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Brief communication

The pharmacokinetic profile of synthetic cathinones in a pregnancy model

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ABSTRACT

In recent years, the abuse of synthetic cathinones or 'bath salts' has become a major public health concern. Although these compounds were initially sold legally and labeled "not for human consumption", the 'bath salts' are psychostimulants, with similar structures and pharmacologic mechanisms to cocaine, the amphetamines, and 3,4 methylendioxymethamphetamine (MDMA, Molly, or Ecstasy). The reported use of these substances by women of child-bearing age highlights the necessity of studies seeking to delineate risks of prenatal exposure. Three popular drugs of this type are methylone, mephedrone, and 3, 4-methylenedioxypyrovalerone (MDPV). Unfortunately, there is currently no information available on the teratogenicity of these compounds, or of the extent to which they cross the placenta. As such, the purpose of this study was to examine the pharmacokinetic profile of the 'bath salts' in a pregnancy model. Pregnant mice (E17.5 gestation) were injected intraperitoneally with a cocktail of 5 mg/kg methylone, 10 mg/kg mephedrone, and 3 mg/kg (MDPV) dissolved in sterile saline. Maternal brain, maternal plasma, placenta, and fetal brain were collected at 30 s, 1 min, 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 8 h following injection. Methylone, mephedrone, and MDPV were extracted from tissue by solid phase extraction, and concentrations were determined using a previously validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Interestingly, all 3 cathinones reached measurable concentrations in the placenta, as well as the fetal brain; in fact, for MDPV, the maximal concentration (Cmax) was highest in fetal brain, while mephedrone's highest Cmax value was achieved in placenta. Additionally, the total drug exposure for all 3 compounds (as represented by area under the curve, AUC) was higher in fetal matrices (placenta and fetal brain) than in maternal matrices (maternal brain and plasma), and the half-lives for the drugs were longer. Given the extensive presence of methylone, mephedrone, and MDPV in the fetal brain following prenatal exposure, fetal risk is definitely a concern. As there are currently no prenatal studies available on the teratogenicity of these agents, pregnant patients should be informed about the potential risks that these substances may have.

1. Introduction

Synthetic derivatives of the psychostimulant cathinone first appeared in Israel in 2004. These substances were sold legally, marketed as 'bath salts' or 'plant food', with the label "not for human consumption". However, abuse of these drugs quickly became a public health threat. In 2012, just 8 years after their discovery, over 20 different combinations of synthetic cathinones had been identified (Mohr et al., 2012). In a 2013 review of 43 synthetic cathinone toxicity cases, three of the most common synthetic cathinones confirmed were 3,4-methylenedioxymethcathinone (methylone), 4-methyl-methcathinone, (mephedrone), and 3,4 methylenedioxypyrovalerone (MDPV) (Mohr et al., 2012; Marinetti and Antonides, 2013). Despite the evolving drug market, the 2015 National Forensic Laboratory Information System and the European Monitoring Centre for Drugs and Drug Addiction reported methylone and MDPV rank among the top 15 most commonly abused cathinone derivatives in the US, while mephedrone and MDPV were among the top in the UK (U.S. Department of Justice/Drug Enforcement Administration Office of Diversion Control, 2015; European Monitoring Centre for Drugs and Drug Addiction, 2015).

The synthetic cathinones have similar pharmacology to the amphetamines, cocaine, and 3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy" or "Molly") leading to a propensity for abuse and addiction. Users report desirable effects including euphoria, increased alertness, and sexual arousal (Mohr et al., 2012; German et al., 2014; Dybdal-Hargreaves et al., 2013; Nelson et al., 2014; Zawilska and Wojcieszak, 2013). However, the drugs may also produce dangerous health effects including hypertension, respiratory distress, violent

Abbreviations: MDPV, 3, 4-methylenedioxypyrovalerone; LC-MS/MS, liquid chromatography-tandem mass spectrometry; C_{max}, maximal concentration; T_{max}, time to maximal concentration; $T_{1/2}$, elimination half-life; AUC, area under the curve; Cl, clearance

