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Brief communication

The pharmacokinetic profile of methylphenidate use in pregnancy: A study in mice

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ABSTRACT

The purpose of this study was to quantify the amounts of the d- and l-*threo* enantiomers of methylphenidate in maternal plasma, placenta, and maternal and fetal brain tissue following prenatal exposure and to establish a pharmacokinetic profile for MPH during pregnancy. Due to increasing rates of use of methylphenidate amongst females of childbearing age, it is important to understand the extent of exposure to the fetus. Briefly, pregnant mice were injected with 5 mg/kg methylphenidate at 18 days gestation, and tissue was collected 1, 5, 10, 30, 60, and 120 min following injection. Methylphenidate was extracted from tissue via solid phase extraction, and concentrations were determined using liquid chromatography–mass spectrometry (LC–MS). Because methylphenidate is administered as a racemic mixture of d- and l-*threo* enantiomers and the d-enantiomer is more pharmacologically active, the enantiomers were quantified separately. Interestingly, we found that methylphenidate does cross the placenta and enter the fetal brain. Although the highest concentrations were achieved in maternal brain, the concentrations of d- and l-methylphenidate in fetal brain were comparable to those of maternal plasma. Additionally, both d- and l-methylphenidate had longer half-lives in placenta than in maternal or fetal brain. Interestingly, there was a bimodal peak in maternal brain concentrations, at 5 min and again at 60 min, which was not observed in maternal plasma. Finally, the total exposure (as represented by area under the curve) was statistically significantly higher for the active d-enantiomer than the l-enantiomer in maternal brain tissue. In conclusion, methylphenidate crosses the placenta and reaches measurable concentrations in fetal brain. Although long-term behavioral and developmental studies are needed to determine specific outcomes of prenatal exposure, discussion with pregnant patients on the potential risks of methylphenidate exposure is warranted.

1. Introduction

Methylphenidate (Ritalin®, Concerta®, Metadate™, Methylin™, Daytrana®, Focalin®) is a psychostimulant currently used in the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy. ADHD affects an estimated 11% of school-aged children (Perou et al., 2013). It is conservatively estimated that approximately 5% of adults suffer from symptomatic ADHD, not including those individuals who were diagnosed as an adolescent (de Graaf et al., 2008). Methylphenidate (MPH) was found to be the most prescribed medication to adolescents ages 12–17, with a reported 46% increase in number of prescriptions from 2002 to 2010 (Chai et al., 2012).

Prescription drug misuse and abuse is a growing problem affecting our nation, both in adult and adolescent populations. Of 12th graders who reported using a prescription stimulant drug in the absence of a physician's order, 59.6% indicated they received the medication for free from a friend or relative (Johnston et al., 2014). With a combination of rising rates of patients continuing stimulant therapy into childbearing age, as well as rising rates of diversion for non-medicinal use, it is important to understand the pharmacokinetic profile of MPH in a maternal-fetal model.

Previous studies have produced various data regarding the impact of prenatal exposure to MPH without actual quantification in tissues. A meta-analysis of studies examining MPH use in pregnancy and fetal malformation rates failed to show any statistical significance, although they report lacking trials with substantial power for analysis (Bolea-Alamanac et al., 2013). However, animal studies suggest that prenatal MPH exposure may result in behavioral abnormalities. Lloyd and colleagues demonstrated that when both male and female mice are exposed to pre- and postnatal MPH, they show greater impulsivity, compulsivity, and motivation for reward when compared to a saline control group. (Lloyd et al., 2013).

The extent to which MPH crosses the placenta and reaches fetal brain tissue is not clearly defined. As with numerous other prescription medications, it is reasonable to suspect altered kinetic properties during pregnancy. Therefore, it is crucial for the foundation and understanding of future behavioral studies to establish the kinetic profile of methylphenidate when used during pregnancy. Of the enantiomers of MPH, the *threo* enantiomers are selectively used in pharmacotherapy, as

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they are the most potent (Ferris and Tang, 1979). Although current formulations of MPH include both d-*threo* and l-*threo* enantiomers, the d-enantiomer is believed to be responsible for mediating the pharmacologic effects of MPH activity in vitro (Markowitz and Patrick, 2008). Therefore, the purpose of this study is to quantify the amounts of the d- and l-*threo* enantiomers of MPH in placenta, maternal plasma, and maternal and fetal brain tissue following prenatal exposure.

2. Materials and methods

2.1. Materials

The methylphenidate HCl reference standard (1 mg/mL in methanol, 99.5% purity) was obtained from Cerilliant (Round Rock, TX, USA). LC–MS grade methanol was procured from Burdick & Jackson (Muskegon, MI, USA). The glacial acetic acid was obtained from Amresco (Solon, OH, USA). Triethylammonium acetate buffer (TEAA) and methylphenidate HCl (powder) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methylene chloride, 2-propanol, ammonium hydroxide, and sodium fluoride were obtained from Fisher Scientific (Fairlawn, NJ, USA).

