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Drug interaction between ethanol and 3,4-methylenedioxymethamphetamine ("ecstasy")

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ABSTRACT

Alcohol (ethanol) and 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) are frequently co-abused, but recent findings indicate a harmful drug interaction between these two agents. In our previous study, we showed that MDMA exposure inhibits the activity of the acetaldehyde (ACH) metabolizing enzyme, aldehyde dehydrogenase2 (ALDH2). Based on this finding, we hypothesized that the co-administration of MDMA and ethanol would reduce the metabolism of ACH and result in increased accumulation of ACH. Rats were treated with MDMA or vehicle and then administered a single dose of ethanol. Liver ALDH2 activity decreased by 35% in the MDMA-treated rats compared to control rats. The peak concentration and the area under the concentration versus time curve of plasma ACH were 31% and 59% higher, respectively, in the MDMA–ethanol group compared to the ethanol-only group. In addition, the MDMA–ethanol group had 80% higher plasma transaminase levels than the ethanol-only group, indicating greater hepatocellular damage. Our results not only support a drug interaction between MDMA and ethanol but a novel underlying mechanism for the interaction.

1. Introduction

Alcohol (ethanol) is the most widely abused legal substance and is often co-abused with other agents such as 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). The co-abuse of ethanol and MDMA is prevalent worldwide (Gammella et al., 1997; Topp et al., 1999; Winstock et al., 2001; Strote et al., 2002; Barrett et al., 2006). MDMA users in the United States engage in more binge drinking than non-users, and in the United Kingdom, over 70% of MDMA users also drink ethanol at hazardous levels (Winstock et al., 2001; Strote et al., 2002). In Spain and Australia, the rates are 64% and 40%, respectively (Gammella et al., 1997; Topp et al., 1999). The extensive co-abuse of ethanol and MDMA is of particular concern since studies indicate that the co-abuse of ethanol and MDMA increases the risk of organ damage. For example, the co-administration of ethanol with MDMA enhanced MDMA-mediated long term neurotoxicity (Izco et al., 2007) as well as hepatotoxicity (Pontes et al., 2008). However, little is known about the mechanism of the drug interaction.

In humans and rats, ethanol is metabolized to acetaldehyde (ACH) primarily by alcohol dehydrogenase2 (ADH2) and to a lesser extent, by cytochrome P4502E1 (Lieber, 2004a,b). ACH is then metabolized to acetate by aldehyde dehydrogenase (ALDH) (Hawkins and Kalant, 1972; Klyosov, 1996; Klyosov et al., 1996). Several members of the ALDH superfamily are expressed in the liver, but the mitochondrial ALDH2 is considered the major ACH metabolizing enzyme due to its very low K_m for ACH ($\leq$1 μM) (Rashkovetsky et al., 1994) as well as the demonstration that mice lacking functional *aldh2* genes have markedly higher levels of ACH subsequent to ethanol administration (Isse et al., 2005). In rodents, in addition to mitochondrial ALDH2, cytosolic ALDH1 may metabolize ACH to some extent, given its relatively low K_m values (14–15 μM). In contrast, human ALDH1 exhibits a high K_m for ACH ($\geq$180 μM) and is considered unimportant in ACH metabolism (Klyosov et al., 1996). ACH is considered to be toxic since it is highly reactive and readily binds to cellular macromolecules such as proteins or DNA (Brooks, 1997; Niemelä, 2001) and ACH–protein adducts can act as auto-immunogens to initiate inflammation (Yokoyama et al., 1993, 1995; Nakamura et al., 2004). ALDH inhibitors such as disulfiram elevate ACH levels in ethanol-treated animals (He et al., 2001; Kinoshita et al., 2002) and humans (Johansson et al., 1991; Johnsen et al., 1992). The increase in ACH level is associated with a wide range of adverse effects, including: hypotension, reflex tachycardia, palpitations, headache, nausea and vomiting (Sauter et al., 1977; Johansson et al., 1991; Johnsen et al., 1992). A polymorphism of the human *ALDH2* gene, designated

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*ALDH2*2*, is common in East Asians and results in a dominantly inactive ALDH2 enzyme (Wolff, 1972; Yoshida et al., 1984). This polymorphism is associated with ethanol intolerance as well as increased risk of cancer attributed to ACH accumulation (Yokoyama et al., 1998, 2002a,b). Collectively, the results described above, as well as others, indicate that ACH is the major causative factor in the carcinogenic, hepatic, and neurological complications of alcoholism and binge drinking.

