ETHYLPHENIDATE FORMATION IN HUMAN SUBJECTS AFTER THE ADMINISTRATION OF A SINGLE DOSE OF METHYLPHENIDATE AND ETHANOL

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ABSTRACT:
Ethylphenidate was recently reported as a novel drug metabolite in two overdose fatalities where there was evidence of methylphenidate and ethanol coingestion. This study explores the pharmacokinetics of ethylphenidate relative to methylphenidate and the major metabolite ritalinic acid, in six healthy subjects who received methylphenidate and ethanol under controlled conditions. Subjects (three males, three females) received a single oral dose of methylphenidate (20 mg; two 10-mg tablets) followed by consumption of ethanol (0.6 g/kg) 30 min later. Methylphenidate, ritalinic acid, and ethylphenidate were quantified using liquid chromatography-tandem mass spectrometry. Ethylphenidate was detectable in the plasma and urine of all subjects after ethanol ingestion. The mean (± S.D.) area under the concentration versus time curve for ethylphenidate was 1.2 ± 0.7 ng/ml/h, representing 2.3 ± 1.3% that of methylphenidate (48 ± 12 ng/ml/h). A significant correlation was observed between the area under the concentration versus time curve of methylphenidate and that of ethylphenidate. In view of the known dopaminergic activity of racemic ethylphenidate, it remains possible that under certain circumstances of higher level dosing, e.g., in the abuse of methylphenidate and ethanol, the metabolite ethylphenidate may contribute to drug effects.

Methylphenidate (Ritalin, (dl)-threo-α-phenyl-2-piperidineacetic acid methyl ester) is the most commonly used psychostimulant in the United States for the treatment of attention-deficit hyperactivity disorder (ADHD)1 and is perhaps the most frequently prescribed psychotropic medication in children (Robison et al., 1999). In humans, the majority of orally administered methylphenidate has been reported to be stereoselectively deesterified (Buggé et al., 1996; see Patrick and Markowitz, 1997) to the inactive metabolite ritalinic acid (Patrick et al., 1981). This metabolite reaches plasma concentrations one to two orders of magnitude greater than that of the parent drug (Redalieu et al., 1991). The toxicology of ethylphenidate has not been established, and the racemic stereoisomers do not exhibit centrally active dopamine transport binding (Schweri et al., 1991). The toxicology of ethylphenidate has not been established.

During cocaine and ethanol abuse, cocaethylene blood concentrations may approach or exceed that of cocaine (Jatlow et al., 1996), and cocaethylene has been reported to be more lethal than cocaine (Hearn et al., 1991). The toxicology of ethylphenidate has not been established.

1 Abbreviations used are: ADHD, attention-deficit hyperactivity disorder; CNS, central nervous system; LC, liquid chromatography; MS, mass spectrometry; AUC, area under the concentration versus time curve; t_{1/2}, terminal phase half-life.

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A racemic ethylphenidate-HCI reference standard was synthesized as previously described (Markowitz et al., 1999). All sample analyses were performed by National Medical Services (Willow Grove, PA) using a novel liquid chromatography (LC)-mass spectrometry (MS)-MS method developed for this study. Both an amino ester and an amino acid internal standard were used to control for the inherent differences in extraction and chromatographic characteristics of the analytes. Thus, after addition of methyl-labeled D₃-methylphenidate (Radian International, Austin, TX) and phenyl-labeled D₉-ritalinic acid (Radian International), 1 ml of plasma or urine was diluted with 1 ml of water and adjusted to pH 6.0 with 2 ml of 0.1 M phosphate buffer. The extraction used a solid-phase procedure using Varian Bond Elute Certify C8 columns (Varian, Harbor City, CA) and the Zymark ZymaPure II vacuum manifold (Zymark, Hopkinton, MA). The final eluent was evaporated under nitrogen and reconstituted with 1 ml of water and adjusted to pH 6.0 with an HCl reference standard.

Detection was by MS-MS using a Micromass Quattro LC/MS/MS (Micromass UK Limited, Manchester, England). The high abundance 384 ions for deuterated methylphenidate and ritalinic acid. Quantitation was achieved by comparison of the chromatographic peak areas of unknowns with those of the standard curves from spiked biological samples. Note that MH⁺ ions were monitored by LC-MS in our previous report of the nanogram per milliliter ethylphenidate values in fatal overdose cases (Markowitz et al., 1999). The peak areas of the m/z 248→84, m/z 234→84, and m/z 220→84 product ions for ethylphenidate, methylphenidate, and ritalinic acid, respectively, were acquired, as were the m/z 237→84 and m/z 225→84 ions for deuterated methylphenidate and ritalinic acid. Quantitation was achieved by comparison of the chromatographic peak areas of unknowns with those of the standard curves from spiked biological samples using the internal standard method. The lower limit of quantitation of methylphenidate was 1.0 ng/ml. The lower limit of quantitation of ritalinic acid was 10 ng/ml. For ethylphenidate, the calibration curve with spiked plasma blanks (0.05, 0.25, 0.5, 2.5, 5 ng/ml) yielded a correlation coefficient (r²) of 0.9999. The limit of quantitation of ethylphenidate was <0.05 ng/ml. Within run c.v. values of methylphenidate and analyzed metabolites ranged from 7 to 10%. Figure 2 illustrates representative LC-MS-MS chromatography from a subject plasma sample after administration of methylphenidate and ethanol.

