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The abuse potential of oxethazaine: Effects of oxethazaine on drug-seeking behavior and analysis of its metabolites in plasma and hair in animal models

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ABSTRACT

Oxethazaine, an over-the-counter (OTC) antacid, is a precursor of phentermine, which is the most abused anorectic by methamphetamine users in Korea. However, no studies have investigated the abuse potential of oxethazaine. Therefore, we examined and compared the consequences of oxethazaine and phentermine treatment on animal models of conditioned place preference (CPP) and self-administration. Furthermore, oxethazaine and its metabolites in rat plasma were monitored using liquid chromatography–tandem mass spectrometry (LC–MS/MS) after oxethazaine administration, and compared with phentermine itself after phentermine administration to clarify the relationship between phentermine production by oxethazaine ingestion and the possible oxethazaine dependence. Oxethazaine metabolites were also determined by LC–MS/MS in rat hair after oxethazaine administration to investigate the possibility of phentermine detection in hair from oxethazaine abusers. In the behavioral experiment, phentermine (3 mg/kg) produced CPP in mice while oxethazaine (5, 10, and 15 mg/kg) did not. Moreover, phentermine (0.25 mg/kg/infusion) was self-administered by rats at 80% of free-feeding weight, whereas oxethazaine was not. In the analytical study, mephentermine and phentermine, both oxethazaine metabolites, were detected below the limit of quantitation or not detected in both plasma and hair from rats that had ingested oxethazaine (10 mg/kg, single dose or for 2 weeks). On the other hand, phentermine was detected in plasma and hair samples from rats that had ingested phentermine (10 mg/kg, single dose or for 2 weeks). Consequently, phentermine induced significant rewarding effects, but oxethazaine did not. Presumably, either oxethazaine does not have any abuse potential or oxethazaine metabolism to phentermine does not result in a pharmacologically active level of psychostimulant in the body. Furthermore, phentermine was not a major metabolite in hair obtained from oxethazaine abusers, which should make it possible to differentiate between chronic oxethazaine and phentermine users.

1. Introduction

Oxethazaine is a topical anesthetic, which has been used clinically as an antacid (Masuda et al., 2002; Yamazak, 1982). It is commercially available in Korea as a complex with aluminum hydroxide gel and magnesium hydroxide as an over-the-counter (OTC) drug to treat gastritis, duodenal ulcer, esophagitis, etc. Recently, oxethazaine was shown to be a source of mephentermine and phentermine, which are prohibited by the World Anti-Doping Agency (2011) (http://www.wada-ama.org/en/World-Anti-Doping-Program/Sports-and-Anti-Doping-Organizations/International-Standards/Prohibited-List/The-2011-Prohibited-List/Prohibited-In-Competition), found in urine (Hsu et al., 2009; Huang et al., 2010). However, little information on oxethazaine metabolism is available in previous literature. While phentermine is listed under Schedule IV by the U.S. Drug Enforcement Administration (DEA) (http://www.justice.gov/dea/pubs/scheduling.html) and its usage is controlled under the Narcotics Control Law in Korea, mephentermine is not currently controlled by the DEA or in Korea. Pharmacologically, mephentermine is an α_1-selective adrenergic receptor agonist and is used clinically to prevent hypotension, particularly during spinal anesthesia (Westfall and Westfall, 2011). Phentermine is an adrenergic reuptake inhibitor and has been widely used as an anorectic drug (Kaplan, 2005).

The distribution, legitimate or otherwise, of precursor drugs that are metabolized to controlled drugs, such as methamphetamine and amphetamine, has recently received attention (Musshoff, 2000). Oxethazaine is a precursor drug and has recently become an issue in the Anti-Doping Program in Taiwan (Hsu et al., 2009). A previous study demonstrated that the ratios of mephentermine and phentermine were different in

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urine samples from five volunteers who ingested oxethazaine, mephentermine, or phentermine (Huang et al., 2010). In Korea, phentermine is the most abused anorectic by methamphetamine users, presumably due to synergistic effects with methamphetamine, as well as economic or legal burdens inherent to the usage of other illegal drugs (Lee et al., 2011a, 2012). Previously, it was shown that phentermine caused variable but reliable self-administration in rhesus monkeys, which suggested moderate abuse potential (Stafford et al., 2001). A phentermine injection (3 mg/kg) has also been reported to produce a significant conditioned place preference (CPP) in mice (Rothman et al., 1998). Even though oxethazaine is a precursor to phentermine, no studies have been conducted to investigate the abuse potential of oxethazaine.

Hair is a disputable specimen for drug analysis as it lacks a dose–concentration relationship and can be affected by additional drug incorporation from environmental contamination; individual variance, such as hair color; and quality control issues in the analytical methods (Amanda, 2002). Nevertheless, hair analysis is widely accepted as an analytical tool to prove drug abuse both in practice and in research (Pragst and Balikova, 2006). Therefore, the possibility of phentermine incorporation into hair by oxethazaine abuse should be examined.

In this study, the effects of oxethazaine and a metabolite, phentermine, which has abuse potential, on drug-seeking behavior were examined and compared by self-administration and CPP methods to investigate the abuse potential of oxethazaine. Moreover, oxethazaine and its metabolites in rat plasma were monitored using liquid chromatography–tandem mass spectrometry (LC–MS/MS) after oxethazaine administration and compared with phentermine itself after phentermine administration to clarify the relationship between phentermine production by oxethazaine ingestion and the possible development of oxethazaine dependence. Oxethazaine metabolites were also determined by LC–MS/MS in rat hair after oxethazaine administration to investigate the possibility of phentermine detection in hair from oxethazaine abusers.