Our previous study, which characterized MDMA-mediated oxidative damage of proteins, revealed that MDMA administration results in the oxidative modification and inactivation of many liver mitochondrial proteins including ALDH2 (Moon et al., 2008). Since ALDH2 is the major ACH metabolizing enzyme, we hypothesized that the co-administration of MDMA with ethanol will result in decreased metabolism of ACH and thus, an increase in its blood level. Our present study was aimed at determining the effect of MDMA administration on the pharmacokinetics of ACH. As part of this study, we have improved an HPLC based method for the quantitation of ACH. Our results indicate that the co-administration of MDMA and ethanol inhibits ALDH1 and ALDH2 enzymes and increases the accumulation of ACH as well as hepatotoxicity.

2. Materials and methods

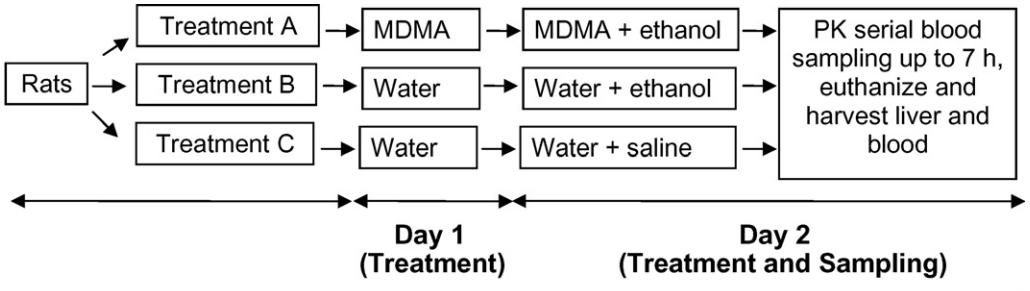
2.1. Chemicals

MDMA as racemate (±) hydrochloride salt, ethanol (absolute), ACH, propionaldehyde 2,4-dinitrophenyl hydrazone (PRO-DNPHO), ACH-2,4-dinitrophenyl hydrazone (ACH-DNPHO), dimethylsulfoxide, sodium acetate, perchloric acid and sodium ethylenediaminetetra acetate were from Sigma–Aldrich. Derivatizing agent 2,4-dinitrophenyl hydrazine (DNPH) was from TCI. Saline (0.9%) was from Baxter. Heparin sodium was from American Pharmaceutical Partners. HPLC grade methanol and acetonitrile were from American Bioanalytical. Purified water (Barnstead) was used for aqueous solutions and the mobile phase.

2.2. Animals

Male Sprague–Dawley rats (200–225 g) with cannulated carotid artery were from Harlan Laboratories. Catheter patency was maintained by daily flushing of the cannula with heparinized saline (100 IU/mL) solution. Animals were maintained in a 12:12 light:dark cycle in a temperature and humidity controlled environment and given *ad libitum* access to food and water. The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Maryland, School of Pharmacy and performed in accordance with the National Institutes of Health Guideline.

2.3. Study design



MDMA (2.5 mg/mL in water) was administered per orally (p.o.) in a volume of 4 mL/kg. Ethanol (25%, w/v) was administered intraperitoneally (i.p.) in a volume of 12 mL/kg. Rats received one of the following treatments: Treatment A (MDMA + ethanol): MDMA (10 mg/kg in 4 mL/kg dose volume, p.o.) × 2 days and 1 h post the second dose, ethanol (3 g/kg in 12 mL/kg dose volume, i.p.); Treatment B (ethanol): water (4 mL/kg, p.o.) × 2days and 1 h post the second dose, ethanol (3 g/kg in 12 mL/kg dose volume, i.p.); Treatment C (vehicle): water (4 mL/kg, p.o.) × 2 days and 1 h post the second dose, saline (12 mL/kg, i.p.). *MDMA dosing regime*: The MDMA dose of 10 mg/kg administered orally in rats results in clinically relevant plasma concentrations of MDMA and is within the dose range used in most MDMA studies (5–20 mg/kg), including our previous study (Upreti and Eddington, 2008). In our other previous study, when MDMA (10 mg/kg) was administered twice, 24 h apart from each other, a significant inhibition of ALDH2 activity was observed (Moon et al., 2008). Accordingly, this MDMA dosing regimen was used in the current study. *Ethanol dosing regime*: In our previous pharmacokinetic study of MDMA in rats, the highest plasma concentration of MDMA was apparent at 1 h post-dose (Upreti and Eddington, 2008). Hence, ethanol was administered to rats at 1 h after the second MDMA dose in this study. The dose of ethanol and the selection of blood sampling time points for measuring ACH levels were based on a previous report in which the pharmacokinetics of ethanol was described in rats (Livy et al., 2003).