Plasma Ethanol Analysis. Plasma ethanol determinations were made using the automated Axisym REA ethanol assay system (Abbott Laboratories, Abbott Park, IL) which uses radiative energy attenuation. The lower limit of sensitivity for this automated assay was 13 mg/dl.

Noncompartmental Pharmacokinetic Analysis. The apparent terminal phase half-lives (t₁/₂) for methylphenidate, ethylphenidate, ritalinic acid, and ethanol were determined from the terminal slope of log-transformed plasma

ml of soda water. Subjects consumed this drink within 15 min to minimize intersubject variability in ethanol pharmacokinetics. This dose of ethanol approximated doses used in recent clinical studies of coacetylene formation (Farr et al., 1997; McCance-Katz et al., 1998). The sequence of methylphenidate and ethanol administration was designed to favor their coabsorption. Additional blood samples were taken at 1, 1.5, 2, 3, 4, and 6 h after methylphenidate dosing. Ten-milliliter plastic syringes were used for this purpose, and catheter lines were cleared of residual heparin solution before sampling. The blood was transferred to green-stopped heparinized glass blood collection tubes (Vacutainers; Becton Dickinson, Rutherford, NJ) previously stored in an ice bath, centrifuged at 4°C for 5 min, and the plasma was immediately aspirated into plastic vials and stored at −80°C until analysis. Cumulative urine was collected for 6 h after methylphenidate dosing and the total volume was recorded. An aliquot of urine was retained for analysis of methylphenidate and metabolites.

Materials and Methods

Subjects. All subjects gave written informed consent before participating in the study. The study population consisted of six individuals (three females, three males) aged 24 to 32 years, who were healthy as assessed by medical history, physical examination, 12-lead electrocardiogram, and routine laboratory tests including complete blood count, serum electrolytes, blood glucose, and liver function indices. Additionally, all subjects were nonsmokers and abstained from the use of caffeine-containing beverages for the duration of the study. The study was conducted in compliance with the current National Institute on Alcohol Abuse and Alcoholism (NIAAA) Recommended Council Guidelines on Ethyl Alcohol Administration in Human Experimentation (June, 1989) and did not involve the administration of ethanol to alcohol-naive subjects. Subjects were specifically questioned about any alcohol or substance use history and were asked to answer questions from the Brief Michigan Alcoholism Screening Test (MAST) with an exclusion criteria of scoring ≥2.

Drug Administration and Sampling. All subjects fasted for 8 h before drug administration, and were then fed a standard breakfast consisting of a bagel, cream cheese, and orange juice. An indwelling venous catheter was placed into a forearm vein for serial blood sampling. After obtaining baseline blood pressure, heart rate, and alcohol breathalyzer (Alco-Sensor III; Intoximeters Inc., St. Louis, MO) readings, subjects voided their bladders and were given 20 mg of methylphenidate administered orally as two 10-mg immediate release tablets (Ritalin; Novartis Pharmaceuticals, Summit, NJ) followed by 180 ml of water.

Blood samples (10-ml) were obtained predose and 0.5 h after methylphenidate administration. Immediately after the 0.5-h blood sample was obtained, patients were given an alcoholic drink containing 0.6 g/kg of body weight of ethanol (0.66 ml/kg 95% ethanol) mixed with 180 ml of orange juice and 60
concentration versus time data. Other pharmacokinetic parameters were noted directly from the data or calculated by standard methods (Rowland and Tozer, 1989). The assumption was made that ethanol elimination in the concentration range observed was first order. Calculating ethanol apparent half-lives on this basis using linear regression could overestimate the true “half-life” of ethanol if this assumption is invalid. However, in comparison to previously published values (Holford, 1987), the assumption of first order elimination appears reasonable for the observed data and allowed a practical means of comparison with pharmacokinetic estimates for the other compounds of interest in this study.

Correlations between parameters for individuals were assessed by linear regression analysis (Instat 3.01; GraphPad Software, San Diego, CA). Differences between means of parameters were assessed by the paired Student’s t test (Instat) or repeated measures ANOVA. The level of significance was set at P < 0.05.

