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Title page

Differential influences of ethanol on early exposure to racemic methylphenidate compared to dexmethylphenidate in humans

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Running title page

Dexmethylphenidate versus methylphenidate-ethanol interactions

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ABBREVIATIONS: MPH, methylphenidate; EPH, ethylphenidate; CES1, carboxylesterase 1; ADHD, attention-deficit/hyperactivity-disorder, C_{max} , maximum plasma concentration; AUC, area under the curve; T_{max}, time to maximum concentration; VAS, visual analog scale; K, elimination rate constant; GeoMean, geometric mean.

Abstract

Enantioselective hydrolysis of oral racemic methylphenidate (dl-MPH) by carboxylesterase 1 (CES1) limits the absolute bioavailability of the pharmacologically active d-MPH isomer to approximately 30%, and that of the inactive l-MPH to only 1-2%. Co-administration of dl-MPH with ethanol results in elevated d-MPH plasma concentrations accompanied by CES1 mediated enantioselective transesterification of l-MPH to l-ethylphenidate (EPH). The present study tested the hypothesis that administration of the pure isomer dexmethylphenidate (d-MPH) will overcome the influence of ethanol on d-MPH absorption by eliminating competitive CES1-mediated presystemic metabolism of l-MPH to l-EPH. Twenty-four healthy volunteers received dl-MPH (0.3 mg/kg) or d-MPH (0.15 mg/kg), with or without ethanol (0.6 g/kg). During the absorption phase of dl-MPH, concomitant ethanol significantly elevated d-MPH plasma concentrations (44-99%; $P<0.005$). Further, immediately following the ethanol drink the subjective effects of “high”, “good”, “like”, “stimulated” and overall “effect” were significantly potentiated ($P\leq0.01$). Plasma l-EPH concentrations exceeded those of l-MPH. Ethanol combined with pure d-MPH did not elevate plasma d-MPH concentrations during the absorption phase and the ethanol-induced potentiation of subjective effects was delayed relative to dl-MPH-ethanol. These findings are consistent with l-MPH competitively inhibiting presystemic CES1 metabolism of d-MPH. Ethanol increased the d-MPH $AUC_{0\text{-inf}}$ by 21% following dl-MPH ($P<0.001$) and 14% for d-MPH ($P=0.001$). In men receiving d-MPH-ethanol, the d-MPH absorption partial $AUC_{0.5\text{-}2\ h}$ was 2.1 times greater

and the T_{max} occurred 1.1 h earlier than in women; consistent with an increased rate of d-MPH absorption reducing hepatic extraction. More rapid absorption of d-MPH carries implications for increased abuse liability.

Introduction

Adult attention-deficit/hyperactivity disorder (ADHD) patients treated with methylphenidate (MPH) commonly use or misuse ethanol (Levin and Kieber 1995; Darredeau et al., 2007). In addition, the diversion and abuse of MPH has been on the rise (Scharman et al., 2007) and many, if not most, MPH abusers report co-abuse with ethanol (Teter et al., 2003; Barrett et al., 2005; Darredeau et al., 2007; Novak et al., 2007; Wilens et al., 2008). In a previous drug interaction study using normal volunteers, ethanol was found to significantly increase positive subjective responses to dl-MPH (Patrick et al., 2007), as consistent with the illicit popularity of this drug combination.

The influence of ethanol on dl-MPH pharmacokinetics (Patrick et al., 2007) includes: (1) an increase in the *rate* of d-MPH absorption which has implications for heightened abuse liability (Volkow et al., 2003; Spencer et al., 2006); (2) an increase in the mean maximum plasma concentration (C_{max}) of d-MPH where threshold brain concentrations associated with reinforcing effects may be reached (Volkow et al., 2003); and (3) an increase in the overall plasma exposure to d-MPH [area under the curve (AUC)]. In addition to these ethanol-mediated influences on the psychoactive d-MPH isomer (Srinivas et al., 1992, Aoyama et al., 1994), the inactive l-MPH isomer (Markowitz and Patrick, 2008) serves as the enantioselective transesterification substrate (Patrick et al., 2007; Zhu et al., 2011) for carboxylesterase 1 (CES1); l-MPH combines with ethanol to yield the inactive metabolite l-ethylphenidate (EPH; Fig.1) (Patrick et al., 2005;

Williard et al., 2007). This biotransformation product can serve as a biomarker for concomitant dl-MPH-ethanol exposure (Markowitz et al., 1999) analogous to the detection of the transesterification metabolite cocaethylene (ethylcocaine) evidencing cocaine-ethanol co-abuse (Herbst et al., 2011).

Over 80% of an oral dose of dl-MPH can be recovered in the urine (Redalieu et al., 1982) as the inactive (Patrick et al., 1981) hydrolysis metabolite ritalinic acid (Fig. 1). Approximately 1% is excreted unchanged (LeVasseur et al., 2008). Both the hydrolysis of dl-MPH to ritalinic acid and the transesterification with ethanol to yield l-EPH are primarily catalyzed by hepatic CES1 (Bourland et al., 1997; Sun et al., 2004; Zhu et al., 2008; 2009). Pre-systemic hydrolysis of oral dl-MPH results in the relatively low oral bioavailability of 25% for the therapeutic d-MPH isomer (Srinivas et al., 1992; Aoyama et al., 1994). However, due to the pronounced enantioselectivity of CES1 only 2-5% (Srinivas et al., 1993; Modi et al., 2000) or less (Patrick, et al., 2007) of the inactive l-MPH isomer (Markowitz and Patrick 2008) reaches the systemic circulation, except in the circumstance of a *CES1* null allele (Patrick et al., 2007; LeVasseur et al., 2008; Zhu et al., 2008).

The single isomer d-MPH became a treatment option for ADHD in 2002. Head-to-head efficacy comparisons of immediate-release d-MPH versus dl-MPH in ADHD children, using twice-daily regimens with d-MPH administered at half the mg/kg dose of dl-MPH, found comparable efficacy over the course of a typical school day (Wigal et al., 2004). The present study extends the

pharmacological comparisons of d-MPH to dl-MPH as it pertains to differential interactions with concomitant ethanol. These results are discussed in the context of abuse liability, pharmacokinetic-pharmacodynamic correlations and adult ADHD drug individualization.

We explored the hypothesis that the ethanol-induced increase in d-MPH bioavailability following dl-MPH administration will be significantly reduced upon substituting enantiopure d-MPH for dl-MPH, the reasoning being that removal from the formulation of the more rapidly metabolized CES1 substrate l-MPH will avoid competitive inhibition of CES1 during d-MPH absorption. In the following normal volunteer study, 12 men and 12 women were administered dl-MPH (0.3 mg/kg) or d-MPH (0.15 mg/kg) with or without ethanol (0.6 g/kg) 0.5 h later in a randomized, 4-way cross-over design. Serial blood samples were drawn for enantiospecific pharmacokinetic analysis of plasma MPH and EPH, as well for blood ethanol determinations. Periodic subjective effects utilized visual analog scale (VAS) questionnaires and hemodynamic effects were recorded.

Materials and Methods

Research subjects. Each subject provided a written informed consent approved by the Medical University of South Carolina's Office of Research Integrity. The study was conducted in the Clinical & Translational Research Center located at the Medical University of South Carolina. The study population consisted of 24 normal volunteers (12 men, 12 women) aged 21-42 years who were healthy as assessed by medical history, physical examination, 12-lead electrocardiogram, and routine laboratory tests. All subjects were within 15% of ideal body weight, were non-smokers and were asked to abstain from the use of caffeine containing beverages beginning at 7:30 p.m. the evening before and continuing through each active study day.

Study design. All subjects were admitted to the clinic on the morning of each active study day. One hour prior to dosing, the subjects received a light breakfast of a plain bagel (36 g total: fat 1 g, carbohydrate 29 g, protein 6 g) with cream cheese (30 g total: fat 9.2 g, protein 4.4 g, carbohydrate 0.03 g) and skim milk (240 ml total: fat 9.2 g, carbohydrate 11.5 g, protein 8.4 g), finished within 15 min. Then an indwelling venous catheter was placed in each subject's arm for serial blood sampling. MPH was administered with 240 ml of water. Ethanol or non-ethanol orange juice drinks were consumed 0.5 h after MPH dosing. The ethanol drink was administered as 0.6 g/kg ethanol (0.66 ml/kg 95% ethanol) in 180 ml of orange juice and 60 ml of soda water, with water added to give a total volume of 450 ml. On alcohol free treatments, the alcohol volume was replaced with water.

