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Drug Interaction Between Ethanol and 3,4-Methylenedioxymethamphetamine (“ecstasy”)

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Abstract

Alcohol and 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 was 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.

Keywords

alcohol; ethanol; 3,4-methylenedioxymethamphetamine (MDMA); ecstasy; metabolism; acetaldehyde; aldehyde dehydrogenase; drug interaction; liver toxicity; transaminase

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.,

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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, 2004b). ACH is then metabolized to acetate by aldehyde dehydrogenase (ALDH) (Klyosov, 1996; Klyosov et al., 1996; Hawkins and Kalant, 1972). 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 value for ACH ($1 \mu\text{M}$) (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 ($14\text{--}15 \mu\text{M}$). In contrast, human ALDH1 exhibits a high K_m for ACH ($180 \mu\text{M}$) 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; Niemela, 2001) and ACH-protein adducts can act as auto-immunogens to initiate inflammation (Yokoyama et al., 1993; Yokoyama et al., 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 *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; Yokoyama et al., 2002a; Yokoyama et al., 2002b). 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.

Materials and methods

Chemicals

MDMA as racemate ($\pm$) 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.

Animals

Male Sprague-Dawley rats (200–225 gm) 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.

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.) $\times$ 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.) $\times$ 2 days 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.) $\times$ 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, 2007). 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, 2007). Hence, ethanol was administered to rats at 1 h after the second MDMA dose in this study. The dose of ethanol and 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 μ L 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 $5000 \times g$, and plasma stored at -80°C . Liver tissue was immediately excised, blotted dry and stored at -80°C .

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.

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 $5000 \times g$. 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, propionaldehyde 2,4-dinitrophenyl hydrazone (PRO-DNPCHO; 100 μM). ACH-DNPCHO and PRO-DNPCHO 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 $\times$ 2) and water (1 mL $\times$ 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-DNPCHO and PRO-DNPCHO were extracted with methanol (1 mL $\times$ 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 (4.6×150 mm, 5 μm particle size) protected by a C-18, guard column (4×3 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).

Calibration curves and quality control

Calibrations were obtained by spiking 200 μL of control blood with PRO-DNPCHO (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).

Analysis of ACH quantitation data

Non-compartmental analysis of blood ACH concentrations versus time data was performed using WinNonLin (Pharsight) that used a linear trapezoidal numerical integration method. The parameters obtained were the peak plasma concentration (C_{max}), the time to reach peak concentration (T_{max}), the area under the plasma concentration versus time curves from time zero to the last measurable concentration (AUC_{0-t}) and when possible using the slope of the terminal elimination phase, the apparent elimination half-life ($t_{1/2}$). Statistical significance of the differences between ACH systemic exposure parameters (C_{max} and AUC_{0-t}) in the different treatment groups was determined via unpaired t-test (Prism; GraphPad Software).

Quantitation of plasma transaminase activity

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured in the rat plasma samples using a clinical chemistry analysis system kit Prochem-V (Drew Scientific) following manufacturer's instructions. Statistical significance of the differences in plasma transaminase levels between vehicle-, ethanol-, or MDMA+ethanol-treated groups were determined using a one-way analysis of variance (ANOVA). When ANOVA results showed significant differences, post-hoc multiple comparisons were performed using Tukey' test (GraphPad Software).

Results

Inhibition of ALDH1 and ALDH2 activities in MDMA and MDMA+ethanol-exposed livers

We recently showed that MDMA exposure leads to decreased ALDH2 activity in rat liver (Moon et al., 2008). Since cytoplasmic ALDH1 may also contribute to the metabolism of ACH in rats (Klyosov et al., 1996), we used liver tissue samples from our previous study (Moon et al., 2008) to assess the effect of MDMA-exposure on ALDH1 activity. In our previous study, rats were treated twice with 10 mg/kg of MDMA, 24 h apart, and then liver tissue isolated 12 h post the second treatment. The results revealed that ALDH1 activity was inhibited 30% in rats treated with MDMA ($p<0.01$) (Fig. 1A). We also retested the effect of MDMA on ALDH2 activity and in agreement with our previous result, ALDH2 activity was inhibited (48% reduction; $p<0.01$). Our data reveal that MDMA-exposure leads to the inhibition of both ALDH1 and ALDH2 enzymes in rat liver. We then compared the activities of liver ALDH1 and ALDH2 in MDMA+ethanol-treated versus vehicle-treated rats in the current study. Exposure of rats to both MDMA and ethanol resulted in reduced activities of cytosolic ALDH1 (65%, $p<0.001$) and mitochondrial ALDH2 activities (35%, $p<0.01$) (Fig. 1B). These results indicate that MDMA treatment alone or together with ethanol (MDMA+ethanol) inhibit cytosolic ALDH1 and mitochondrial ALDH2 activities in rat liver.

