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Transdermal and Oral *d*-Methylphenidate–Ethanol Interactions in C57BL/6J Mice: Transesterification to Ethylphenidate and Elevation of *d*-Methylphenidate Concentrations

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Abstract

We tested the hypothesis that C57BL/6J mice will model human metabolic interactions between *dl*-methylphenidate (MPH) and ethanol, placing an emphasis on the MPH transdermal system (MTS). Specifically, we asked: (1) will ethanol increase *d*-MPH biological concentrations, (2) will MTS facilitate the systemic bioavailability of *l*-MPH, and (3) will *l*-MPH enantioselectively interact with ethanol to yield *l*-ethylphenidate (*l*-EPH)? Mice were dosed with MTS (¼ of a 12.5 cm² patch on shaved skin) or a comparable oral *dl*-MPH dose (7.5 mg/kg), with or without ethanol (3.0 g/kg), and then placed in metabolic cages for 3 h. MPH and EPH isomer concentrations in blood, brain, and urine were analyzed by gas chromatographic–mass spectrometry monitoring of N-(*S*)-prolylpiperidyl fragments. As in humans, MTS greatly facilitated the absorption of *l*-MPH in this mouse strain. Similarly, ethanol led to the enantioselective formation of *l*-EPH and to an elevation in *d*-MPH concentrations with both MTS and oral MPH. Although only guarded comparisons between MTS and oral MPH can be made due to route-dependent drug absorption rate differences, MTS was associated with significant MPH–ethanol interactions. Ethanol-mediated increases in circulating concentrations of *d*-MPH carry toxicological and abuse liability implications should this animal model hold for ethanol-consuming attention-deficit hyperactivity disorder patients or coabusers.

Keywords

methylphenidate; ethanol; ethylphenidate; transesterification; carboxylesterase; transdermal; drug interactions; metabolism; phase I enzyme; absorption

INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is the most commonly diagnosed childhood neuropsychiatric condition. The stimulant drug *dl*-methylphenidate (*dl*-MPH; Fig. 1,) has remained a first-line pharmacotherapeutic agent to treat ADHD since the 1950s.^{1–3} Further, the persistence of ADHD into adulthood is increasingly recognized.^{1,4–8} In the adult ADHD population, *dl*-MPH is also the most widely prescribed psychotherapeutic agent.⁷ As a consequence, this controlled substance has become more widely available for abuse and diversion,^{9–11} especially among high school¹² and college students.^{13,14}

Appropriate drug therapy for this older ADHD population requires a special consideration of lifestyle and lifespan comorbidities⁷ such as hypertension.^{13,15} Optimized adult ADHD pharmacotherapy may be complicated by alcohol consumption, alcohol use disorder (AUD), or other substance use disorder (SUD). Both AUD and SUD are over-represented in adult ADHD,^{4,16,17} especially in women.¹⁸ Not surprisingly, given the clinical nature of adult ADHD,¹⁹ and the susceptible population for which MPH is prescribed,⁴ *dl*-MPH-related emergency department visits have totaled in the thousands each year, for example, 8000 in 2004.¹¹ Moreover, emergency room presentations for incidents involving alcohol in combination with drugs have risen 63% for persons aged 18–19 years, and have increased 100% for persons age 45–54 years.²⁰ Poison center data reveal how extensive *dl*-MPH abuse has become.^{11,21–24} In a drug diversion context, ADHD stimulants are often coabused with ethanol, for example, in 53% of those surveyed,²⁵ and *dl*-MPH in particular has been reported to be coabused with ethanol in 92% of those surveyed.¹⁰ Accordingly, prescribing or diverting psychostimulants has generated special concern regarding concomitant ethanol use or abuse.^{26–28}

These statistics are consistent with MPH being classified as a Drug Enforcement Agency (DEA) schedule II controlled substance,²⁹ that is, a medication of very high abuse potential.^{30–32} Accordingly, the prevalence and inherent danger of concomitant *dl*-MPH and ethanol warrant research into the pharmacology of this drug combination.

Coadministration of ethanol and *dl*-MPH orally to humans^{33,34} results in a drug–drug interaction, wherein the methyl ester of MPH is transesterified to yield ethylphenidate (EPH; Fig. 2)³³ in addition to being hydrolyzed to the inactive³⁵ metabolite ritalinic acid.³⁶ Both EPH and ritalinic acid formation appear to be primarily mediated by the actions of carboxylesterase 1 (CES1),^{37–39} which exhibits *l*-MPH substrate enantioselectivity in both the transesterification and hydrolysis pathways.^{33,40}

The metabolic transesterification of *dl*-MPH with ethanol to yield EPH was first reported *in vitro* using rat microsomes.⁴¹ Subsequently, EPH was detected in human tissues from two fatal drug overdoses in which unknown amounts of MPH and ethanol were consumed.⁴² These findings prompted a normal human volunteer pilot study of the

dl-MPH–ethanol interaction⁴³ followed by a larger human study wherein enantiospecific methodology for plasma analysis was utilized.³² In this latter study, it was established that the *dl*-MPH–ethanol transesterification pathway primarily yields the *l*-enantiomer of EPH (Fig. 2).