The drinks were consumed over 15 min at 30 ml/min. Subjects received a standard lunch 3.5 h after MPH dosing. Lunch consisted of a turkey sandwich on whole wheat bread (2 slices bread, 3 slices turkey), 31 g (1 1/8 oz bag) of baked potato chips (Baked Lay's®), 120 g of canned fruit in light syrup (Dole® mixed fruit cups), and 360 ml of water. A standardized dinner was provided 10.5 h after dosing. There was at least a 6-day washout period between treatment regimens and negative urine drug screens and urine pregnancy results (females) were obtained at the beginning of each active study session.

An open label, randomized, crossover study design was employed. Four treatment schedules were used: dl-MPH with or without ethanol (treatments A and B) using oral immediate-release dl-MPH HCl (0.3 mg/kg) administered as 10 and 5 mg tablets (Ritalin®, Novartis Pharmaceuticals, Summit, NJ), and the 5 mg tablets cut to the nearest 2.5 mg using a tablet cutter as appropriate; d-MPH with or without ethanol, using oral immediate-release d-MPH hydrochloride (0.15 mg/kg) administered as 5 and 2.5 mg tablets (Focalin®, Novartis Pharmaceuticals, Summit, NJ), and the 2.5 mg tablets cut to the nearest 1.25 mg using a tablet cutter as appropriate.

A total of 12 blood samples were taken over each active study period from the indwelling venous catheter. These blood collection times correspond to 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 h after MPH dosing. Blood collection tubes (Vacutainers®, Becton Dickinson, Rutherford, NJ) were previously stored in an ice bath and contained sodium oxalate to minimize post-sampling MPH and EPH

hydrolysis. Heparin treated stoppered tubes (2 ml) were used to collect whole blood for blood ethanol analysis by the hospital clinical laboratory per standard procedures. Venous catheter lines were flushed of residual heparin solution prior to sampling. Samples were promptly centrifuged at 4ºC for 5 min and the plasma immediately aspirated into separate labeled polypropylene vials and stored at -70ºC until analysis.

Vital signs. Blood pressure, heart rate, temperature and respiratory rate were obtained at the screening visit and recorded at the beginning and end of each of the three active sessions. Blood pressure and heart rate were periodically recorded at 0, 0.75, 1.75, 2.75, 3.75, 4.75, 5.75, and 12 h after MPH dosing.

Visual Analog Scales. A nine question drug subjective effects questionnaire used visual analog subscales for the following questions: (1) Do you feel any drug effect?; (2) How high are you?; (3) Do the drugs have any good effects?; (4) Do the drugs have any bad effects?; (5) Do you like the drugs?; (6) Do you feel depressed; (7) How anxious are you?; (8) How stimulated do you feel? and (9) How intoxicated do you feel?. A questionnaire was administered before (baseline) dosing with MPH and repeated at 0.75, 1.25, 2.25, 3.25, 4.25, 5.25, and 11 h after MPH dosing. The subscales allowed rating of the degree to which the subject was experiencing each effect by making a vertical mark on a graduated (0-10) solid line ranging in drug effect from “not at all” (0) to “extremely” (10).

Recovery Period. Following each study period, subjects remained at the study site until blood ethanol concentrations were below 10 mg% (mg/dl) as measured by a breathalyzer test.

d-MPH, l-MPH, d-EPH and l-EPH plasma analysis. Plasma analyses were conducted at MEDTOX Laboratories (St. Paul, MN) using liquid chromatography-tandem mass spectrometry and a vancomycin based chiral stationary phase Chirobiotic V column (50 x 2.1mm) from Advanced Separation Technologies (Whippany, NJ). A deuterated internal standard provided analytical control, with a range of spiked plasma calibrators run in parallel. The lower limit of quantitation was 0.05 ng/ml for each isomer (see Patrick et al., 2007 for details).

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated by standard methods (Rowland 1989). The non-compartmental, analysis of enantiospecific MPH and EPH plasma concentrations was performed using WinNonlin v 5.1 (Pharsight, Cary, NC).

Statistical analysis. The mean and the least square geometric means of the two test treatments (d-MPH) and the reference (dl-MPH) were calculated for the Cmax and AUC. Ratios of the test geometric means to the reference, as well as the 90% confidence intervals about the reference, were determined. Comparisons between the male and female pharmacokinetic parameters were made using the student t-test assuming equal variance. Correlations between parameters for individuals were assessed by linear regression analysis (Instat® 3.01, GraphPad Software, San Diego, CA). The primary endpoint variables were

compared using analysis of variance (ANOVA) with Treatment (A,B,C,D) as a between (repeated measures) factor and sex as a between subjects factor using the Latin square design to take into account sequence (carry-over) effect as described by Winer (1962). The level of significance was set at P= 0.05.

Results

Human subjects. Twenty-four normal volunteers [12 men aged 22-30 years: mean (+/- S.D.) 25.8 (2.4), weight 82.2 (11.1) kg, all Caucasian; and 12 women aged 21-42 years: mean 26.9 (4.5), weight 59.6 (6.8) kg, 11 Caucasian, 1 Asian] completed the entire protocol. Treatment emergent sinus tachycardia resulted in one subject being removed from the study. Another subject withdraw due to a headache, nausea and vomiting. Dropped subjects were replaced. Nine occurrences of headaches were reported and were treated with ibuprofen (400 mg administered at the earliest 3.5 h following the MPH dose thus after d-MPH T_{max} and after time to peak subjective effects). No subject had any clinically significant findings on post-study "exit" laboratory tests.

Influence of ethanol on d-MPH pharmacokinetics: dl-MPH versus d-MPH. Figure 2A profiles the mean plasma concentration time course for the d-MPH isomer following oral dl-MPH (0.3 mg/kg; reference) with or without ethanol (0.6 g/kg); and Figure 2B profiles the mean d-MPH plasma concentration following oral d-MPH (0.15 mg/kg; test) with or without ethanol (0.6 g/kg) in 24 normal volunteers. The corresponding pharmacokinetic parameters are reported in Table 1, while the statistical comparisons are given in Table 2. Ethanol elevated the mean d-MPH C_{max} , and $AUC_{0\text{-}inf}$values when dosing with dl-MPH, as well as the C_{max} and $AUC_{0\text{-}inf}$ values when dosing with the pure isomer d-MPH. In the dl-MPH treatment, ethanol increased the mean C_{max} (CV%) and $AUC_{0\text{-}inf}$ (CV%) values from 10.1 (31) ng/ml and 52.1 ng•h/ml (29), to 12.0 (22) ng/ml and 62.8

(26) ng•h/ml, respectively. The corresponding increases in d-MPH C_{max} and $AUC_{0\text{-}inf}$ values by ethanol for the pure d-MPH treatments were 10.7 (21) ng/ml and 53.7 (22) ng•h/ml without ethanol, increasing to 12.4 (23) ng/ml and 61.8 (25) ng•h/ml with ethanol.

Table 2 shows the pharmacokinetic statistical comparisons of the treatments. For the two dl-MPH treatments, ethanol significantly increased the C_{max} by 22% (i.e., GeoMean ratio 1.22), the $AUC_{0\text{-}inf}$ by 21% and the MPH absorption phase partial $AUC_{0.5\text{-}2h}$ 72% relative to dosing with dl-MPH alone. In the pure isomer d-MPH treatments, ethanol significantly increased the C_{max} by 15% and the $AUC_{0\text{-}inf}$ by 14% compared to d-MPH alone, but ethanol did not significantly influence the $AUC_{0.5\text{-}2h}$.

In the comparison of dl-MPH versus d-MPH alone, or dl-MPH combined with ethanol versus d-MPH combined with ethanol, the 90% confidence interval for the geometric mean (GeoMean) ratio for both C_{max} and $AUC_{0\text{-}inf}$ demonstrated bioequivalence (i.e., CI's within 80-125; Table 2) (Metzler CM, 1991). In the dl-MPH-ethanol treatment, ethanol not only significantly elevated d-MPH (Fig. 2A) plasma concentrations but also l-MPH (Fig. 3) plasma concentrations during the absorption phases of the drug isomers and ethanol (Fig. 4). Compared to dl-MPH alone, ethanol increased the mean d-MPH plasma concentrations at 1 h by 99%, (P<0.000), 1.5 h by 57% (P=0.001) and 2.0 h by 44%. The corresponding d-MPH partial $AUC_{0.5\text{-}2h}$ increased 72% (P=0.001) during d-MPH absorption (Table 2). In contrast, ethanol did not significantly alter d-MPH early exposure

when dosing with the pure d-MPH isomer; partial $AUC_{0.5\text{-}2h}$ decreased -5% (*P*=0.75).