2. Material and methods

2.1. Chemicals

All solvents were high-performance liquid chromatography grade. 3,4-Methylenedioxyamphetamine-d_5 (MDA-d_5) and phentermine for the analytical study were purchased from Cerilliant (Round Rock, TX, USA). Mephentermine was kindly provided by Dr. Yao Yi Ju of the Health Science Authority in Singapore. Oxethazaine and phentermine for the animal study were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals

Male Wistar rats, 7-weeks-old, and male lean Zucker rats, 5-weeks-old, were obtained from Orient Bio Co. Ltd. (Seoul, Republic of Korea) and male ICR mice were purchased from Koatech Co., Ltd. (Pyeongtaek, Republic of Korea). The animals were allowed a 1-week acclimation period in the laboratory animal facility. The Wister rats, lean Zucker rats, and ICR mice weighed 300–400 g, 350–450 g, and 22–26 g, respectively, when each test started. All animals, except the Wister rats, were provided with tap water and a commercial diet ad libitum. The Wistar rats had a-food restricted diet in order to maintain 80% of their free-feeding weight (FFW) during the food-training experiment to motivate food-reinforced responses and self-administration. After the food training test and surgery, however, they were given ad libitum access to food for 2 days. The animal room was maintained at a temperature of 24 ± 2 °C and at a relative humidity of 50 ± 20%, with a 12 h light/dark cycle. The Wistar rats were housed alone and the ICR mice were housed 10 per cage. The lean Zucker rats were kept in separate metabolic cages to prevent urine and saliva contamination of the rat hair. All procedures were approved by the Animal Care and Use Committee at the National Forensic Service.

2.3. CPP apparatus and procedure

The CPP apparatus consisted of two compartments ($15 \times 15 \times 15$ cm) separated by closable guillotine doors. One compartment was white with a striated floor, and the other was black with a smooth floor. The compartments were dimly illuminated (12 lx). The CPP procedure consisted of preconditioning (days 1–2), pre-tracking (day 3), conditioning (days 4–11), and post-tracking (day 12). On days 1–2, the mice were allowed to explore both compartments. On day 3, the amount of time spent on each side was recorded for 900 s. On days 4–11, conditioning (40 min per session) was conducted using a biased and counter-balanced procedure. The mice were injected with oxethazaine (5, 10, or 15 mg/kg, i.p.), phentermine (3 mg/kg, i.p.), or the vehicle and underwent conditioning in alternate compartments on subsequent days during days 4–11. To test the effect of oxethazaine on sub-threshold doses of methamphetamine induced CPP, mice were pretreated with oxethazaine (5, 10 or 15 mg/kg, i.p.) 30 min prior, then conditioned with 0.3 mg/kg of methamphetamine (i.p.). On day 12, the mice were given free access to both compartments for 900 s, and the time spent on each side was recorded. The time spent in the CPP apparatus and movements within the boxes were tracked with a computer-based video-tracking system (NeuroVision, Pusan National University, Busan, Korea). A CPP was determined by comparing the preferences for the less preferred side (i.e., the drug-paired side) during the post-tracking and pre-tracking sessions.

2.4. Self-administration

2.4.1. Apparatus

During the experimental sessions, each Wister rat was placed in a standard operant chamber, which was placed in a light- and sound-attenuating cubicle ($28 \times 26 \times 20$ cm; Med Associates Inc., St. Albans, VT, USA). The front door and back wall of the chamber were made of transparent plastic, and the other walls were opaque metal. The chamber had two retractable response levers mounted on one side of the opaque walls and a food hopper located between the levers. A stimulus light was mounted above each lever. Drugs were injected by a syringe pump (Razel Scientific Instruments, Georgia, VT, USA) on top of the cubicle. Experimental sessions were controlled and recorded by a computer in the experimental room, with both a custom interface and software. At the start of a session, two response levers were present in the chamber; pressing the right lever delivered 0.1 ml of a drug solution over 4 s. During the injection, a stimulus light above the active lever was illuminated and lasted over the course of a time-out period (20 s) that followed each injection. Presses of the left lever were counted but had no other programmed consequences. The session ended by withdrawing of the levers.

2.4.2. Procedure

To acquire operant responses, the Wister rats were initially trained to press a lever for 45 mg food pellets (Bio-Serv, Frenchtown, NJ, USA) until the criteria were achieved (100 food pellets for the 3 consecutive days) in 3 h daily sessions. After food training, food and water were available ad libitum for at least 2 days. The detailed surgical methods were previously described (Wee et al., 2007). To summarize, the rats were implanted with silastic catheters (0.3 mm ID × 0.64 mm OD; Dow Corning Co. Midland, MI, USA) into the right external jugular vein. The rats were flushed with 0.2 ml of the antibiotic gentamicin sulfate (0.32 mg/ml; Kukje Pharma Co. Seongnam, Republic of Korea) in heparinized saline (20 IU/ml) and injected with Procillin (20,000 IU/ml) in saline by intramuscular injection, followed by 0.2 ml of heparinized saline alone. The rats were allowed to recover for 5 days before beginning self-administration.