Any *l*-MPH, or the metabolite *l*-EPH, which reaches the bloodstream, is unlikely to contribute directly to the pharmacodynamics of the *dl*-MPH–ethanol interaction in view of the findings that only the *d*-isomers of MPH and EPH possess potent effects on dopaminergic and noradrenergic systems.^{40,44} In spite of this, ethanol consumed with *dl*-MPH by normal human volunteers resulted in a significant elevation of maximum plasma *d*-MPH concentrations ($C_{\max}$) and overall *d*-MPH exposure.³³ Elevated plasma *d*-MPH concentrations increase the potential for adverse cardiovascular events, especially in ADHD patients with comorbid hypertension.^{45,15}

In addition to the influence of ethanol on *dl*-MPH pharmacokinetics, the above-mentioned normal human volunteers reported an increase in pleasurable effects when combining *dl*-MPH with ethanol.³² Such positive subjective effects may predispose individuals to greater abuse liability.^{26,27,47} The enhanced likability of this drug combination may be based on interactive effects of these two psychoactive drugs on excitatory neural systems as recently reported using a C57BL/J6 (C57) mouse behavioral model.⁴⁸ However, the increased likability may also pertain to the elevated rate at which *d*-MPH reaches the bloodstream.^{49–51} When *dl*-MPH is combined with ethanol, the time to maximum concentration ($T_{\max}$) occurs at the same time as *dl*-MPH dosed alone. However, the $C_{\max}$ is much higher at this time following concomitant *dl*-MPH and ethanol than when *dl*-MPH is dosed alone.³³

In 2006, the US Food and Drug Administration approved the first transdermal patch for the administration of *dl*-MPH (Daytrana®, Noven Therapeutics LLC, Miami, FL, USA). This *dl*-MPH transdermal delivery system (MTS) relies on a high load of *dl*-MPH free base incorporated within a uniform blend of acrylic polymers and silicone adhesives to drive drug absorption based on the drug concentration gradient without the need for permeability enhancers (for review, see Ref. 3). Using transdermal delivery, *dl*-MPH circumvents the extensive and highly enantioselective presystemic metabolism associated with oral dosing.^{33,52} Accordingly, MTS results in approximately 50 times higher plasma *l*-MPH concentrations than those that occur following oral dosing.⁵³

The present preclinical study investigated aspects of MTS and oral MPH absorption and disposition as influenced by the coadministration of ethanol. Special attention was given to the formation of *l*-EPH in view of the relatively large amount of *l*-MPH anticipated to reach the bloodstream following MTS delivery. The C57 mouse strain was chosen based on its frequent use as a reference strain in preclinical psychopharmacology of stimulant agents, including MPH and ethanol.^{40,44,48,54,55} Further, like human MPH metabolism, the C57 mouse has previously been reported to favor *l*-MPH as a substrate in the transesterification of ethanol to yield *l*-EPH after intraperitoneal (i.p.) dosing.⁴⁰

Blood, brain, and urine concentrations of *d*-MPH, *l*-MPH, *d*-EPH, and *l*-EPH were analyzed. The mean MTS dose delivered from a quarter of a 12.5 cm² patch (smallest of four sizes available) after a 3.25 h wear was calculated by quantifying the residual MPH content in the used patches. This dose was then administered for oral studies, while clearly recognizing the limitations of any direct drug dispositional comparisons of a bolus oral *dl*-MPH dose to that of the MTS in mice where prolonged release of drug occurs from the patch. A modification

of an established gas chromatographic (GC)–mass spectrometric (MS)–electron impact (EI)–selected ion monitoring (SIM) method was used for these enantiospecific determinations.^{44,56} MPH and EPH enantiomers were derivatized with (*S*)-*N*-trifluoroacetylpropyl chloride (TFP-Cl) to yield GC-resolvable diastereomers. Piperidine-deuterated *dl*-MPH was incorporated for analytical control.

MATERIALS AND METHODS

Materials

Ethanol used for oral animal studies was from AAPER Alcohol and Chemical Co. (Shelbyville, Kentucky; 95%). *dl*-MPH·HCl used for oral animal studies was from Sigma–Aldrich (St. Louis, Missouri; lot #118K1052) and the 12.5 cm² size MTS was from Shire US (Wayne, Pennsylvania; lot #2616811; smallest of four sizes available). Laboratory tape used to secure MTS or placebo was from VWR International (Radnor, Pennsylvania; white, 12.7 mm). *dl*-MPH·HCl in methanol (1 mg/mL calculated as free base; Cerilliant, Round Rock, Texas) was used as the analytical reference standard. The *dl*-EPH·HCl standard in ethanol (1 mg/mL calculated as free base) was synthesized in-house.⁴⁴ TFP-Cl in dichloromethane (1 M; Sigma-Aldrich, St. Louis, Missouri), sodium carbonate (Fischer Scientific, Fair Lawn, New Jersey), *n*-butyl chloride (Burdick & Jackson, Muskegon, Michigan), and acetonitrile (Sigma-Aldrich, St. Louis, Missouri) were also used. Piperidine-deuterated *dl*-MPH·HCl was synthesized in-house⁵⁷ and contained approximately 25% of the D₅-isotopolog for SIM and containing no D_{0–1}-MPH. It is noted that piperidine-deuterated D₉-MPH·HCl is commercially available (Cerilliant, Round Rock, Texas).