Ethanol elevated the mean C_{max} (CV%) for l-MPH nearly two-fold, with C_{max} concentrations rising from 0.18 (86) ng/ml without ethanol to 0.32 (86) ng/ml with ethanol. The lower limit of detection for l-MPH (0.05ng/ml) was reached at ~5 h post dosing. No l-MPH was detected in the d-MPH treatments.

Sex differences in d-MPH, l-MPH and ethanol pharmacokinetics. While a trend was found for men having a greater overall exposure ($AUC_{0\text{-}inf}$) to d-MPH, the $AUC_{0.5\text{-}2h}$ for d-MPH was significantly greater for men compared to women in the respective d-MPH-ethanol and d-MPH alone treatments: 14.5 vs. 6.9 ng·h/ml; (*P*<0.005) and 12.5 vs. 7.4 ng·h/ml (*P*<0.05). For the dl-MPH-ethanol and dl-MPH without ethanol treatments the corresponding values were 12.8 vs. 8.9 (*P*=0.17) and 9.4 vs. 7.3 (*P*=0.14), respectively. The T_{max} values for men were less than for women in all 4 treatments, reaching significance again for the dl-MPH only, d-MPH-ethanol and d-MPH alone treatments: 3 h vs. 1.9 h (*P*<0.05); 2.6 h vs. 1.6 h (p=0.01) and 2.3 h vs. 1.7 h (*P*<0.05), respectively..

The T_{max} for ethanol in was 1.3 h for both men and women. However, the overall exposure to ethanol was 31% and 34% greater in men than women in the dl-MPH-ethanol and d-MPH-ethanol treatments, respectively (*P*<0.01). The mean C_{max} and $AUC_{0\text{-}12}$ values (S.D.) was 63.2 (15) mg% and 184 (23) ng•ml/h, respectively, in men compared to 53.9 (26) mg% and 133 (33) ng•ml/h for women (Fig. 5).

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Unlike the time course for d-MPH where d-MPH exposure was greater in men than women, the l-MPH isomer exposure was significantly greater in women than men in the racemic MPH treatments ($P<0.05$), especially when dl-MPH was combined with ethanol, e.g., at post dl-MPH dosing times 1 h and 1.5 h where plasma concentrations of l-MPH were nearly twice that in men compared to women. For this treatment, the AUC of l-MPH for females was approximately twice that for males (*P<0.05*), with mean C_{max} values of 0.3 ng/ml and 0.17 ng/ml, respectively (Fig. 6).

Enantioselective transesterification of l-MPH to l-EPH. Metabolic transesterification of MPH to EPH was enantioselective in the formation of l-EPH (Fig. 1); and was only detected in the dl-MPH-ethanol treatments. l-EPH reached a mean Cmax of 0.45 ng/ml with a T_{max} of 2 h (Fig. 3). No significant sex difference was found in the extent of l-EPH formation. The highest individual concentration of plasma l-EPH was 1.28 ng/ml. No d-EPH was detected in any sample owing to the very limited extent to which d-MPH serves as a transesterification substrate and our lower limit of detection being 0.05 ng/ml of plasma (see Zhu et al., 2011 for d-EPH findings using increased sensitivity).

Subjective effects. Table 3 summarizes the maximum VAS subscale responses from “any drug effect” in general, as well as those more directly serving as surrogates for abuse liability, i.e., the positive subjective effects of “high”, “good”, “like” and “stimulated”. These maximum effects occurred either at 0.75 h following MPH or more frequently at 1.25 h post-MPH. Other than “intoxicated”, the negative effects were generally below the effect scale of 1. In

the dl-MPH-ethanol treatment, immediately following the consumption of the ethanol drink (0.75 h after dl-MPH dosing), study subjects reported significantly increased “drug effect” and positive subjective effects when compared to receiving dl-MPH alone. In the pure isomer d-MPH-ethanol treatment only the subscale of “high” reached significance (*P*=0.044) at this early time when compared to d-MPH alone. However, the VAS questionnaire administered 0.5 h later (1.25 h following MPH dosing) revealed that the positive subjective effects produced by the d-MPH-ethanol regimen had then become more prominent for the d-MPH-ethanol treatment compared to the dl-MPH-ethanol treatment.

The baseline values did not differ significantly from the 10 h questionnaires and baseline values were subtracted from the post-dosing responses. There were no statistically different sex differences in subjective effects though there was a trend toward greater effects in men than in women.

Hemodynamic effects. Concomitant ethanol significantly elevated heart rates in both the dl-MPH and d-MPH drug combination treatments (Fig. 7). There was a trend toward a greater effect on heart rate for the dl-MPH-ethanol treatment than for the d-MPH-ethanol treatment. The greatest time point increase in heart rate for any treatment was at the 0.75 h reading for the dl-MPH-ethanol treatment (14 beats/min); the time corresponding to completion of the ethanol drink. The mean diastolic and systolic pressures were elevated more in the two ethanol combination treatments than in the MPH only treatments but these difference did not reach statistical significance.

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Discussion

Ethanol 0.5 h following a dose of the racemic drug dl-MPH significantly increased early exposure to d-MPH. The absorption phase partial (see Chen et al., 2010) $AUC_{0.5\text{-}2h}$ for d-MPH was elevated by 72% when compared to dosing with dl-MPH alone (P<0.005; Fig. 2; Table 2). Contrasting these findings, ethanol did not significantly influence the $AUC_{0.5\text{-}2h}$ for the pure d-MPH form (-5%; P=0.311). However, after the absorption phase of MPH and ethanol, ethanol significantly elevated d-MPH exposure for both enantiopure and racemic MPH (Fig. 2; Table 2).

The time interval of 0.5-2 h was of special interest in this study because it brackets the time from when subjects first began ethanol consumption until the approximate end of the absorption phases for d-MPH (Fig. 2), l-MPH (Fig.3) and ethanol (Fig. 4). In the dl-MPH-ethanol treatment, ethanol significantly increased the rate at which d-MPH and l-MPH reached the systemic circulation. The most rapid rise in plasma d-MPH occurred between the 0.5 and 1 h sampling times where at 1 h the mean d-MPH concentration had increased to twice that of the treatment receiving dl-MPH alone (P<0.005).

Within the 0.5-1.0 h period, one VAS questionnaire was administered (0.75 h following dl-MPH; Table 3). The responses to the VAS subscales “any drug effect” and the positive subjective effects “high”, “good”, “like” and “stimulated” were all significantly greater at 0.75 h (immediately after ethanol consumption) for the racemic dl-MPH-ethanol combination compared to dl-MPH given alone. With the pure d-MPH-ethanol treatment, only “high” was significantly greater than

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for d-MPH alone (*P*=0.044). Importantly however, the mean *peak* positive subjective effect VAS responses to either dl-MPH or d-MPH were all significantly increased by ethanol, most frequently occurring at the subsequent 1.25 h post-MPH VAS questionnaire. By that time, ethanol potentiated positive subjective responses to the pure d-MPH to a greater extent than even for the racemate (Table 3).

Correlations between an increase in d-MPH absorption rate and enhanced positive subjective effects have served as surrogates for abuse liability. Increasing the rate of d-MPH absorption (Kollins et al., 1998; Spencer et al. 2012), and the subsequent rate of delivery to the central nervous system (Swanson et al., 2003); Volkow et al., 2003; Stoops et al., 2004; Spencer et al., 2006) strongly influence stimulant abuse liability. The present findings extend these pharmacokinetic-pharmacodynamic relationships to the early d-MPH exposure period following concomitant dl-MPH and ethanol while contributing to an understanding of the special reward value and high incidence of MPH-ethanol co-abuse (Darredeau et al., 2007; Novak et al., 2007; Wilens et al., 2008).