Blood sample (0.2 mL) was collected via carotid cannula at 0.25, 0.75, 1.25, 2 and 7 h after the ethanol or saline dose and added to 15 μL of ice cold sodium EDTA (38 mg/mL). Blood samples were snap frozen in dry ice and stored at −80 °C. After the last blood collection, rats were euthanized by carbon dioxide asphyxiation and blood was collected by cardiac puncture. The blood was centrifuged for 10 min at 5000 × *g*, and plasma stored at −80 °C. Liver tissue was immediately excised, blotted dry and stored at −80 °C.

2.4. Measurement of ALDH2 and ALDH1 activities

Liver mitochondrial ALDH2 activity was measured using a previously described method (Tank et al., 1981; Moon et al., 2005) where the production of NADH by solubilized mitochondrial proteins (0.5 mg/assay) is determined spectrophotometrically at 340 nm in the presence of 10 μM propionyl aldehyde, 2 mM NAD^+ and 5 mM pyrazole in 60 mM Na-pyrophosphate buffer (pH 8.5). Cytosolic ALDH1 activity was determined by the same method, except that cytosolic proteins (0.5 mg/assay) were used and 60 μM propionyl aldehyde was used as the substrate, as described (Moon et al., 2007). The reaction rate was linear for up to the initial 3 min of incubation. One unit represents a reduction of 1 μmole NAD^+/min/mg protein at room temperature.

2.5. Quantitation of ACH

Proteins in a 200 μL aliquot of blood sample were precipitated with 300 μL of 3 M perchloric acid. The reaction was immediately neutralized with 800 μL of 3 M sodium acetate and then centrifuged for 5 min at 5000 × *g*. All steps were performed in a cold room to minimize ACH evaporation. The derivatizing agent, 2 mM solution of DNPH, prepared in mixture of DMSO and 0.1 M acetate buffer (pH 4) (36:64, v/v), was added to the supernatant and incubated for 30 min at room temperature. The reaction mixture was spiked with the internal standard, PRO-DNPHO; 100 μM. ACH-DNPHO and PRO-DNPHO were purified with SPE using reverse phase (C-18) extraction cartridges Sep-Pak Vac from Waters. The column was first equilibrated with methanol (1 mL 2×) and water (1 mL 2×), and the reaction mixture loaded, then washed with 1 mL of water and 1 mL of water:methanol (50:50, v/v). The ACH-DNPHO and PRO-DNPHO were extracted with methanol (1 mL 2×) and evaporated to dryness under nitrogen at 37 °C. The residue was reconstituted in 100 μL of mobile phase and 40 μL was used for HPLC analysis. The chromatographic system consisted of a Waters 1525 pump coupled with a 717-autosampler. The analytical column used was a Symmetry C-18 (4.6 mm × 150 mm, 5 μm particle size) protected by a C-18, guard column (4 mm × 3 mm, 5 μm) (Phenomenex). Isocratic mobile phase consisted of water (40%) and acetonitrile (60%) at a flow rate of 1 mL/min. Eluate was monitored by a 2487 dual-wavelength detector from Waters with absorbance wavelength at 365 nm. The acquired data were processed with the Empower software (Waters).

2.6. Calibration curves and quality control

Calibrations were obtained by spiking 200 μL of control blood with PRO-DNPHO (100 μM) and increasing amounts of ACH on the day of analysis. Stock solutions of ACH (20–5000 μM) were prepared fresh in water via serial dilutions of the primary stock solution (1 M). The concentrations of ACH in the blood used for calibration were: 2, 5, 10, 20, 50, 100 and 250 μM. Acquired data were calibrated by plotting the peak area ratio of the analyte and the internal standard against the concentration of calibration standards followed by a weighted linear regression analysis using Sigma Plot. Quality control (QC) samples were prepared by spiking control blood with ACH on the day of the study. QC samples were spiked with: low quantity control, (LQC; 20 μM), medium quantity control, (MQC; 125 μM), and high quantity control, (HQC; 250 μM).