Animals

Male C57 mice aged 8–10 weeks (25–35 g) were obtained from Jackson Laboratories (Bar Harbor, Maine). They were individually housed in a temperature- and humidity-controlled colony room on a 12-h light/dark cycle (light: 07:00–19:00 h) with free access to food and water for at least 7 days before the start of any tests. All experiments were approved by and conducted within the guidelines of the Institutional Animal Care and Use Committee at the Medical University of South Carolina and followed the guidelines of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication no. 80-23, revised 1996). Animal studies were conducted in the Institute of Psychiatry at the Medical University of South Carolina.

Drug Administration

Mice were randomly placed into one of four test groups as shown in Table 1. All mice, regardless of group assignment, were treated similarly. This included the use of active (MTS) or placebo patches and delivery of ethanol or water by gastric intubation (gavage). To this end, mice were lightly anesthetized by placement into a chamber containing 5% isoflurane for 8–10 min. The mice were taken out and their hair was clipped along their abdomen and back, from shoulders to hips.

Immediately after hair clipping, one-fourth of a 12.5 cm² MPH transdermal patch, or a placebo patch (band-aid adhesive resembling the MTS), was applied to the lower left hip

area. The patch was secured by applying tape over the patch and around the mouse for one full loop to ensure a constant skin interface and to prevent the mice from disturbing the patch. Mice were returned to their home cage for 15 min to recover from anesthesia, then dosed by gavage, according to their assigned group, that is, 3.0 g/kg ethanol and 7.5 mg/kg (calculated as the free base) *dl*-MPH·HCl, or deionized water (dH₂O) using a standard volume of 0.02 mL/g body weight.

Sample Collection

Following gavage, mice were individually placed for 3 h in single metabolic chambers designed to separate urine from solid waste. Urine was collected and measured to the nearest microliter value. Mice were then deeply anesthetized using isoflurane. Venous blood was collected using cardiac puncture and stored in heparinized tubes. The brain was removed, separated along the sagittal line, weighed, and stored as two separate samples. Used patches were collected and later extracted for residual *dl*-MPH to calculate the dose delivered to the cutaneous site. Blank urine, blood, and brain used for calibration curves were collected from mice not exposed to any drugs. All matrices were kept on dry ice until stored in a -70°C freezer.

Sample Preparation

Urine—All urine samples were thawed immediately prior to analysis. Blank mouse urine (150 µL) was fortified over a range of concentrations with *dl*-MPH (0, 0.5, 0.75, 1.5, 3, 4.5 µg/mL) and *dl*-EPH (0, 0.15, 0.3, 0.45, 0.6, 0.9 µg/mL). These calibrators were run in parallel with experimental urine samples (150 µL). The internal standard, piperidine-deuterated *dl*-MPH, was dissolved in dH₂O such that 200 µL aliquots provided a concentration of 5 µg D₅-*dl*-MPH per 150 µL of urine. Sodium carbonate (50 µL, 1.2 M) was added to each urine sample to adjust the pH to approximately 9.5. Samples were extracted with *n*-butyl chloride/acetonitrile (2 mL, 4:1) by vortexing for approximately 0.5 min.

Blood—All blood samples were thawed immediately prior to analysis and used in the freezer-hemolyzed state in view of MPH having previously been reported to distribute nearly equally between serum and the red cell fraction.⁵⁸ Blank mouse blood (200 µL) was fortified over a range of concentrations with *dl*-MPH (0, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0 µg/mL) and *dl*-EPH (0, 0.01, 0.025, 0.05, 0.075, 0.1 µg/mL). These were run in parallel with experimental blood (200 : L) as calibrators. The internal standard, piperidine-deuterated *dl*-MPH, was dissolved in dH₂O such that 200 µL aliquots provided a concentration of 5 µg D₅-*dl*-MPH per 200 µL of blood. Sodium carbonate (2 mL, 1.2 M) was added to each blood sample to adjust the pH to approximately 9.5. Samples were extracted with *n*-butyl chloride/acetonitrile (2 mL, 4:1) by vortexing for approximately 0.5 min.

Brain—All brain samples were thawed immediately prior to analysis. Blank mouse brain (1/2, left hemisphere) was fortified over a range of concentrations with *dl*-MPH (0, 0.025, 0.05, 0.1, 0.2, 0.5, 1.0, 1.5 µg/g) and *dl*-EPH (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 µg/g) and run in parallel with experimental brains (left hemisphere). The internal standard, piperidine-deuterated *dl*-MPH, was dissolved in dH₂O such that 200 µL aliquots provided a

concentration of 5 μ g D₅-*dl*-MPH per 150 μ L of urine. The internal standard, piperidine-deuterated *dl*-MPH, was dissolved in dH₂O such that 200 μ L aliquots provided a concentration of 2.5 μ g D₅-*dl*-MPH per brain sample. Sodium carbonate (2 mL, 1.2 M) was added to each brain sample to adjust the pH to approximately 9.5. Samples were homogenized (Polytron PT1200) for 10 s, then 0.5 g sodium chloride was added and the samples were vortexed for 20 s. Samples were extracted with *n*-butyl chloride/acetonitrile (2 mL, 4:1) by vortexing for 30 s, then centrifuged at 1137 g for 7 min.