The mean d-MPH partial $AUC_{0.5\text{-}2h}$ for men was greater than for women in all treatments, and reached statistical significance for the two pure d-isomer treatments. This early d-MPH exposure period for the d-MPH ethanol combination and for d-MPH alone was 110% and 71% greater in men than women, respectively (Fig. 5). Also a significantly longer d-MPH T_{max} occurred in men compared to women (see Results). Contrasting the sex differences in d-MPH exposure, the AUC for l-MPH (Fig. 6) was significantly greater in women

than men. Regardless of sex, the plasma l-MPH concentrations remained an order of magnitude less than the corresponding d-MPH concentrations. There was a tendency toward greater positive subjective effects in men than in women, though these subscales did not reach statistical significance. An earlier MPH-ethanol study found women to experience a greater “stimulated” response than men. However, those results included data from ethanol-*then*-dl-MPH treatments, and dosing was delayed 1.5 h relative to breakfast to reduce food effects (Patrick et al., 2007).

The mechanism by which ethanol prominently influences the absorption phase for d-MPH in the dl-MPH treatment is consistent with the l-MPH component competitively inhibiting CES1 as the enzyme enantioselectively transesterifies l-MPH to l-EPH (Fig. 3; Patrick et al., 2007; Zhu et al., 2011). First-pass hepatic metabolism of l-MPH is so extensive that, in effect, the racemic drug is biocatalytically resolved before reaching the systemic circulation. Thus, following dl-MPH absorption the elimination phase of d-MPH-ethanol interactions with CES1 become more comparable to that of the enantiopure d-MPH formulation. Thereafter, CES1 reverts to the more limited transesterification of d-MPH to d-EPH competing with hydrolysis (Zhu et al., 2011). In support of this competitive inhibition mechanism, human liver incubations of dl-MPH and ethanol in the presence or absence of the CES1 substrate cocaine demonstrated that cocaine significantly reduced both the rate of MPH deesterification to ritalinic acid as well as the rate of MPH transesterification to EPH (Koehm et al., 2010).

Ethanol also elevated plasma concentrations of the inactive enantiomer l-MPH though these values did not reach l-EPH concentrations (Fig. 3). In an analogous fashion, ethanol has been reported to elevate cocaine exposure (Perez-Reyes et al., 1994; Farre et al., 1997) while serving as a transesterification substrate yielding CES1 mediated cocaethylene in humans (Herbst et al., 2011) and in other species (Roberts et al., 1993; 1995; Hedaya et al., 1996; Parker et al., 2010).

The significant elevation of heart rate upon combining ethanol with either dl-MPH or d-MPH (Fig.7) is consistent with the resulting increase in exposure to the pressor agent d-MPH. In addition, this cardiovascular response may be attributable to additive catecholaminergic influences of MPH and ethanol. The dose of ethanol used in the present study has been reported to elevate heart rate by 5.7 beats/min (Spaak et al., 2008). Similarly, combining ethanol with cocaine has been shown to significantly elevate heart rate in humans compared to cocaine alone (Herbst et al., 2011).

The pharmacokinetics of the dl-MPH and d-MPH treatments without ethanol provides evidence (Table 2) of d-MPH (0.15 mg/kg) bioequivalence to dl-MPH (0.3 mg/kg), with the caveat that the d-MPH tablets were not designed to be cut to the nearest one-half as was conducted in the present study. These results are consistent with extent of absorption comparisons between immediate-release d-MPH and modified-release MPH formulations (Tuerck et al., 2007).

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Authorship contributions:

Participated in research design: Patrick, Straughn.

Conducted experiments: Bernstein, Malcolm. Patrick

Contributed new reagents or analytic tools: Patrick

Performed data analysis: Straughn, Reeves, Bell, Anderson, Patrick.

Wrote or contributed to the writing of the manuscript: Patrick, Straughn, Bell

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Footnotes

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b) Masters thesis, Owen Reeves III: Ethanol Interactions with dl-Methylphenidate versus d-Methylphenidate in Humans, Apr 16, 2011; Citation of meeting abstract: Reeves O, Straughn A, Bell G, Bernstein H, Malcolm R, Patrick K. Ethanol Elevates Plasma Dexmethylphenidate and *dl*-Methylphenidate Concentrations and Potentiates Subjective Effects. Am Col Clin Pharmacol 40th annual meeting, Chicago, Il, Sept 10-12, 2011, J Clin Pharmacol 51: 1332, 2011.

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Figure Legends

FIG. 1. CES1 mediated enantioselective deesterification of dl-MPH to the primary metabolite ritalinic acid and to the enantioselective transesterification to l-EPH with concomitant ethanol (from Patrick et al., 2007).

FIG. 2. (A) Mean d-MPH plasma concentrations (+/-S.D.) after dosing with dl-MPH (0.3 mg/kg) alone or dosing with dl-MPH (0.3 mg/kg) followed by ethanol (0.6 g/kg) 0.5 h later; **(B)** Mean d-MPH plasma concentrations after dosing with d-MPH alone or dosing with d-MPH (0.15 mg/kg) followed by ethanol (0.6 g/kg;) 0.5 h later.

FIG. 3. Mean l-MPH and l-EPH plasma concentrations (+/-S.D.) after dosing with dl-MPH (0.3 mg/kg) alone or dosing with dl-MPH (0.3 mg/kg) followed by ethanol (0.6 g/kg) 0.5 h later.

FIG. 4. There was significantly greater ethanol exposure (AUC_{0-12}) in men than in women (+/-S.D.). In the dl-MPH-ethanol treatment the AUC was 31% higher for men (*P<0.01*) and in the d-MPH-ethanol treatment the AUC was 34% higher for men (*P<0.01*).

FIG. 5. Sex based differences in d-MPH plasma concentrations (+/-S.D.). The absorption phase partial $AUC_{0.5\text{-}2h}$ for the d-MPH-ethanol treatment (lower left) revealed the most prominent sex dimorphism, where d-MPH exposure in men was over twice that of the women (*P*<005).

FIG. 6. l-MPH exposure (+/-S.D.) was significantly greater in women than in men (*P<0.05*).

FIG. 7. Ethanol significantly elevated heart rate when combined with dl-MPH Ritalin®) or d-MPH (Focalin®) with trends toward greater heart rates during the dl-MPH-ethanol treatment than for the d-MPH-ethanol treatment.

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Tables

TABLE 1

		MEAN VALUES		CV% in ( )	
		dl-MPH		**d-MPH**	
Component	**Parameter**	**Ethanol**	**dl-MPH**	**Ethanol**	**d-MPH**
d-MPH	K (h-1)	0.247 (18)	0.248 (20)	0.259 (15)	0.254 (23)
d-MPH	$T_{1/2}$ (h)	2.9 (19)	2.9 (19)	2.7 (17)	2.8 (20)
d-MPH	C_{max} (ng/ml)	12.0 (22)	10.1 (31)	12.4 (23)	10.7 (21)
d-MPH	Tmax (h)	2.2 (34)	2.4 (47)	2.1 (48)	2.0 (40)
d-MPH	AUC_{last} (ng*h/ml)	58.0 (24)	47.9 (29)	57.5 (24)	50.1 (22)
d-MPH	$AUC_{0\text{-}inf}$ (ng*h/ml)	62.8 (26)	52.1 (29)	61.8 (25)	53.7 (22)
d-MPH	$AUC_{0.5\text{-}2}$ (ng*h/ml	11.8 (36)	8.5 (56)	11.7 (47)	11.1 (34)
l-MPH	K (h-1)	0.477 (36)	0.393 (41)	a	a
l-MPH	$T_{1/2}$ (h)	1.7 (37)	2.1 (41)	a	a
l-MPH	C_{max} (ng/ml)	0.32 (89)	0.18 (86)	a	a
l-MPH	T_{max} (h)	1.7 (41)	1.8 (49)	a	a
l-MPH	AUC_{last} (ng*h/ml)	0.86 (86)	0.45 (121)	a	a
l-MPH	$AUC_{0\text{-}inf}$ (ng*h/ml)	1.08 (68)	0.77 (73)	a	a
l-EPH	K (h-1)	072 (23)	a	a	a
l-EPH	$T_{1/2}$ (h)	1.0 (28)	a	a	a
l-EPH	C_{max} (ng/ml)	0.53 (81)	a	a	a
l-EPH	T_{max} (h)	1.9 (91)	a	a	a
l-EPH	AUC_{last} (ng*h/ml)	1.19 (65)	a	a	a
l-EPH	$AUC_{0\text{-}inf}$ (ng*h/ml)	1.61 (66)	a	a	a

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Ethanol	C_{max} (mg%)	58.2 (25)	a	58.7 (19)	a
Ethanol	T_{max} (h)	1.8 (17)	a	1.9 (20)	a
Ethanol	AUC_{last} (mg%*h/ml)	158 (34)	a	158 (29)	a

a = all plasma assay for all subjects below limit of detection

d-MPH arithmetic mean (SD%), least square geometric mean (90% CI) and geometric mean ratio of pharmacokinetic parameters for the 24 subjects. Subjects received either dl-MPH (0.3 mg/kg) or d-MPH (0.15 mg/kg) with or without ethanol (0.6 g/kg) 0.5 h later.