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behavior, seizures, suicidality, and even death (Karila et al., 2015; Prosser and Nelson, 2012; DEA, 2011). Previous reports indicate an onset of action of these cathinones within 30–45 min and duration of several hours to days (Mohr et al., 2012). While users may take the drugs through ingestion, injection, smoking, and rectal administration, the most common route of administration is insufflation (snorting) (Khan et al., 2013; Fideral Register, 2011). According to DEA statistics, the dose of synthetic cathinones varies widely from 25 mg to 250 mg (up to 5 g per session, accounting for repeated dosing), and the substances are commonly administered in combination with each other as a "cocktail", or in combination with other neurologically acting agents like cocaine, the amphetamines, or MDMA (Angoa-Perez et al., 2017; Araujo et al., 2015; DEA, 2011; Fideral Register, 2011).

Another alarming trend associated with the 'bath salts' is the abuse of these compounds in women of child-bearing age. Even after the classification of many of the synthetic cathinones as Schedule I substances by the DEA, the lack of international control laws has allowed abuse to continue, with the internet serving as a central marketplace for these compounds (Dybdal-Hargreaves et al., 2013; Karila et al., 2015). Additionally, despite the numerous news reports of the dangers of these drugs, the use of 'bath salts' by middle and high school students has remained steady since 2013, with approximately 0.9% of 8th graders reporting past year use and 0.8% of 10th and 12th graders reporting past year use (Johnston et al., 2017). Although these data do not stratify the statistics by gender, it is likely that a number of women of child-bearing age are taking these drugs. Given the addictive properties of the 'bath salts', women who initiate use will likely have difficulty stopping use, even in the event of pregnancy. In fact, there have been reports of the use of synthetic cathinones by pregnant women (Gray and Holland, 2014; Pichini et al., 2014). As such, it is important to understand the potential risks these compounds might have on the developing fetus, and the first logical step is an investigation of the extent to which these compounds cross the placenta and enter the fetal brain. Given the relatively small molecular weight and lipophilicity of methylone, mephedrone, and MDPV, we hypothesize that the drugs will cross the placental barrier and enter the fetal brain. In this study, we examine the placental transfer of the 'bath salts' and investigate the pharmacokinetics of these compounds when administered in combination in a pregnancy model using a previously validated high pressure liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (Peters et al., 2016).

2. Materials and methods

2.1. Materials

The reference standards for 3,4-methylenedioxymethcathinione HCl (methylone), 4-methyl-methcathinone HCl (mephedrone), and 3,4-methylenedioxypyrovalerone HCl (MDPV) (1 mg/mL) in methanol were obtained from Cerilliant (Round Rock, TX, USA). The deuterium-labeled standards used were 3,4-methylenedioxymethcathinone-D_3 HCl (D_3-methylone), 4-methyl-methcathinone-D_3 HCl (D_3-methedrone), and 3,4-methylenedioxypyrovalerone-D_8 HCl (D_8-MDPV), and at concentration of 0.1 mg/mL in methanol. These were also purchased from Cerilliant. LC-MS grade methanol was procured from Burdick & Jackson (Muskegon, MI, USA) and the glacial acetic acid from Amresco (Solon, OH, USA). Methylene chloride, 2-propanol, ammonium hydroxide, and sodium fluoride were purchased from Fisher Scientific (Fairlawn, NJ, USA), while the ammonium formate, MDPV HCl, mephedrone HCl, and methylone HCl (powder) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals, tissue collection, sample preparation, and analysis

