NeuroToxicology 63 (2017) 21–32

Contents lists available at ScienceDirect

NeuroToxicology

Full Length Article

Behavioral intoxication following voluntary oral ingestion of tetramethylenedisulfotetramine: Dose-dependent onset, severity, survival, and recovery

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ARTICLE INFO

Article history:
Received 3 February 2017
Received in revised form 22 August 2017
Accepted 23 August 2017
Available online xxx

Keywords:
Tetramethylenedisulfotetramine (tetramine, TETS)
Variable-interval schedule of reinforcement
Median lethal dose (LD_{50})
Oral toxicity
Operant behavior
Rat

ABSTRACT

Tetramethylenedisulfotetramine (tetramine, or TETS) is a highly toxic rodenticide that has been responsible for over 14,000 accidental and intentional poisonings worldwide. Although the vast majority of TETS poisonings involved tainted food or drink, the laboratory in vivo studies of TETS intoxication used intraperitoneal injection or gavage for TETS exposure. Seeking to develop and characterize a more realistic model of TETS intoxication in the present study, rats were trained to rapidly and voluntarily consume a poisoned food morsel. Initially, the overt toxic effects of TETS consumption across a large range of doses were characterized, then a focused range of doses was selected for more intensive behavioral evaluation (in operant test chambers providing a variable-interval schedule of food reinforcement). The onset of intoxication following voluntary oral consumption of TETS was rapid, and clear dose-dependent response-rate suppression was observed across multiple performance measures within the operant-chamber environment. At most doses, recovery of operant performance did not occur within 30 h. Food consumption and body weight changes were also dose dependent and corroborated the behavioral measures of intoxication. This voluntary oral-poisoning method with concomitant operant-behavioral assessment shows promise for future studies of TETS (and other toxic chemicals of interest) and may be extremely valuable in characterizing treatment outcomes.

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1. Introduction

Tetramethylenedisulfotetramine (tetramine, or TETS) is a highly potent convulsant commonly used as a black market rodenticide in Asia and is one of the most dangerous food and water contaminants that could be used in an intentional poisoning scenario (Whitlow et al., 2005). TETS is easily synthesized (Croddy, 2004), tasteless, odorless, remarkably stable (Voss et al., 1961), environmentally persistent (Croddy, 2004), and particularly toxic to mammalian species (Whitlow et al., 2005; Voss et al., 1961; Esser et al., 1991), and it can remain in the body for up to six months (Chau et al., 2005). TETS was once marketed as a rodenticide, and despite its use being banned worldwide since 1984 (Roberts and Reigart, 1999), it remains widely available in some regions. It has been responsible for large numbers of accidental and intentional mass poisonings and is perhaps responsible for more intentional fatal poisonings than any other chemical in recent history (Li et al., 2014). These incidents are particularly common in China, where over 14,000 poisonings and 900 fatalities attributable to TETS occurred between 1991 and 2010 (Li et al., 2012). TETS, as an agent of terror, has gained increased attention from Western countries (Jett and Yeung, 2010) and has been used in over 50 poisonings outside of China (Zhang et al., 2011). Although an intentional poisoning involving TETS has not yet been reported in the U.S., a child was accidentally poisoned at her home in New York City by TETS brought back from China. Within 15 min of exposure the child experienced seizures that persisted for 4 h despite aggressive therapy with lorazepam, phenobarbital, and pyridoxine. The child remained hospitalized for several days and at discharge appeared to have multiple neurological deficits, including absence seizures and possible cortical blindness (CDC, 2003). There is currently no standard treatment for TETS poisoning in the United States, but a common Chinese protocol for TETS poisoning involves gastric lavage and high-volume or charcoal-filter hemoperfusion to remove as much TETS from the body as possible (Chau et al., 2005; Yu et al., 2005). Several potential drug treatments have also been identified for TETS poisoning, including diazepam (Shakarjian et al., 2012, 2015; Bruun et al., 2015; Vito et al., 2014), ketamine

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http://dx.doi.org/10.1016/j.neuro.2017.08.009
0161-813X/Published by Elsevier B.V.

(Shakarjian et al., 2012), MK-801 (Shakarjian et al., 2012, 2015), high-dosage γ-aminobutyric acid (GABA) (Sun et al., 2007), allopregnanolone (Bruun et al., 2015; Sun et al., 2007), and sodium dimercaptopropane sulfonate (Na-DMPS) (Sun et al., 2007). However, no drug treatment (single or combinatorial) has been completely effective at preventing behavioral intoxication and delayed lethality following TETS poisoning, possibly due to the mechanism by which TETS toxicity occurs.