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TABLE 2

Statistical comparisons between treatment groups by geometric mean (GeoMean) ratio

		dl-MPH + Ethanol to **dl-MPH**	**d-MPH + Ethanol** to **d-MPH**	**dl-MPH + Ethanol** to **d-MPH + Ethanol**	**dl-MPH** to **d-MPH**
Parameter	**Statistical Test**				
K d-MPH	GeoMean Ratio	1.00	1.03	0.95	0.98
	90% CI	0.92-1.08	0.95-1.11	0.88-1.03	0.91-1.06
	P-value	0.928	0.582	0.283	0.667
C_{max} d-MPH	GeoMean Ratio	1.22	1.15	0.97	0.91
	90% CI	1.13-1.32	1.06-1.24	0.90-1.04	0.84-0.98
	P-value	<.001	0.005	0.462	0.041
$AUC_{0\text{-inf}}$	GeoMean Ratio	1.21	1.14	1.01	0.95
	90% CI	1.14-1.30	1.07-1.22	0.94-1.08	0.89-1.01
	P-value	<0.001	0.001	0.846	0.193
$AUC_{(0.5\text{-}2h)}$ d-MPH	GeoMean Ratio	1.72	0.95	1.09	0.6
	90% CI	1.32-2.24	0.73-1.24	0.84-1.42	0.46-0.79
	p-value	0.001	0.75	0.57	0.002
K l-MPH	GeoMean Ratio	1.26			
	90% CI	1.08-1.48			
	P-value	0.018			
C_{max} l-MPH	GeoMean Ratio	1.78			
	90% CI	1.50-2.11			
	P-value	<0.001			
$AUC_{0\text{-inf}}$ l-MPH	GeoMean Ratio	1.5			

	90% CI	1.18-1.91	
	P-value	0.009	
C_{max} Ethanol	GeoMean Ratio		0.99
	90% CI		0.91-1.06
	P-value		0.744
AUC_{0-12h} Ethanol	GeoMean Ratio		0.99
	90% CI		0.90-1.08
	P-value		0.795

TABLE 3

VAS Treatment Comparisons

A = dl-MPH + Ethanol; B = dl-MPH alone; C = d-MPH + Ethanol;

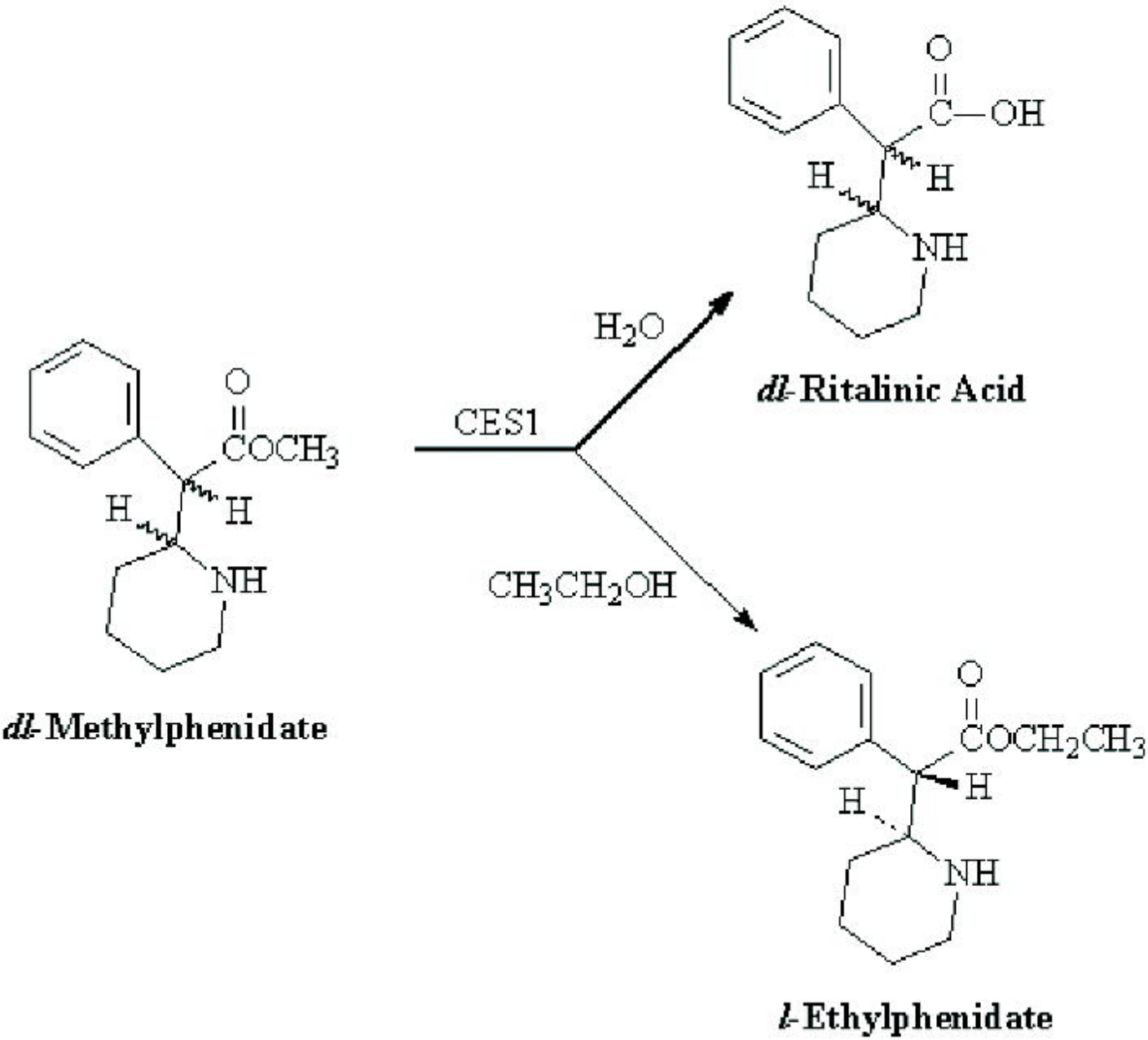
D = d-MPH alone

Subscale	Comparison	Ratio	*p*-value	Lower_CI	Upper_CI
Effect 0.75 h	A - B	2.46	0.000	1.69	3.58
Effect 0.75 h	C - D	1.42	0.114	0.99	2.05
High 0.75 h	A - B	2.11	0.005	1.38	3.23
High 0.75 h	C - D	1.60	0.044	1.09	2.34
Good 0.75 h	A - B	2.26	0.001	1.56	3.27
Good 0.75 h	C - D	1.37	0.128	0.97	1.94
Like 0.75 h	A - B	2.07	0.008	1.33	3.22
Like 0.75 h	C - D	1.54	0.092	1.01	2.35
Stimulated 0.75 h	A - B	1.73	0.035	1.13	2.64
Stimulated 0.75 h	C - D	1.42	0.166	0.94	2.14
Effect 1.25 h	A - B	1.39	0.064	1.04	1.86
Effect 1.25 h	C - D	1.86	0.001	1.38	2.50

High 1.25 h	A - B	2.40	0.003	1.51	3.82
High 1.25 h	C - D	3.05	0.000	1.92	4.85
Good 1.25 h	A - B	1.61	0.039	1.11	2.36
Good 1.25 h	C - D	1.87	0.008	1.28	2.74
Like 1.25 h	A - B	1.44	0.102	1.00	2.08
Like 1.25 h	C - D	1.30	0.248	0.89	1.91
Stimulated 1.25 h	A - B	1.24	0.354	0.84	1.84
Stimulated 1.25 h	C - D	1.88	0.010	1.26	2.81

Overall “effect” and positive subjective effects of racemic dl-MPH compared to enantiopure d-MPH as influenced by ethanol within the drug absorption phase: 0.75 h and 1.25 h following MPH (T=0) with or without ethanol (0.6 g/kg; consumed at a constant rate from 0.5-0.75 h). Baseline values were subtracted from post-dosing VAS ratings. All maximal effects occurred either at 0.75 h post-MPH dosing or more frequently at 1.25 h post-MPH.