Quantitation of ACH in blood

To quantify the highly reactive and volatile ACH, we adopted a previously described HPLC method that involves the derivatization of ACH to a stable product (ACH-DNPPO) (Kozutsumi et al., 2002). The ACH-DNPPO is retained on a reverse phase separation column and detectable using an ultraviolet detector. We have made significant changes to the assay, including the use of a new internal standard (PRO-DNPPO) and sample preparation procedures and HPLC conditions to improve sensitivity. Typical overlaid chromatograms of ACH-DNPPO and PRO-DNPPO in spiked blood analyzed using our methods are shown in Fig. 2A. Nominal retention times for ACH-DNPPO and PRO-DNPPO were 5.0 and 7.5 min, respectively.

Linearity, accuracy and precision

Calibration curves for blood ACH levels were prepared on three separate days and were linear between the concentration of 5 to 250 μM , with a mean correlation coefficient of $r^2=0.9971$. The lowest spiked concentration with the percentage deviation (DEV) and relative standard deviation (RSD) $<20\%$ was accepted as the lowest limit of quantification (LLOQ) of ACH (5 μM). Fig. 2B displays the linearity of measured ACH levels in rat blood with mean $\pm$ standard deviation (SD) values for the constants in the regression equations used to calculate ACH concentrations in blood samples. The QC samples, spiked with ACH, stored and analyzed together with the study samples, displayed high accuracy and precision. Table 1 shows the accuracy and precision values for ACH quantitation in the QC samples. The mean measured QC concentrations deviated from the true spiked

concentrations in the range of –10.2% to 4.63%. The precision in the measurement of QC samples at the three tested concentrations was in the range of 2.02% to 4.96% RSD.

Effect of MDMA on pharmacokinetics of acetaldehyde

ACH levels in the blood samples from the three treatment groups were quantified and the time course (Fig. 3A), the peak concentration (C_{max}) (Fig. 3B), and the area under the curve (AUC_{0-t}) (Fig. 3C) determined. The results indicate a clear trend towards higher blood ACH levels in rats treated with both MDMA and ethanol as compared to ethanol alone. In the MDMA and ethanol co-treated rats, the means for C_{max} and AUC_{0-t} of blood ACH increased by 31% and 59%, respectively. Blood ACH concentrations increased rapidly after administration of ethanol in the control group (ethanol alone) and the peak blood ACH levels were reached within the first sampling time point (T_{max} ; 0.25 h–0.75 h; mean 0.38 h). Thereafter, ACH concentrations declined rapidly and approached the lowest limit of quantification by 7 h (Fig. 3A). On the other hand, rats treated with MDMA+ethanol displayed greater variability in the time to reach peak ACH concentration (T_{max} ; 0.25 h–7 h; mean 2.7 h) and the ACH levels persisted up to the last sampling time point of 7 h (Fig. 3A). At the 7 h time point, the mean ACH concentration in MDMA+ethanol-exposed rats was 2-fold higher than in ethanol-only exposed rats. The delayed ACH peak concentration observed in MDMA+ethanol-exposed rats prevented the accurate estimation of the terminal elimination slope of blood ACH concentration-time curve and hence the elimination $t_{1/2}$ could not be calculated. The mean elimination $t_{1/2}$ of ACH in rats administered ethanol alone was 2.8 h.

Liver damage in rats treated with MDMA+ethanol versus ethanol alone

To assess liver damage in the three treatment groups, plasma ALT and AST levels were measured 7 h after dosing with ethanol (Fig. 4). In the ethanol only-treated rat group, statistically insignificant increases in plasma ALT and AST levels were observed. However, when both MDMA and ethanol were administered, significant increases in plasma transaminase levels (2.7-fold increase for ALT, $p < 0.001$ and 3-fold increase for AST, $p < 0.01$) were observed, relative to vehicle treated rats as well as the ethanol only group (80% higher, $p < 0.01$) for ALT. These results indicate greater hepatocellular damage in the MDMA+ethanol-group compared to the ethanol only-treated and vehicle groups.