MPH Extraction from Used Patches—Used patches were analyzed for residual content of *dl*-MPH to establish the cutaneous dose delivered. Before being placed on the animal, whole patches (including the backing) were weighed and then cut into quarters. Each quarter was then weighed and used to determine what percent of the whole patch it represented.

In advance of analyzing the used patches for their *dl*-MPH content, a method for *dl*-MPH recovery from unused patches was developed. The unused patches were placed in scintillation vials with methanol (1 mL/calculated mg of *dl*-MPH) and sonicated over a range of times from 1 min to more than 20 min to determine the time required for near-complete extraction/recovery. An unused 12.5 cm² patch contains 27.5 mg of *dl*-MPH free base, whereby a quarter patch contains 6.875 mg of *dl*-MPH. For specific quarter patch cuttings, the exact *dl*-MPH content was calculated as follows: [(weight of quarter MTS/weight of whole MTS) $\times$ 27.5 = mg *dl*-MPH]. Accordingly, for the used study patches, residual *dl*-MPH was determined by taking a 100 μ L aliquot after 15 min of sonication and adding D₅-*dl*-MPH (10 μ g) as the internal standard.

Chiral Derivatization—The organic phases from all matrix extractions were transferred into 4 mL screw-cap silanized vials (Supelco) and the solvent was evaporated to dryness under nitrogen. TFP-Cl (1 M, 250 μ L) was added to each vial, sealed with Teflon[®] lined caps (Supelco) and heated at 58°C for 45 min. Aliquots of these samples were then transferred to silanized microvial inserts within auto sampler vials for GC–MS analysis.

Instrumental Analysis—All analyses were conducted using an Agilent Model 6890 GC-5973N MS with ChemStation and a modification of published methods.^{44,56} GC separations were carried out on a 30 m $\times$ 0.32 mm, 0.25 μ m film thickness, 5% phenylmethylpolysiloxane fused silica column (DB-5; J & W Scientific, Folsom, California). Pulsed-splitless injections (2 μ L) were used. The injector port was fit with a deactivated glass wool protected sleeve operated at 250°C and the helium carrier gas linear velocity was 50 cm/s. The GC was held at 70°C for 1.5 min, then ramped to 315°C at 10°C/min and held for 4 min for a total run time of 30 min. Detection was carried out by EI ionization (70 eV) and SIM, acquiring the N-TFP-piperidyl fragment ions of *d*-MPH, *l*-MPH, *d*-EPH, and *l*-EPH (m/z 277) with D₅-*d*-MPH and D₅-*l*-MPH monitored at m/z 282 (Fig. 3).

The lower limit of quantitation was based on a signal-to-noise ratio of at least 10 for all analytes. The signal-to-noise ratios for the lowest calibrators were at least 25. It is noted that calibrator concentrations are indicated as racemic (*dl*-) MPH and EPH, whereas analyte concentrations are reported for each enantiomer. All calibration plots provided a linearity of r^2 above 0.99.

Statistical Methods—A two-way analysis of variance followed by pairwise comparisons using the Student's *t*-test method was used in the analysis of all data. Samples were analyzed as independent samples and were assumed to have equal variances. Statistical analysis was conducted using SPSS 12.0 (SPSS Inc., Chicago, Illinois).

RESULTS

MPH Dose Delivered from MTS

The *dl*-MPH dose received by the MTS test animals over the 3.25 h wear time was determined by extracting the remaining *dl*-MPH from used patches and back calculating from the initial *dl*-MPH content in a quarter of a 12.5 cm² MTS. Sonication for 15 min was necessary to extract a mean no less than 95% of the labeled *dl*-MPH content of unused quarter patches and, accordingly, 15 min of sonication was used to calculate the 3.25 h dose delivered by difference. Shorter sonication times did not allow for complete *dl*-MPH extraction, whereas using later time points caused the MTS matrix to significantly degrade. This resulted in the extractant becoming cloudy, and GC–MS of such aliquots were found to foul the injector port and resulted in unacceptable chemical noise in the chromatograms. The mean *dl*-MPH dose delivered using the MTS over 3.25 h was 0.23 mg or 7.5 mg/kg. This dose was used for oral dosing (gastric intubation) in a parallel study of oral *dl*-MPH–ethanol interactions. The 7.5 mg/kg oral dose is likely to over-represent the bioavailable fraction of the mean *dl*-MPH MTS dose calculated as above in view of the likelihood of some residual *dl*-MPH remaining in the skin prior to circulatory absorption, for example, in humans dosed with MTS, residual *dl*-MPH results in a biphasic decay of the drug from plasma following patch removal.⁵⁹