Figure 1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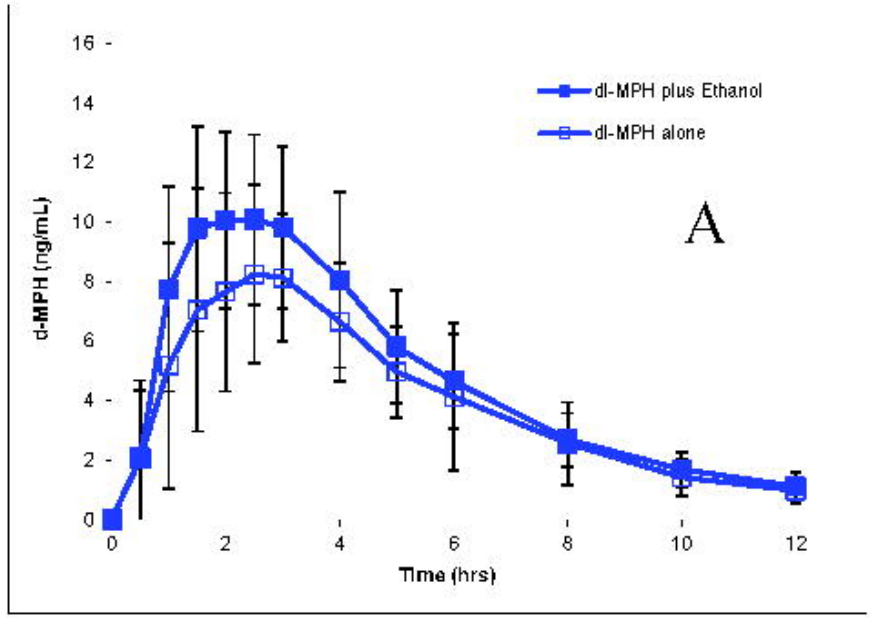
l-Ethylphenidate

Figure 2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dl-MPH plus Ethanol
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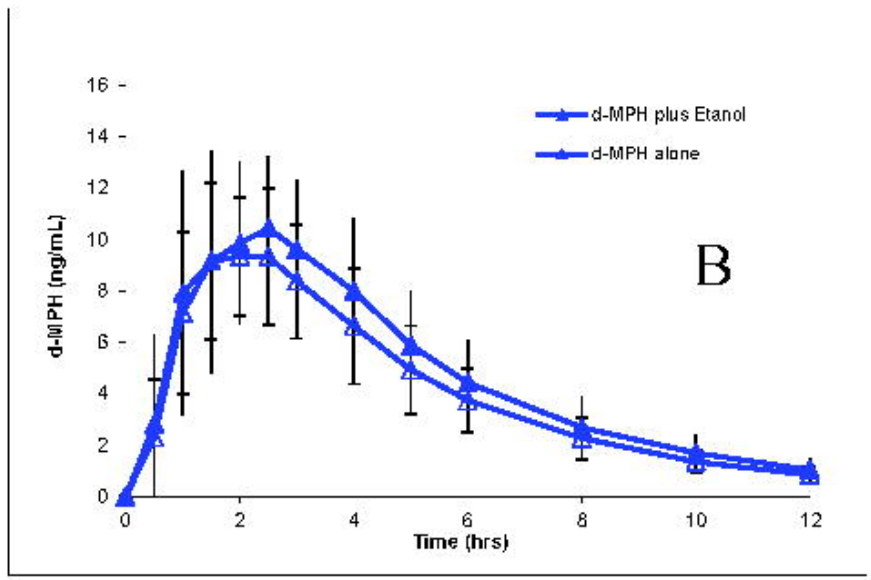
Time (hrs)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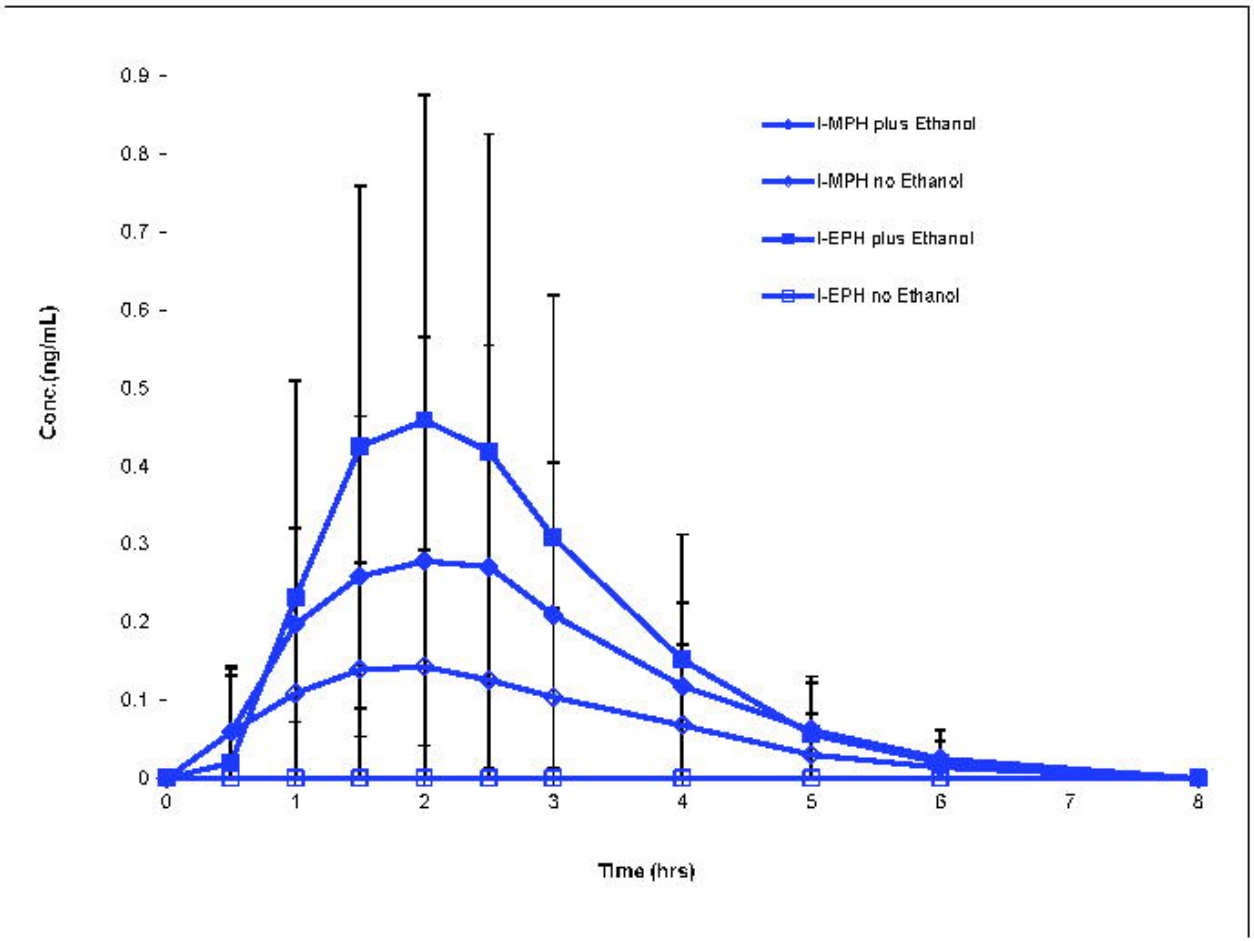
Time (hrs)