All animals were housed in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AALAC) where animals received food and water *ad libitum*. The East Tennessee State University Committee on Animal Care approved all implemented procedures using the National Institutes of Health (NIH) guidelines. Briefly, pregnant Swiss-Webster mouse dams were injected intraperitoneally with a cocktail containing 3 mg/kg MDPV, 5 mg/kg methylone, and 10 mg/kg mephedrone in sterile saline at E17.5 gestation. Drug concentrations were chosen based on binging doses reported in DEA statistics and those utilized in previous rodent studies (Kamata et al., 2006; Robinson et al., 2012; Colon-Perez et al., 2016; DeLarge et al., 2017; Hicks et al., 2017). Mice were sacrificed *via* exsanguination followed by cervical dislocation, and tissues, including maternal brain, maternal plasma, placenta, and fetal brain, were collected at the following times post-injection: 30 s, 1 min, 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 8 h. For plasma collection, maternal blood samples were collected in heparinized tubes, centrifuged at 12, 000 rpm for 3 min, and the supernatants (plasma) were saved. All tissues were flash-frozen in liquid nitrogen and stored at −70 °C until analysis (n = 5–6 per time point). The concentration of methylone, mephedrone, and MDPV in the various tissue samples was determined with liquid chromatography-tandem mass spectrometry using the previously validated method (Peters et al., 2016). Briefly, brain and placenta were homogenized in 0.1 M phosphate buffer with 1% NaF [volume of buffer added (mL) = brain weight (g) × 5], and homogenate or plasma samples were spiked with internal standards at the following concentrations: 1 ng/mL D8-MDPV, 2 ng/mL D3-mephedrone, and 10 ng/mL D3-methylone. All tissue homogenate samples (brain or placenta) were vortexed and centrifuged (5 min; 1500 rpm), and then supernatants or plasma were collected and subjected to solid-phase extraction using Clean Screen DAU extraction columns (UCT, Bristol, PA, USA) as detailed in Peters et al., 2016. Separation was achieved using an Atlantis HILIC Silica column (2.1 × 100 mm, 3 µm particle size) (Waters; Milford, MA, USA) and a mobile phase, consisting of acetonitrile (B) and 5 mM ammonium formate, pH 3.0 adjusted with formic acid (A), at a flow rate of 0.2500 mL/min. A linear gradient of 90% B to 70% B over 6 min facilitated separation. Mass spectrometric detection utilized a Shimadzu IT-TOF system with a positive electrospray (+ ESI) source. Data acquisition utilized the following precursor ions: MDPV at 276.14 *m/z*, D8-MDPV at 284.17 *m/z*, mephedrone at 178.15 *m/z*, D3-mephedrone at 181.14 *m/z*, methylone at 208.12 *m/z*, and D3-methylone at 211.14 *m/z*. This method allows for lower limits of detection (LLODs) of 1 ng/mL for MDPV and 5 ng/mL for both mephedrone and methylone, with lower limits of quantification (LLOQs) of 5 ng/mL for MDPV and 10 ng/mL for mephedrone and methylone. In addition, this validated method has desirable intra- and inter-day precision and accuracy (%RSD and % error were ≤ 15% for every calibration point) (Peters et al., 2016). Furthermore, extraction efficiency exceeded 80% at both low (10 ng/mL) and high (100 ng/mL) calibration concentrations (Peters et al., 2016).

2.3. Data analysis and statistics

Phoenix 64/WinNonLin software (Certara USA, Inc., Princeton, NJ) was used to calculate pharmacokinetic parameters using calculated concentrations corrected for tissue weight obtained from LC-MS data from all the matrices, including maternal plasma, maternal brain, fetal brain, and placenta. Calculated parameters for each matrix included maximal concentration (C_{max}, ng/g (tissue) or ng/mL (plasma)), area under the curve (AUC, ng x min/g (tissue) or ng x min/mL (plasma)), clearance (Cl, g/min (tissue) or mL/min (plasma)), time to maximal concentration (Tmax, min), and elimination half-life ($T_{1/2}$, min). An *a priori* significance level was set at < 0.05. The C_{max} and AUC standard errors were compared by an ANOVA, followed by Tukey's post-hoc analysis using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).