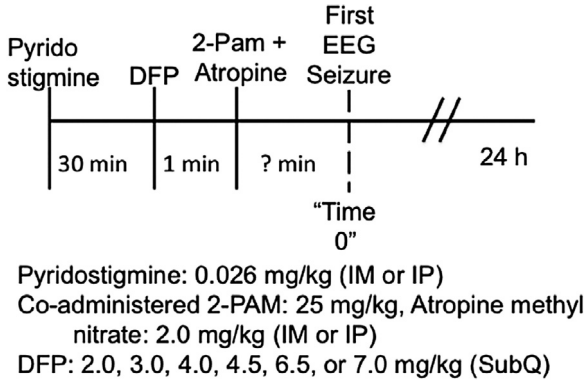

Fig. 1. Schematic summary of protocol.