Figure 3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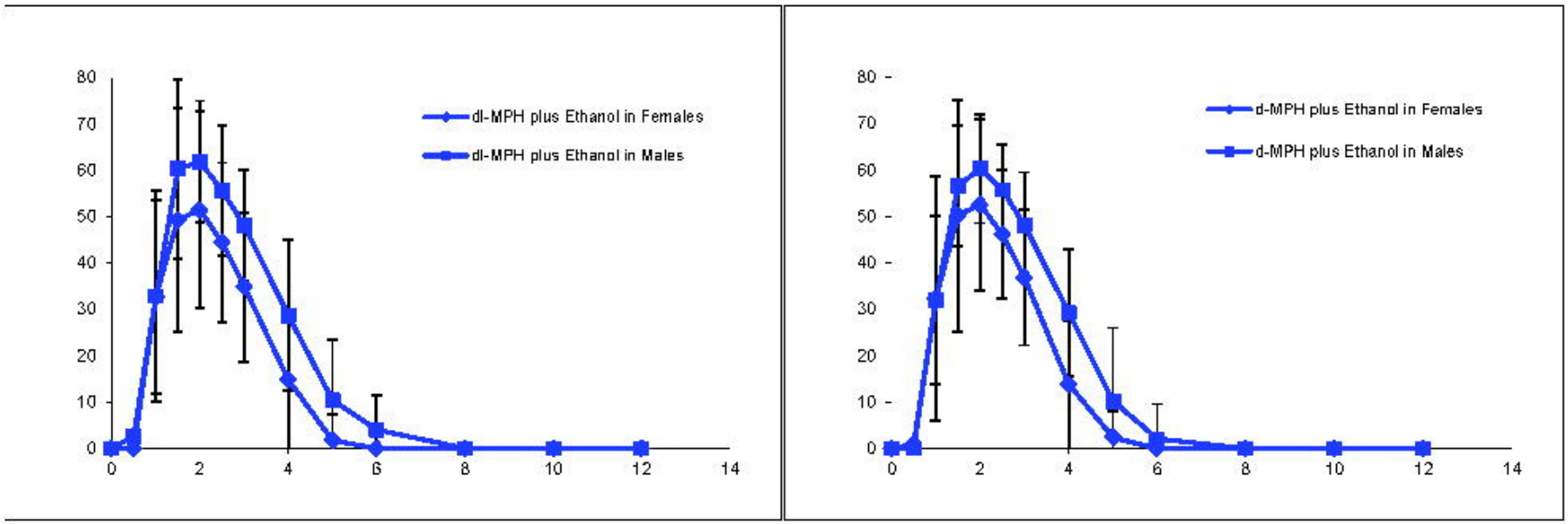
Time (hrs)
l-MPH plus Ethanol
l-MPH no Ethanol
l-EPH plus Ethanol
l-EPH no Ethanol

Figure 4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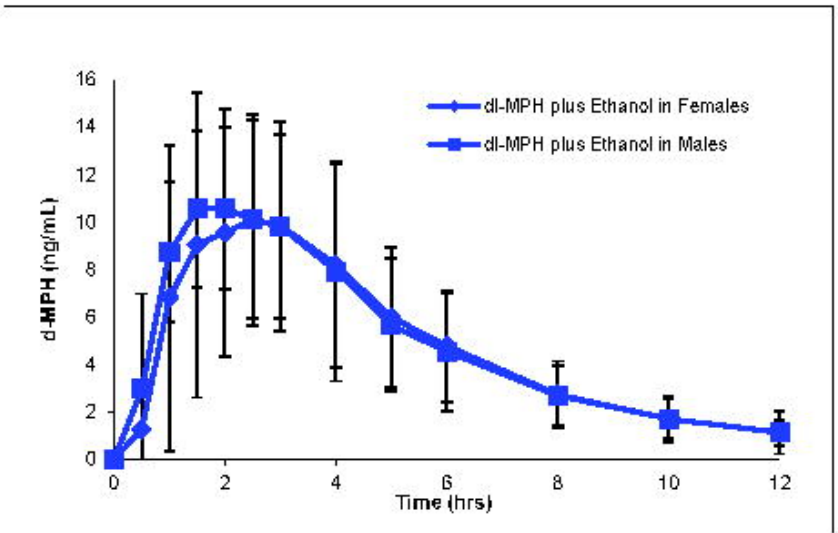
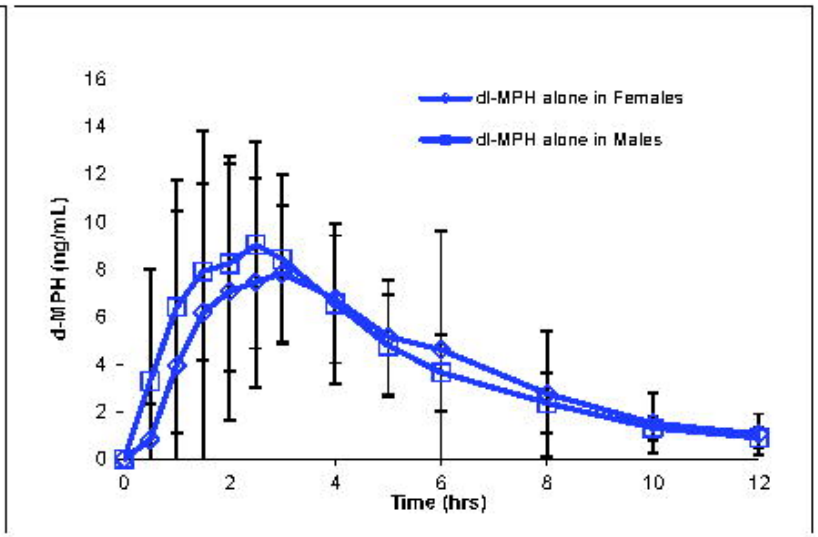
dl-MPH plus Ethanol in Males
d-MPH plus Ethanol in Females
d-MPH plus Ethanol in Males

Figure 5


dl-MPH plus Ethanol in Females
dl-MPH plus Ethanol in Males
d-MPH (ng/mL)
Time (hrs)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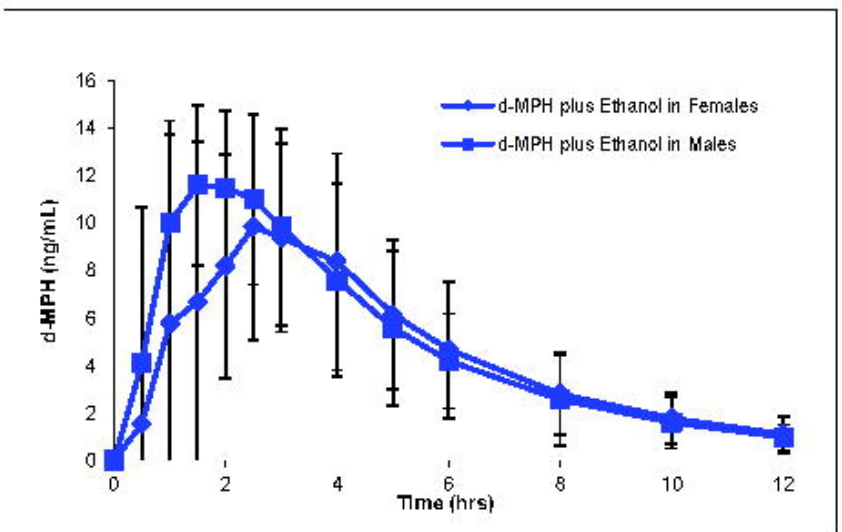
dl-MPH alone in Males
d-MPH (ng/mL)
Time (hrs)


d-MPH plus Ethanol in Females
d-MPH plus Ethanol in Males
d-MPH (ng/mL)
Time (hrs)