Results

The methylphenidate and ethanol combination was well tolerated by all research subjects with no adverse events noted. Ethylphenidate was only detected in plasma after ethanol intake (post 0.5 h), whereas ritalinic acid was detected at the 0.5-h time point immediately before ethanol dosing (Fig. 3). Noncompartmental pharmacokinetic parameters for the compounds of interest are shown in Table 1. As with other pharmacokinetic studies of methylphenidate, ritalinic acid was the major metabolite of methylphenidate (see Patrick and Markowitz, 1997). The mean area under the concentration versus time curve (AUC) for ritalinic acid was 23 ± 4 times greater than that of methylphenidate, whereas the ethylphenidate AUC was only 2.3% ± 1.3 of the mean methylphenidate AUC (Table 1).

There were highly significant correlations between plasma methylphenidate and both metabolite plasma concentrations (r² = 0.48, P < 0.001 for ethylphenidate and r² = 0.70, P < 0.001 for ritalinic acid). Hysteresis was observed in three subjects (one clockwise, two counterclockwise) in time series plots of plasma concentration data for methylphenidate and ethylphenidate. Significant correlations between the AUC values of methylphenidate/ethylphenidate and methylphenidate/ritalinic acid (r² = 0.70, P = 0.037 and r² = 0.66, P = 0.048, respectively) were also found (Table 1). Ethylphenidate and ethanol plasma concentrations were not significantly correlated (r² = 0.11, P = 0.14). Five of the six subjects showed clear hysteresis but not consistent between individuals (two clockwise, three counterclockwise) in time series plots of plasma ethylphenidate versus ethanol plasma concentration data.

Furthermore, there was no significant correlation between 1) eth-
ethylphenidate and ethanol AUC values (r² = .05, P = .86); 2) the mean half-lives of ethanol and ethylphenidate (P = .76, paired Student’s t test); 3) the individual half-life values (r² = .0008, P = .96); 4) differences between tmax values for the analytes of interest (P = .52, repeated measures ANOVA). The t1/2 of ethylphenidate was significantly shorter than that of methylphenidate (P = .0123), but there was no significant correlation between the individual values (r² = .0966, P = .55). The amounts of methylphenidate, ethylphenidate, and ritalinic acid excreted in the urine from 0- to 6-h postmethylphenidate were 1.4 ± 0.8, 0.02 ± 0.1, and 19.9 ± 10.8% of the methylphenidate dose, respectively.

**Discussion**

These data confirm the presence of ethylphenidate as a minor metabolite of methylphenidate when given at a normal clinical dose, followed by a moderate intake of ethanol. The concentration of ethylphenidate detected after ethanol administration appeared to be dependent more on methylphenidate plasma concentration than ethanol concentration. Similarly, cocaethylene formation after cocaine and ethanol intake correlates poorly with blood ethanol concentrations (Bailey, 1996; Brookoff et al., 1996). Surprisingly, the mean t1/2 of ethylphenidate was significantly lower than that of methylphenidate. This result may be partly an artifact of a brief sampling time after single dose administration, as methylphenidate concentration in plasma rapidly declines below the level of assay sensitivity. The elimination rate of ethylphenidate may be dependent on its rate of formation from methylphenidate. In this situation the apparent elimination of ethylphenidate should occur with a half-life similar to methylphenidate. However, after a single oral dose of methylphenidate, the observed plasma concentration of ethylphenidate is the sum of the metabolite formed by first-pass elimination during absorption of methylphenidate and metabolite continuously formed from the absorbed drug. The result is the temporary disappearance of the metabolite at a rate seemingly faster than that of the parent compound. A separate administration of preformed ethylphenidate would be required to clarify the issue of whether the elimination of the metabolite is rate-limited by its formation from methylphenidate. Finally, until chiral chromatographic methods are applied to ethylphenidate determinations, the influence of enantioselective transesterification and/or deesterification on pharmacokinetic parameters cannot be definitively described. The potential significance of this consideration has precedents in the disparate rates of methyl ester hydrolysis for both methylphenidate (see Patrick and Markowitz, 1997) and cocaine (Gatley et al., 1990) enantiomers.

The elimination of ethanol was not significantly different from that of ethylphenidate. These data also imply that increasing the intake of ethanol may not necessarily increase the amount of ethylphenidate formed at a given dose of methylphenidate. However, in the case of cocaethylene formation, it has been suggested that continual intake of ethanol in the presence of cocaine could prolong the elimination of cocaethylene by providing an ongoing source of ethanol for ester exchange (Bourlaid et al., 1998).