Influence of Ethanol on Urinary Analytes

Transdermal DI-MPH—The total urinary elimination of *d*-MPH following the 3.25 h MTS wear time was significantly greater in the animals dosed with ethanol compared with those given dH₂O [Fig. 4a; *t* = 5.52, degree of freedom (df) = 10, *p* < 0.001]; rising from 0.48 to 1.39 μg to account for 0.04% of the total dose of *d*-MPH calculated to be cutaneously delivered. Further, in animals dosed with MTS, total urinary excretion of *l*-MPH was significantly increased, rising from 0.43 μg for animals dosed with dH₂O to 0.96 μg for animals dosed with ethanol (Fig. 5a; *t* = 4.07, df = 10, *p* < 0.01). There was not a significant difference between the urinary excretion of *d*-MPH compared with *l*-MPH in animals dosed with dH₂O; however, in animals dosed with ethanol, the urinary excretion of *d*-MPH was significantly greater than *l*-MPH (*t* = 2.13, df = 10, *p* < 0.05). Both enantiomers of MPH were detectable in animals gavaged with ethanol; however, *l*-MPH was enantioselectively formed with a significantly greater total elimination found relative to *d*-MPH (Fig. 6a; *t* = 5.74, df = 10, *p* < 0.001). The total urinary elimination of *l*-MPH was 0.2 μg, which represents 0.01% of the total dose of *l*-MPH calculated to be cutaneously delivered, whereas the total urinary elimination of *d*-MPH was 0.05 μg. The total urine volume excreted following ethanol treatment was significantly greater than following dH₂O treatment (*t* = 4.81, df = 10, *p* < 0.001) as consistent with the diuretic effect of ethanol.

Oral DI-MPH—The total urinary elimination of *d*-MPH following oral *dl*-MPH over the 3 h collection period was significantly greater in the animals dosed with ethanol compared with those given dH₂O (Fig. 4b; $t = 7.56$, $df = 10$, $p < 0.001$); rising from 0.09 to 0.46 μ g and accounting for 0.012% of the total dose of *d*-MPH gavaged. Further, in animals dosed with oral *dl*-MPH, the total urinary excretion of *l*-MPH was significantly increased, rising from 0.07 μ g for animals dosed with dH₂O to 0.31 μ g for animals dosed with ethanol (Fig. 5b; $t = 5.45$, $df = 10$, $p < 0.001$). There was not a significant difference between the urinary excretion of *d*-MPH compared with *l*-MPH in animals dosed with dH₂O; however, in animals dosed with ethanol, the urinary excretion of *d*-MPH was significantly greater than *l*-MPH ($t = 2.23$, $df = 10$, $p < 0.05$). Both isomers of EPH were detectable in animals gavaged with ethanol; however, *l*-EPH was enantioselectively formed with a significantly greater total urinary elimination of *l*-EPH relative to *d*-EPH (Fig. 6b; $t = 3.71$, $df = 10$, $p < 0.01$). The total urinary elimination of *l*-EPH was 0.02 μ g, whereas the total urinary elimination of *d*-EPH was 0.005 μ g. Again, the total urine volume excreted followed ethanol (a diuretic) treatment was significantly greater than following dH₂O treatment ($t = 4.39$, $df = 10$, $p < 0.001$).

Influence of Ethanol on Blood Analytes

Transdermal DI-MPH—The blood concentration of *d*-MPH after MTS dosing was significantly greater in animals dosed with ethanol compared with dH₂O; increasing 72% from 0.36 to 0.61 μ g/mL (Fig 4a; $t = 4.22$, $df = 10$, $p < 0.01$). Further, in animals dosed with MTS, concentrations of *l*-MPH significantly increased from 0.29 μ g/mL for animals dosed with dH₂O to 0.51 μ g/mL for animals dosed with ethanol (Fig. 5a; $t = 2.82$, $df = 10$, $p < 0.05$). There was no significant difference between the blood concentration of *d*-MPH and *l*-MPH in animals dosed with dH₂O or in animals dosed with ethanol. Both enantiomers of EPH were formed in animals gavaged with ethanol; however, *l*-EPH was enantioselectively formed with a significantly greater concentration found relative to *d*-EPH (Fig. 6a; $t = 2.99$, $df = 10$, $p < 0.05$). The blood concentration of *l*-EPH was 0.04 μ g/mL, whereas the concentration of *d*-EPH was 0.03 μ g/mL.

Oral DI-MPH—The blood concentration of *d*-MPH following oral *dl*-MPH was significantly greater in the animals dosed with ethanol compared with those given dH₂O; increasing 59% from 0.018 to 0.03 μ g/mL (Fig. 4b; $t = 2.95$, $df = 10$, $p < 0.05$). Further, in animals dosed with oral *dl*-MPH, concentrations of *l*-MPH were significantly increased from 0.015 μ g/mL for animals dosed with dH₂O to 0.05 μ g/mL for animals dosed with ethanol (Fig. 5b; $t = 4.56$, $df = 10$, $p < 0.001$). There were no significant differences between the blood concentration of *d*-MPH and *l*-MPH in animals dosed with dH₂O or in animals dosed with ethanol. Neither isomer of EPH was detectable in animals gavaged with oral *dl*-MPH and ethanol.