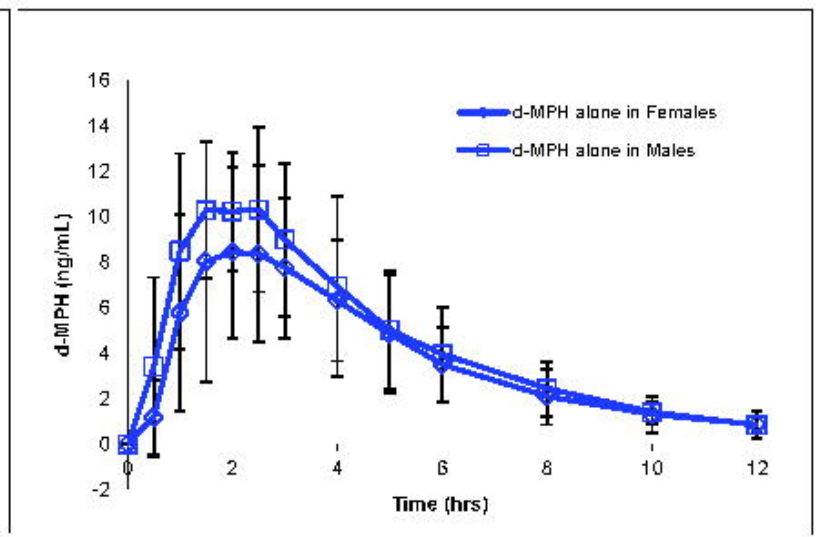
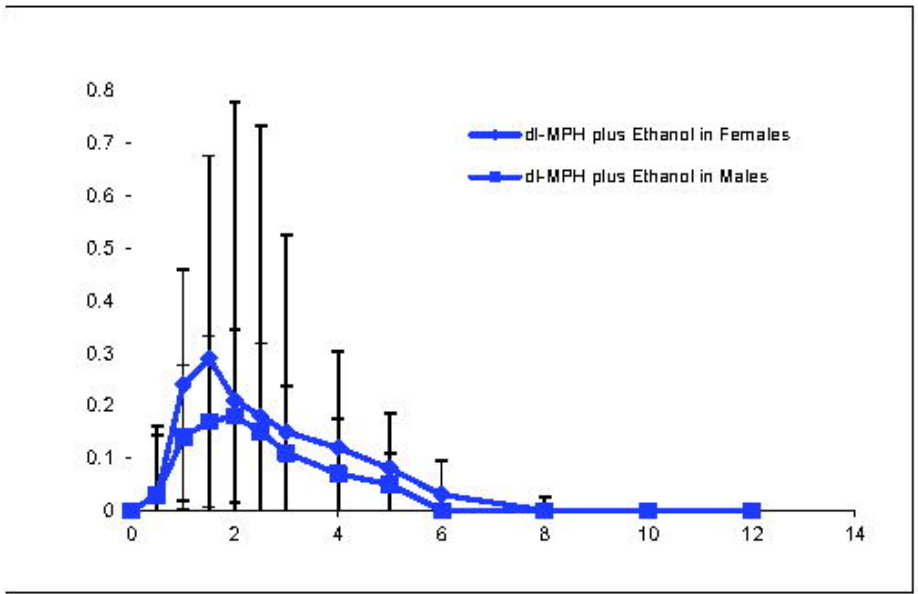

d-MPH alone in Females
d-MPH alone in Males
d-MPH (ng/mL)
Time (hrs)

Figure 6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12
14
dl-MPH plus Ethanol in Females
dl-MPH plus Ethanol in Males

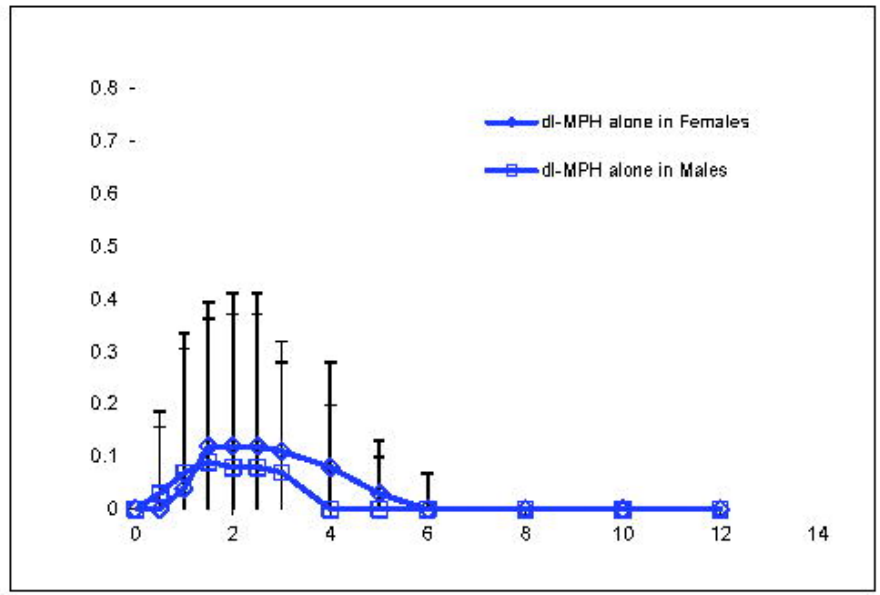
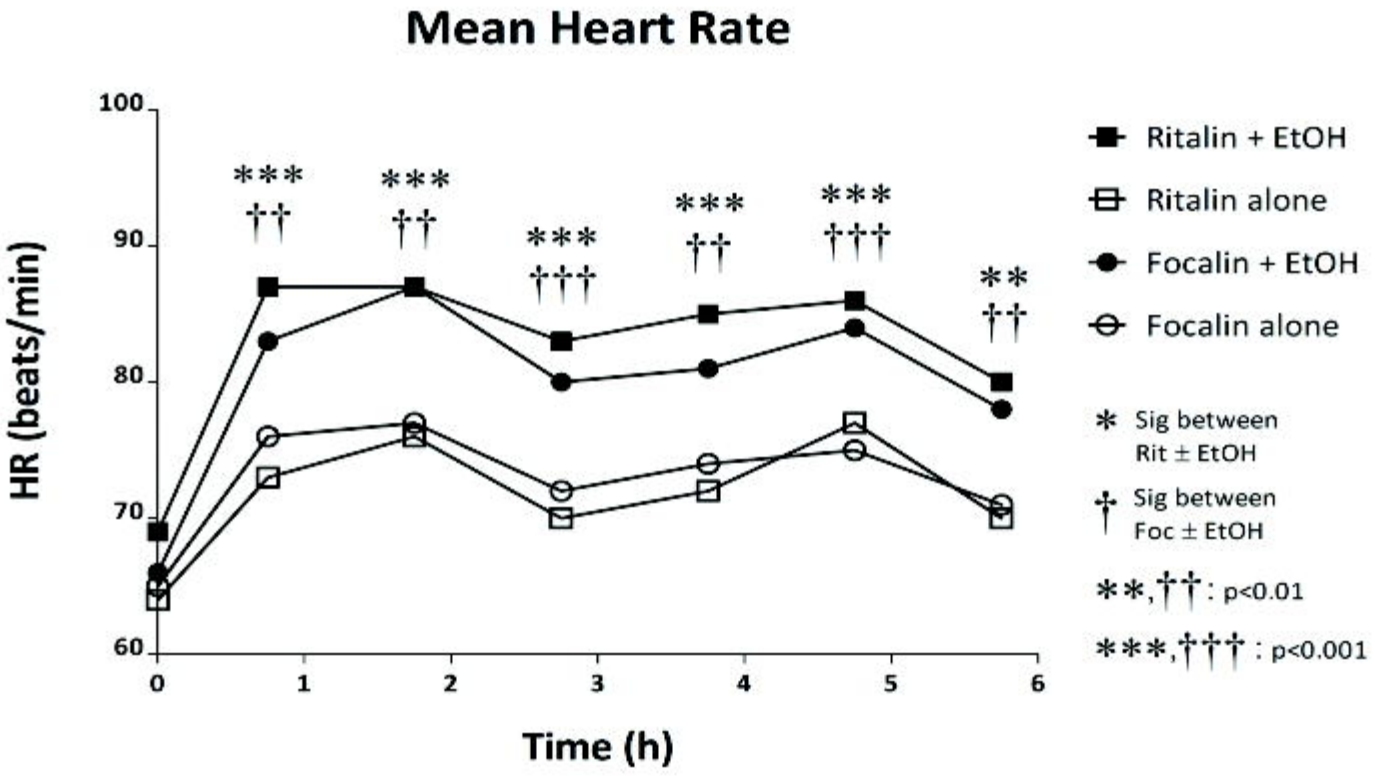

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
dl-MPH alone in Females
dl-MPH alone in Males

Figure 7


Mean Heart Rate
HR (beats/min)
100
90
80
70
60
0
1
2
3
4
5
6
Time (h)
***
††
***
††
***
†††
***
††
***
†††
**
††
Ritalin + EtOH
Ritalin alone
Focalin + EtOH
Focalin alone
* Sig between Rit ± EtOH
† Sig between Foc ± EtOH
**,††: p<0.01
***,†††: p<0.001