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

All animals were housed in an Association for the Assessment and Accreditation of Laboratory Animal Care (AALAC) accredited facility with food and water provided ad libitum. All procedures were carried out according to NIH guidelines and were approved by the East Tennessee State University Committee on Animal Care. Pregnant Swiss-Webster mouse dams were injected with 5 mg/kg MPH into the peritoneal space at 18 days gestation, and tissue was collected at 1, 5, 10, 30, 60, and 120 min following injection (n = 5–6 per time point). Animals were sacrificed via exsanguination under deep anesthesia with 5% isoflurane, as blood was collected from the heart using a heparin-coated syringe. Blood samples were collected in heparinized tubes and centrifuged at 12,000 rpm for 3 min. The supernatants (plasma) were removed, flash-frozen in liquid nitrogen, and stored at −70 °C in heparinized tubes until analysis. Additionally, whole fetal and maternal brains and placenta were collected, flash-frozen, and stored at −70 °C. Because MPH is administered as a racemic mixture of d- and l-*threo* enantiomers and the d-enantiomer is more pharmacologically active, the enantiomers were quantified separately using liquid chromatography–tandem mass spectrometry. Chiral separation was achieved using a Chirobiotic V column with a methanol mobile phase containing 0.375 mM triethylammonium acetate and flow rate of 1.0 mL/min. Mass spectrometric detection utilized a Shimadzu IT-TOF system with the APCI source running in positive mode. The precursor ion was set at a mass to charge ratio of 234.15, as the molecular weight of MPH is 233.15, plus the addition of a proton. This method allows for a lower limit of detection (LLOD) of 0.5 ng/mL and a lower limit of quantification (LLOQ) of 7.5 ng/mL, with desirable intra- and inter-day precision and accuracy (%RSD and % error were <15% for every calibration point) (Combs et al., 2013). MPH was extracted from tissue via solid phase extraction using Clean Screen DAU Columns and quantified utilizing a previously validated method which allows for 72–75% recovery of both d- and l-*threo* MPH at both low (10 ng/mL) and high (100 ng/mL) calibration concentrations (Combs et al., 2013).

2.3. Data analyses and statistics

Pharmacokinetic data from all matrices including maternal brain, maternal plasma, placenta, and fetal brain were subjected to non-compartmental analyses using the Phoenix 64/WinNonLin software (Certara USA, Inc., Princeton, NJ). Parameters calculated for each matrix included area under the curve (AUC, ng × min/g (tissue) or ng × min/mL (plasma)), maximal concentration (C_{max}, ng/g (tissue) or ng/mL (plasma)), time to maximal concentration (T_{max}, min), clearance (Cl, g/min (tissue) or mL/min (plasma)), elimination half-life ($T_{1/2}$, min), and volume of distribution (V_d, g (tissue) or mL (plasma)). For AUC and C_{max}, standard errors were reported and thus, these values were compared via an ANOVA followed by Tukey's post-hoc analyses. An a priori level of significance was assigned at 0.05. Since the other parameters were calculated based on a theoretical model created by the software, no standard errors were reported and statistical analyses were not possible.

3. Results

Our results demonstrate that MPH does cross the placenta and enter the fetal brain in measurable concentrations (Fig. 1). Concentrations detected in fetal brain were comparable to those in maternal plasma, while maternal brain concentrations were the highest observed. Interestingly, a bimodal peak in the concentration of d-MPH is observed in maternal brain, with peak concentrations occurring at both 5 min and 60 min. This phenomenon was not observed in any other matrices or in a non-pregnant female model (Fig. 1) (Bentley et al., 2015; Combs et al., 2013).

Pharmacokinetic data was subjected to non-compartmental analysis using the Phoenix 64/WinNonLin software. These data are presented in Table 1. Notably, there were no statistically significant differences between the C_{max} of d- or l-MPH in any of the matrices; however, there were some notable differences in the C_{max} between matrices. The highest concentrations were achieved in maternal brain (Table 1); for d-MPH, the C_{max} for maternal brain was significantly higher than in plasma and fetal brain and for l-MPH, the C_{max} for maternal brain was significantly greater than the C_{max} for all other matrices (plasma, fetal brain, and placenta) ($p < 0.05$, ANOVA followed by Tukey's post-hoc analyses, n = 5 for each time point in each matrix).

Although, as noted above, no statistically significant differences were seen between the C_{max} of the d- and l-enantiomers of MPH, total brain exposure (as represented by AUC) was statistically significantly higher for d-MPH than l-MPH in maternal brain tissue (see Table 1; $p < 0.05$, ANOVA followed by Tukey's post-hoc analyses, n = 5). Furthermore, total exposure is likewise statistically significantly higher in maternal brain for d-MPH than all other matrices, and for l-MPH, the AUC for maternal brain was greater than for plasma and fetal brain ($p < 0.05$, ANOVA followed by Tukey's post-hoc analyses, n = 5).

Interestingly, T_{max} for d-MPH occurred at 60 min, whereas d-MPH achieves C_{max} at 10 min in a non-pregnant female brain (Bentley et al., 2015; Combs et al., 2013). Furthermore, l-MPH did follow the non-pregnant model and achieved T_{max} at 10 min (Table 1).

When examining fetal brain parameters, d-MPH demonstrates a much longer half-life when compared to l-MPH, which could be of consequence considering d-MPH is the established pharmacologically active enantiomer (Markowitz and Patrick, 2008).

4. Discussion

Although long-term behavioral and developmental studies are needed to determine specific outcomes of prenatal exposure, discussion with pregnant patients on the potential risks of methylphenidate exposure is warranted. As described above, of primary concern are two variations in the pharmacokinetic profile of methylphenidate when used during pregnancy: the bimodal peak observed in maternal brain and subsequently delayed T_{max} and the decrease in the maternal brain C_{max} and AUC. In non-pregnant females, maximal concentrations of MPH in brain tissue is reported to be 1018 ± 169.3 ng/g (d-MPH) and 914.4 ± 160.4 ng/g (l-MPH) (Bentley et al., 2015). However, in our study, we observed an almost 2 fold decrease in C_{max} levels (508.7 ± 64.12 ng/g (d-MPH) and 573.9 ± 27.22 ng/g (l-MPH)), which, when paired with a significantly delayed T_{max}, is of potential concern, as there is now opportunity for altered therapeutic response to MPH therapy.