TETS is a neurotoxin which reversibly binds non-competitively to the α1 and γ2 subunits of the γ-aminobutyric acid$_A$ ($GABA_A$) receptor ionophore complex (Bowery et al., 1975; Cao et al., 2012), preventing chloride ion influx and acting as a potent antagonist (Bowery et al., 1975; Zhao et al., 2014). The acute toxicity of TETS antagonism of the GABA system results from an over-excitement of central nervous system neurons. The disruption of the central nervous system caused by acute TETS poisoning leads to severe and widespread neurological and physical symptoms including convulsions, arrhythmias, hematological changes, coma, respiratory failure, refractory status epilepticus, and death (Li et al., 2012; Zhang et al., 2011; Lu et al., 2008). Sub-lethal or chronic doses of TETS can lead to long-term multiple organ failure and permanent neurological impairment (Zhang et al., 2011; Deng et al., 2012). Because of the inherent difficulty in identifying the source and nature of seizures in a clinical setting, treatment of TETS toxicity with typical regimens of anticonvulsant and antiepileptic drugs can prove inadequate. Due to the nature of convulsive status epilepticus, a time-dependent loss of efficacy and potency for many first-line drugs, such as benzodiazepines, quickly develops, and seizures can become self-sustaining (Arif and Hirsch, 2008). Studies suggest that this time-dependent pharmacoresistance is due in part to gradual endocytosis of synaptic $GABA_A$ receptors along with the concurrent movement of excitatory α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors to the synaptic membrane. Further, there is evidence of depletion of some inhibitory neuropeptides during prolonged status epilepticus, which contributes to the self-sustaining nature of the seizure activity (Chen and Wasterlain, 2006).

Previous studies examining TETS intoxication and potential treatments (Voss et al., 1961; Shakarjian et al., 2012, 2015; Bruun et al., 2015; Vito et al., 2014; Flannery et al., 2015; Zolkowska et al., 2012) have relied upon TETS dosing methods that do not mimic the many historical real-world exposures. By far the most common route of poisoning from TETS in humans is via voluntary consumption of TETS-adulterated foods or liquids (Whitlow et al., 2005; Li et al., 2014; Deng et al., 2012). Despite this fact, previous studies of TETS intoxication used gastric gavage or intraperitoneal (IP) injection as a route of administration. Obviously, IP injection is not the same as voluntary eating or drinking. Additionally, results from gavage studies may be misleading because a disproportionately large volume of liquid is forced into the animal's stomach at a rapid rate, causing faster absorption of the toxic chemical (Ferguson, 1962). Likewise, changes in the volume and concentration of IP administrations can affect the pharmacokinetics of the compound being administered (Barrett et al., 1991; Bredberg et al., 1994). TETS is also rapidly absorbed through the saliva and mucosa of the mouth and pharynx (Haskell and Voss, 1957), further changing the potential pharmacokinetics for different routes of TETS exposure. To better characterize the toxic profile of ingested TETS in a manner consistent with real-world scenarios, we developed a voluntary-ingestion model in adult male Sprague-Dawley rats. In this model, rats were trained to reliably consume a known quantity of food (a single piece of Froot Loops® cereal) in a short period of time. After rats were trained to consume the food quickly and reliably, a quantity of TETS in an acetone solution was applied to the food, and the acetone vehicle was allowed to completely evaporate, leaving only the TETS on the food morsel. Rats were then allowed to eat the poisoned food, which they did promptly and reliably.

The current study sought to characterize the toxicity of TETS following voluntary consumption, with an emphasis on behavioral intoxication across a range of doses. Such behavioral measures are intended to inform the onset, type, and degree of intoxication to allow for signs to be rapidly recognized and treatment administered (a so-called "trigger to treat"). Additionally, one can determine the extent and duration over which intoxication persists when left untreated, establishing a baseline for future studies searching for effective treatments against TETS.

2. Methods

2.1. Chemicals

Acetone (≥99.5%) was purchased from Sigma-Aldrich and stored at room temperature. The TETS (anhydrous) was obtained from the Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD) at ~78% purity and stored at 4 °C. The primary impurity (approximately 16% of the sample) within the powder was hexamethylenetrisulfohexamine (HEXS), a common TETS contaminant (Zolkowska et al., 2012; Owens et al., 2009) believed to be approximately 50-fold less toxic than TETS (Esser et al., 1991). The remaining impurities were unable to be characterized at the time of this writing, but appear to be consistent with those noted by the Edgewood Chemical Biological Center in their review of TETS synthesis (Hondrogiannis and Cullinan, 2011).

The TETS powder was dissolved into acetone solution by institutional chemical surety specialists to a concentration of 2 mg/mL. These multiple aliquots (1–2 mL) of TETS were stored securely at room temperature in amber vials in a dark, locked cabinet. An aliquot was typically discarded after a single use, but the initial cohorts used aliquots across multiple cohorts and therefore the solution was kept for up to 6 weeks after being opened and then resealed. All handling of the TETS occurred within the confines of a certified chemical fume hood, and personnel wore a face mask, safety goggles, lab coat, and double nitrile gloves.

2.2. Subjects

One hundred five (105) male Sprague-Dawley rats (SAS SD 400) were obtained from Charles River Laboratories (Wilmington, MA, USA). Forty-five (45) rats were assigned to the overt-toxicity assessment, and 60 rats were assigned to the behavioral-toxicity assessment (n = 12 rats × 5 doses). Rats in both assessments weighed between 201 and 225 g at the time of shipping and were allowed five days to acclimate to our facility. All subjects were housed individually in a vivarium with free access to water under a 12 h light/dark cycle (lights on at 0600). During acclimation all rats were fed *ad libitum*, after which food regulation was implemented and maintained for the remainder of the study. During food regulation rats were given a measured amount of food every afternoon (at least 30 min after behavioral assessment) to ensure their weights were approximately 85% of the *ad libitum* growth-curve weights provided by the vendor.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense (USAMRICD), and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The USAMRICD is a research facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.