Effect of Ethanol on Brain Analytes

Transdermal DI-MPH—The brain concentration of *d*-MPH after MTS dosing was significantly greater in animals dosed with ethanol compared with the dH₂O group; increasing 65.3% from 0.81 to 1.34 μ g/g (Fig. 4a; $t = 2.89$, $df = 10$, $p < 0.05$). Further, in animals dosed with MTS, concentrations of *l*-MPH were significantly increased by ethanol,

rising from 0.84 μ g/g for animals dosed with dH₂O to 1.33 μ g/g for animals dosed with ethanol (Fig. 5a; $t = 2.18$, $df = 10$, $p < 0.05$). There were no significant differences between the brain concentration of *d*-MPH and *l*-MPH in animals dosed with dH₂O or in animals dosed with ethanol. Both isomers of MPH were formed in animals gavaged with ethanol; however, *l*-MPH was enantioselectively formed with a significantly greater concentration found relative to *d*-MPH (Fig. 6a; $t = 8.57$, $df = 10$, $p < 0.001$). The brain concentration of *l*-MPH was 0.14 μ g/g, whereas that of *d*-MPH was 0.005 μ g/g.

Oral DI-MPH—The brain concentration of *d*-MPH following oral dl-MPH was significantly greater in the animals dosed with ethanol compared with those given dH₂O; increasing 40.6% from 0.03 to 0.05 μ g/g (Fig. 4b; $t = 3.67$, $df = 10$, $p < 0.01$). Further, in animals dosed with oral dl-MPH, concentrations of *l*-MPH were significantly increased from 0.02 μ g/g for animals dosed with dH₂O to 0.06 μ g/g for animals dosed with ethanol (Fig. 5b; $t = 3.83$, $df = 10$, $p < 0.01$). There were no significant differences between the brain concentration of *d*-MPH and *l*-MPH in animals dosed with dH₂O or in animals dosed with ethanol. Both isomers of MPH were formed in animals gavaged with ethanol; however, *l*-MPH appeared to have been enantioselectively formed, although the mean concentration was not significantly different from that of *d*-MPH (Fig. 6b).

DISCUSSION

Oral dl-MPH in humans is subject to pronounced enantioselective first-pass metabolism that limits *l*-isomer systemic exposure to approximately 1% that of *d*-MPH.⁵² The mean absolute bioavailability of dl-MPH has been reported to be 30%, but ranges from 11% to 51%.^{60,61} In effect, first-pass metabolism biocatalytically “resolves” oral dl-MPH,⁶² resulting in only the *d*-isomer appreciably reaching the blood-stream. The *d*-isomer component of dl-MPH is generally regarded as the pharmacologically active isomer, responsible for efficacy in the treatment of ADHD.^{63,64} The low oral bioavailability of dl-MPH is largely due to the facile hydrolysis of the constituent methyl ester to yield the inactive³⁵ metabolite *dl*-ritalinic acid and catalyzed primarily through the actions of CES1.^{37,38,39,65} This facile pathway limits the half-life of dl-MPH to only 2–3 h.⁶⁶ Approximately 1% of MPH is excreted in urine unchanged in humans over 24 h, and excreted predominantly as the *d*-isomer.⁵⁶

Our studies with mice dosed with oral dl-MPH (7.5 mg/kg) and dH₂O, while being limited to a single 3-h time point for blood and brain sampling, suggest a lower degree of metabolic enantioselectivity relative to humans, whereby the *d*-MPH-to-*l*-MPH ratio for blood and brain were 1.22 and 1.36, respectively. This apparent greater oral bioavailability of *l*-MPH in the C57 mouse than in man is in general agreement with plasma results using CD1 mice dosed at 5.0 mg/kg⁶⁷ or pregnant rats dosed at 7.0 mg/kg.⁶⁸ Further, the extent of accumulation in brain relative to blood is expected to be less dramatic at 3 h than at earlier time points, especially after oral administration if the decay time course resembles that of the Sprague–Dawley rat.⁵⁸

A primary aim of the present study was to model transdermal MPH–ethanol metabolic interactions. A quarter of the smallest commercially available MTS patch was used and this delivered a mean dose of approximately 7.5 mg/kg of dl-MPH over the 3.25 h wear period

based on the difference between drug content before and after application. Although the MTS is not designed to be cut into portions for clinical applications, the *dl*-MPH content in each patch is evenly distributed throughout the patch³ and required apportioning when using such a small species as the mouse. *dl*-MPH delivery has been reported to occur in a manner directly proportional to the patch surface area in humans.^{3,69} Accordingly, the drug content in the quarter 12.5 cm² patches used in the present study was 25% of 27.5 mg, that is, 6.88 mg. The mean dose of 0.23 mg of *dl*-MPH delivered to the mice ($n = 12$) over the 3.25 h wear period represents 3.3% of the quarter patch content of *dl*-MPH and ranged from 1.9% to 5.1%. In humans, the uncut 12.5 cm² patch size is designed to deliver a mean *dl*-MPH dose of 10 mg over the recommended 9 h wear period. This dose represents 36% of the patch *dl*-MPH content, although ranging between subjects from 15% to 72%.⁷⁰

These apparent transdermal *dl*-MPH absorption differences reflect many factors including: (1) the shorter wear time of 3.25 h for the mouse, (2) the faster rate of ester substrate metabolism expected with rodents relative to humans,⁷¹ (3) the hair follicle-rich shaved skin of the mice opposed to the skin surface of the recommended hip placement in clinical applications, and (4) the potential for a greater relative absorption lag time for the 3.25 h wear period in mice versus 9 h in humans. In this latter context, the average lag time for detectable *d*-MPH in plasma after applying MTS to humans is 3.1 h (ranging from 1 to 6 h).⁷² The above factors notwithstanding, it is recognized that the percutaneous absorption rate for a range of drugs in mice and other rodents has generally been found to be more rapid than in humans or pigs.⁷³