Discussion

Ethanol and MDMA are frequently co-abused (Gammella et al., 1997; Topp et al., 1999; Winstock et al., 2001; Strote et al., 2002; Boyd et al., 2003; Barrett et al., 2006) and evidence supports a drug interaction between these two agents (Izco et al., 2007; Pontes et al., 2008). It is important that we understand the mechanism by which MDMA and ethanol interact to cause tissue damage in order to develop therapeutics or intervention measures to minimize damage. In this study, we have utilized the rat model system to identify a potential mechanism for the drug interaction between these two substances. In both humans and rodents, mitochondrial ALDH2 is the major metabolizing enzyme of ACH (Svanas and Weiner, 1985; Mitchell and Petersen, 1991; Tsai and Senior, 1991; Rashkovetsky et al., 1994) as demonstrated by the fact that the inhibition of ALDH by compounds such as disulfiram and the inactivation of *aldh2* gene by targeted gene disruption in mice increase blood ACH levels after ethanol exposure (Deitrich et al., 1976; He et al., 2001; Kinoshita et al., 2002; Isse et al., 2005). A minor difference between humans and rats in ACH metabolism is that unlike in humans, rat cytosolic ALDH1 may also contribute to ACH metabolism (Klyosov et al., 1996). Since our previous study demonstrated that ALDH2 activity was significantly inhibited in rats treated twice with MDMA (10 mg/kg) 24 h apart (Moon et al., 2008), we used the same dosing regimen in our current study.

Our current results demonstrate that MDMA exposure not only suppresses mitochondrial ALDH2 activity, as previously shown, but cytosolic ALDH1 as well. In addition, the activities of ALDH1 and ALDH2 were inhibited when rats were treated with both MDMA and ethanol. These results validate the use of the rat model to characterize the effects of MDMA-mediated ALDH inhibition on ACH metabolism. Furthermore, the observed suppression of ALDH1 and ALDH2 activities in MDMA and MDMA+ethanol-exposed rats clearly indicates a potential for decreased metabolic elimination of ACH in MDMA+ethanol-treated rats.

Our ACH pharmacokinetic study reveals a strong trend towards increased blood ACH exposure in MDMA+ethanol-treated rats compared to ethanol only-treated rats. Both the peak concentration ($C_{\max}$) and the area under the curve (AUC_{0-t}) were higher in the MDMA+ethanol group compared to the vehicle control group. These results are supportive of our hypothesis, whereby the MDMA mediated inhibition of liver mitochondrial ALDH2 and cytosolic ALDH1 activities is translated into decreased ACH metabolism and higher blood ACH levels. Rats treated with MDMA+ethanol displayed greater variability in the time to reach peak ACH concentration ($T_{\max}$; 0.25 h–7 h) as compared to rats treated with ethanol alone ($T_{\max}$; 0.25 h–0.75 h). The greater variability in the time to reach peak concentration within the ethanol+MDMA group, relative to within the ethanol only group, was likely due to the differential level of ALDH2 and ALDH1 inhibition by MDMA among the individual rats in the ethanol+MDMA group. Importantly, the mean $T_{\max}$ for ACH was delayed (mean $T_{\max}$ 2.7 h in MDMA+ethanol treated rats versus 0.38 h in rats treated with ethanol alone). The delayed $T_{\max}$ provides further evidence for a reduced rate of metabolism of ACH in MDMA+ethanol treated rats as a result of MDMA mediated inhibition of liver mitochondrial ALDH2 and cytosolic ALDH1 activities.

The *p* value for the noted differences in $C_{\max}$ ($p=0.05$) and AUC_{0-t} ($p=0.1$) between the MDMA+ethanol and the ethanol-only groups are either at or above the traditionally accepted value of 0.05. It is evident that the observed high variability of the blood ACH measurements contributed to the lack of statistical significance. Furthermore, since it was not within the scope of this study to investigate the kinetics of ALDH1 and ALDH2 inhibition by MDMA, the duration in between the last MDMA administration and ethanol dosing was not optimized for maximal inhibition of ALDH1 and ALDH2. The use of a larger sample size as well as the optimization of the duration between the last MDMA treatment and ethanol dosing would most likely result in statistically significant increases in blood ACH levels in the group treated with both MDMA and ethanol.