Ethanol has been reported to increase circulating plasma concentrations of cocaine in human subjects (McCance-Katz et al., 1993; Roberts et al., 1993; Farrow et al., 1997; McCance-Katz et al., 1998). In view of what may be a prolonged long half-life (mean = 3.6 h) observed for methylphenidate in this study (otherwise typically reported to be in the 2- to 3-h range; see Patrick and Markowitz, 1997), it may be possible that the ethanol interaction with esterases could reduce the rate of conversion of methylphenidate to ritalinic acid, i.e., the process primarily responsible for the short half-life of methylphenidate. A similar hypothesis has recently been advanced by Farrow et al. (1997) to explain the observed elevating effects of ethanol on plasma cocaine concentrations. A crossover study design with a methylphenidate only phase, which includes a greater number of subjects, may be appropriate to confirm such a drug-drug interaction.

Ethylphenidate is probably best recognized as an internal standard for methylphenidate quantitation from biological samples, as has been reported in numerous pharmacokinetic studies (see Patrick et al., 1985). In view of these findings, any ethanol consumption by subjects whose samples were subsequently assayed using ethylphenidate as the internal standard could potentially lead to an underestimation of methylphenidate concentrations.

Ethylphenidate has not been pharmacologically well characterized. However, it is known that ethylphenidate possesses significant CNS activity. Schweri et al. (1985) found that relative to methylphenidate, (dl)-three-ethyphenidate exhibits approximately 50% the potency of methylphenidate in the inhibition of [3H]methylphenidate binding to rat striatal synaptosomal membranes. Portoghese and Malepsis (1961) reported that (dl)-three-ethylphenidate was 80% as active as methylphenidate in inducing locomotor activity in mice. However, it is important to consider that these comparisons were based on the synthetic racemate of ethylphenidate, which may not serve appropriately as an authentic reference standard of metabolically formed ethylphenidate. In that methylphenidate is subject to enantioselective deesterification, which greatly reduces the oral bioavailability of l-methylphenidate relative to d-methylphenidate (see Patrick and Markowitz, 1997), and that the d-methylphenidate enantiomer is primarily responsible for CNS and peripheral activity (Patrick et al., 1987), it is quite possible that such esterase stereoselectivity general-
izes to substrate transesterification as well. Accordingly, the enantio-meric disposition of circulating ethylphenidate may be distorted (from 50:50) and thus prevent any definitive correlation between the established pharmacology of racemic ethylphenidate and the potential pharmacological contribution of the metabolically formed ethylpheni-date of unknown stereochemistry.

Although the toxicity of ethylphenidate has not been examined, the low concentrations of ethylphenidate detected in this study indicate that a single clinically relevant dose of methylphenidate in combination with moderate intake of ethanol is unlikely to result in substantial generation of this metabolite relative to the parent drug. However, this study raises the possibility that at higher doses of methylphenidate, larger ethylphenidate concentrations might contribute to pharmacological effects. Similarly, some controlled studies of cocaethylene formation in humans have found 80% lower concentrations of cocaethylene than those detected in clinical and forensic cases, a disparity likely attributable to the binge use of cocaine and the longer elimination half-life of cocaethylene (Jatlow et al., 1996).

Among alcohol consuming individuals, ethylphenidate formation may have clinical implications in patients receiving doses of methylphenidate in the upper range of clinically useful doses (>1.5 mg/kg/ day), patients with a relatively low clearance of methylphenidate compared with the population average, or, finally, persons abusing methylphenidate. With regard to the latter group, there are a number of documented cases of intranasal abuse of methylphenidate (Jaffe, 1991; Garland, 1998; Massello and Carpenter, 1999), some with fatal consequences (Falzon and Ward, 1996; Massello and Carpenter, 1999). Although never examined under controlled conditions, intranasal methylphenidate would be expected to allow for a much more rapid and perhaps more complete absorption, resulting in much higher blood concentrations (Falzon and Ward, 1996) than by the oral route, where bioavailability is low (see Patrick and Markowitz, 1997).

In conclusion, the detection of ethylphenidate as a metabolite in plasma and urine of the six human subjects studied opens the possibility that such a CNS active metabolite may contribute to the cathcholaminergic effects in certain individuals, depending on their methylphenidate dose and ethanol consumption. The increased recogni-tion, diagnosis, and pharmacological treatment of adult ADHD is well documented (Spencer et al., 1995; Elia et al., 1999). Many of these individuals may consume moderate amounts of ethanol in social situations. Additionally, it is known that a greater risk for sub-stance abuse exists in this population (Biederman et al., 1995). Forthermore, methylphenidate is well recognized as a drug of high abuse potential in the general population (Drug Enforcement Adminis-tration, 1995), and cases of ethanol and methylphenidate coabuse have been documented (Jaffe et al., 1991). Taken together, it appears that coingestion of methylphenidate and ethanol may frequently occur on an acute or chronic basis. Accordingly, toxicoological studies of ethylphenidate may be warranted in the future.

References