Although the present investigation appears to represent the first MTS study to use mice, previous preclinical studies have shown that shaved mice serve to model transdermal drug delivery.⁷⁴ Hairless or nude mice are more typically used for transdermal delivery studies across the range of patch technologies⁷⁵; however, the neuropharmacological reference strain status of the C57 mouse provided the justification for its use in investigating *dl*-MPH–ethanol interactions (see *Introduction* section). Maintaining the mice in the metabolic chambers for a total of 3 h allowed for the collection of adequate urine volume for analysis, while still permitting quantification of analytes from blood and brain. In this context, the mean elimination half-life of *dl*-MPH in mice (B6C3F1 strain; 3 mg/kg orally) has been reported to be 1.1 h,⁷⁶ whereas that of ethanol (2 g/kg i.p.) in C57 mice appears to be approximately 1.3 h.⁷⁷

Enantioselective *l*-EPH Transesterification

As with oral dosing in humans,³³ coadministration of ethanol and transdermal or oral *dl*-MPH in C57 mice resulted in the enantioselective transesterification of *dl*-MPH, favoring *l*-MPH over *d*-MPH as a substrate. EPH was detectable in the brain, blood, and urine of these mice. Selection of an appropriate species to model esterase-mediated metabolism of *dl*-MPH was an important consideration in our study design. For instance, beagle dogs have been used in pioneering *dl*-MPH metabolism studies,⁷⁸ and in subsequent toxicokinetic studies.⁷⁹ However, esterase-mediated hydrolysis of *dl*-MPH in beagle dogs exhibit the opposite enantioselectivity, preferentially deesterifying *d*-MPH over *l*-MPH.⁸⁰ Further, on the basis of both human investigations³³ and the present findings with C57 mice, the enantioselective

formation of *l*-EPH with coadministration of *dl*-MPH and ethanol is accompanied by an elevation in *d*-MPH concentrations relative to dosing with *dl*-MPH alone. Although *l*-EPH formation was found to be enantioselective, this metabolic pathway was not enantiospecific, that is, *l*-EPH concentrations significantly exceeded *d*-EPH values, although *d*-EPH was readily detectable and quantifiable in C57 mouse samples following MTS and ethanol, as well as in the urine of animals dosed orally with *dl*-MPH. In humans dosed orally with *dl*-MPH and ethanol, *d*-EPH rarely exceeded 10% of the concentration of *l*-EPH.³³

In potential forensic medicine applications,⁴² detection of EPH from biological samples could serve as a biomarker to demonstrate combined consumption of *dl*-MPH and ethanol, analogous to the detection of cocaethylene as evidence of cocaine–ethanol coabuse.⁸¹

The high degree of hepatic localization of CES1 compared with its low level of intestinal expression implicates hepatic transesterification as the primary site of EPH formation after oral dosing of *dl*-MPH.³⁸ However, when dosing *dl*-MPH by the transdermal route, presystemic esterase metabolism may also occur, as has been reported during percutaneous disposition of ester-containing drugs. Transdermal presystemic hydrolysis has been especially associated with the cutaneous fat layer, where methyl ester- and ethyl ester-containing drugs are reported to be readily deesterified in skin during transdermal transport.^{73,82–85} Some degree of presystemic transesterification of *dl*-MPH to EPH may also occur. In the presence of ethanol, transesterification of methyl esters to ethyl esters has been reported in skin.⁸⁶ For instance, the methyl ester methylparaben is rapidly hydrolyzed in skin,⁸³ although in the presence of ethanol, hydrolysis of methylparaben is inhibited by competitive esterase-mediated transesterification of methylparaben to ethylparaben in pig⁸⁷ or human⁸⁸ skin.

As with hepatic esterase substrates, skin esterase activity has also been reported to exhibit enantioselectively, for example, during prodrug ester activation by hydrolysis.⁸⁹ The possibility of cutaneous esterase-mediated biotransformation resulting in transesterification of transdermal *dl*-MPH with ethanol may be favored by the mildly basic cutaneous pH expected at the MTS application site considering the high concentration of *dl*-MPH free base found in MTS.³ Mild cutaneous basicity has been reported to accelerate the rate of ester xenobiotic hydrolysis. For instance, esterase activity toward transdermal drug substrates was accelerated at a pH of 8, but was inhibited at the lower pH of 5.⁸⁸ *dl*-MPH is an especially weak organic base even though it contains a secondary aliphatic amine; it exhibits a p*K*_a of 8.4 versus the p*K*_a of 9.6 for the stimulant methamphetamine.⁹⁰ This relatively low basicity of *dl*-MPH has been theorized to be a consequence of an intramolecular hydrogen bonding interaction between the amine and the methyl ester carbonyl within the MPH structure.⁹¹

Still considering the potential for some degree of cutaneous EPH formation, in addition to subsequent hepatic metabolism, oral ethanol is rapidly distributed throughout mammalian tissue, and a portion of the nonmetabolized dose is excreted cutaneously (sweat), in addition to ethanol excretion by the lungs and kidney.⁹² Finally, even oral MPH reaches skin, as demonstrated using commercial sweat patches placed on the back.⁹³