The organotoxicity of MDMA by itself (Andreu et al., 1998; Beitia et al., 2000; Ben-Abraham et al., 2003; Ellis et al., 1996; Fidler et al., 1996; Garbino et al., 2001) and chronic ethanol intake (Tuma et al., 1996; Yokoyama et al., 1995), has been well documented. However, up to 70% of MDMA users co-abuse ethanol and evidence indicates that the combination of MDMA and ethanol further increases the risk of cell and organ damage. For example, Izco et al. (2007), showed that ethanol enhanced the MDMA-mediated long term serotonergic neurotoxicity in rats. It is of note that in this study, when an inhibitor of ALDH (cyanamide) was co-administered with ethanol, a further increase in MDMA-induced neuronal damage and neurotoxicity of the cortex were observed, concurrent with elevated ACH concentrations (Izco et al., 2007). Ethanol also increased the suppression of immune function by MDMA (Pacifici et al., 2001). In addition, MDMA and ethanol interaction has been shown for psychomotor performance and pharmacokinetics of ethanol (Hernandez-Lopez et al., 2002). Although the drug interaction between MDMA and ethanol may have several underlying mechanisms, our results indicate that the elevation of ACH levels caused by the inhibition of ALDH1 and ALDH2 activities can represent one potential mechanism. Increased cell and organ damage could result directly from the higher ACH level, or

alternatively, the inhibition of ALDH2 by MDMA may lead to the accumulation of toxic lipid peroxidation products such as 4-hydroxynonenal and malondialdehyde, both of which are substrates of ALDH2 (Hartley et al., 1995). Our findings represent a significant advance in the understanding of the mechanism of drug interaction between ethanol and MDMA and our proposed model for drug interaction may provide insight into the mechanism of drug interactions of other chemicals.

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Fig. 1. Inhibition of cytosolic ALDH1 and mitochondrial ALDH2 activities in MDMA-exposed rat livers. Rats were treated with either (1A) MDMA (10 mg/kg, twice) or water or (1B) MDMA+Ethanol (MDMA 10 mg/kg, twice and after the second dose, ethanol 3 g/kg or water). Liver ALDH1 and ALDH2 activities were measured and graphed. Different from vehicle: ** $p < 0.01$, *** $p < 0.001$).

Fig. 2. Selectivity, suitability and linearity of the bioanalytical method for quantitation of blood ACH. (2A) Overlaid HPLC chromatograms of (a) rat control blood (RCB); (b) RCB spiked with 500 μM of derivatized ACH (ACH-DNPCHO); (c) RCB spiked with ACH (500 μM) and (d) RCP spiked with the internal standard (PRO-DNPCHO; 500 μM). Peaks corresponding to derivatized ACH and the internal standard eluted at 5.0 and 7.5 min, respectively. (2B) Calibration curves for quantitation of ACH in blood generated on three different days show low inter-day variability (mean $\pm$ SD). Shown in the inset are mean $\pm$ SD values for the constants in the regression equation used to calculate ACH concentration.

Fig. 3.

Trend towards an increase in blood ACH levels upon treatment with MDMA+Ethanol (MDMA, 10 mg/kg twice and after the second dose, ethanol, 3 g/kg) or Ethanol (water twice and after the second dose, ethanol, 3 g/kg) as shown by (3A) time course of blood ACH levels, (3B) peak ACH concentration achieved (C_{max}) and (3C) area under the concentration versus time curve (AUC_{0-7}). Results are shown as the mean $\pm$ SEM (n=4).

Fig. 4.

Liver damage in rats following co-administration of MDMA and ethanol as indicated by elevated plasma levels of ALT (4A) and AST (4B). Rats were treated with either MDMA +Ethanol (MDMA, 10mg/kg twice and after the second dose, ethanol, 3 g/kg) or Ethanol (water, 4 mL/kg twice and after the second dose, ethanol, 3 g/kg) or Vehicle (water, 4 mL/kg twice and after the second dose, saline, 12 mL/kg). Different from vehicle: ** $p < 0.01$, *** $p < 0.001$. Different from ethanol treated $\Psi = p < 0.01$.

Table 1

Accuracy and precision of blood acetaldehyde quantitation

QC level	Spiked concentration (μM)	Measured concentration (μM)		
		Mean	SD	Precision (RSD)
LQC	20.0	22.0	0.44	2.02
MQC	125	119	5.91	4.96
HQC	250	251	6.31	2.52

LQC, low quality control; MQC, medium quality control; HQC, high quality control;

Triplicates at each concentration;

RSD, relative standard deviation= $(SD \times 100 / \text{Mean})$;%DEV, % deviation= $(\text{Spiked concentration} - \text{Measured concentration} \times 100 / \text{Spiked concentration})$