Significant Increases In *d*-MPH Concentrations by Ethanol

The concentrations of *d*-MPH in blood, brain, and urine were significantly greater in mice dosed with ethanol than those dosed with dH₂O. These findings occurred when dosing either transdermally or orally. *d*-MPH elevation following concomitant MPH–ethanol administration was especially pronounced under the conditions used when dosing *dl*-MPH by the transdermal route. However, any direct comparisons between the extent to which ethanol influences either *d*-MPH concentrations or EPH formation as a function of dosing route cannot be reasonably made due to the inherent disparities of comparing an oral bolus dose of *dl*-MPH with that of the ongoing release of *dl*-MPH from the MTS. It is possible that the elevated *l*-MPH levels associated with transdermal dosing in C57 mice relative to oral dosing could be relevant to the extent to which ethanol elevates *d*-MPH in the course of ethanol interacting with CES1 to form *l*-EPH.

Approximately 50 times more of *l*-MPH reaches the systemic circulation in humans when *dl*-MPH is dosed transdermally than when dosed orally,⁵³ and *l*-MPH is the isomer that enantioselectively serves as a CES1 substrate in the presence of ethanol.^{33,38,39,94,95} If ethanol facilitates *d*-MPH absorption from the MTS through esterase inhibition at the level of the skin and/or liver, the resulting higher drug concentrations, and potentially more rapid rate of absorption of MPH, may influence pleasurable effects³³ of this drug combination, and contribute to additional abuse liability.^{49–51} Further, elevated *d*-MPH plasma concentrations pose the potential for adverse or lethal⁴² cardiovascular effects.^{45,15} In view of the significant influence of ethanol on *d*-MPH concentrations in the C57 mouse model reported here, transdermal *dl*-MPH used to treat adult ADHD may be associated with clinical considerations unique to this route of administration. Should drug interaction findings from of this animal model hold for humans?

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Figure 1.
Enantiomers of MPH: *d*-MPH (left) and *l*-MPH (right).

Figure 2.
Enantioselective transesterification of *dl*-MPH to *l*-EPH following concomitant ethanol.

Figure 3.

Representative GC–MS–SIM chromatogram of *d*-MPH, *l*-MPH, and *l*-EPH from a C57 mouse brain extract (upper ion profile). The sample was collected 3.25 h after dosing with one quarter of a 12.5 cm² MTS and 3 h after dosing with 3.0 g/kg ethanol by gavage. Enantiospecific analysis used chiral derivatization and a deuterated internal standard (lower ion profile).

Figure 4.

(a) In mice treated with one quarter of a 12.5 cm² MTS for 3.25 h, ethanol (3.0 g/kg, gavaged at 0.25 h) increased total excretion of *d*-MPH in urine and increased *d*-MPH concentrations in blood and brain relative to dH₂O. (b) In mice gavaged with *dl*-MPH (7.5 mg/kg), concomitant ethanol (3.0 g/kg) increased total 3 h urinary excretion of *d*-MPH, and increased 3 h *d*-MPH concentrations in blood and brain, relative to gavage dosing with *dl*-MPH (7.5 mg/kg) and dH₂O. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Figure 5.

(a) In mice treated with one quarter of a 12.5 cm² MTS for 3.25 h, ethanol (3.0 g/kg, gavage at 0.25 h) increased total excretion of *l*-MPH in urine and increased *l*-MPH concentrations in blood and brain relative to dH₂O gavage. (b) In mice gavaged with *dl*-MPH (7.5 mg/kg), concomitant ethanol (3.0 g/kg) increased total 3 h urinary excretion of *d*-MPH and increased 3 h *l*-MPH concentrations in blood and brain, relative to gavage dosing with *dl*-MPH (7.5 mg/kg) and dH₂O. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Figure 6.

(a) Ethanol (3.0 g/kg, gavage at 0.25 h) and one quarter of a 12.5 cm² MTS resulted in enantioselective *l*-EPH formation as quantified in 3.25 h urine, blood, and brain. (b) Concomitant gavage of ethanol (3.0 g/kg) and *dl*-MPH (7.5 mg/kg) resulted in greater 3 h urinary elimination of *l*-EPH than for *d*-EPH. EPH was not detectable (ND) in 3 h blood using dosing regimen. In brain, the mean *l*-EPH concentration was greater, but not significantly (NS) different from that of *d*-EPH. EPH offers the potential of serving as a biomarker for combined *dl*-MPH–ethanol exposure. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Table 1

Dosing Regimens for C57 Mice

<i>dl</i> -MPH and Ethanol	<i>dl</i> -MPH and dH ₂ O
1/4 12.5 cm ² MTS	1/4 12.5 cm ² MTS
+3.0 g/kg ethanol (gavage)	+dH ₂ O (gavage)
<i>n</i> = 8	<i>n</i> = 8
Placebo patch	Placebo patch
+7.5 mg/kg <i>dl</i> -MPH (gavage)	+7.5 mg/kg <i>dl</i> -MPH (gavage)
+3.0 g/kg ethanol (gavage)	+dH ₂ O (gavage)
<i>n</i> = 8	<i>n</i> = 8