DMD Fast Forward. Published on October 11, 2006 as doi:10.1124/dmd.106.011973
This article has not been copyedited and formatted. The final version may differ from this version.

TITLE PAGE

An Evaluation of the CYP450 Inhibition Potential of Lisdexamfetamine in Human Liver Microsomes

Authors: Suma Krishnan, Scott Moncrief

Affiliations: New River Pharmaceuticals, Blacksburg, VA (S.K., S.M.)

Laboratory of Origin: New River Pharmaceuticals

DMD Fast Forward. Published on October 11, 2006 as DOI: 10.1124/dmd.106.011973
This article has not been copyedited and formatted. The final version may differ from this version.

RUNNING TITLE PAGE

Running Title: Evaluation of the CYP450 Inhibition Potential of Lisdexamfetamine

Corresponding Author's Name/Addresses/Telephone & Fax Numbers:
Suma Krishnan, MS
1861 Pratt Drive, Suite 1090
Blacksburg, VA 24060
Telephone: (540) 953-0237
Fax: (540) 953-3407
E-mail: skrishnan@nrpharma.com

Number of Text Pages: 24 (includes acknowledgment, references, footnotes, tables)
Number of Tables: 4
Number of Figures: 0
Number of References: 23 (40 max)
Abstract Word Count: 234 (250 max)
Introduction Word Count: 686 (750 max)
Discussion Word Count: 382 (1500 max)

Abbreviations: acetaminophen (APAP), adverse event (AE), attention-deficit/hyperactivity disorder (ADHD), d_4-acetaminophen (d_4-APA), dextrorphan (DRR), human cytochrome P450 (CYP450), hydroxybupropion (OHBP), 7-hydroxycoumarin (7OHCMN), 4′-hydroxymephenytoin (4HMPN), 1′-hydroxymidazolam (1OHMDZ), 6β-hydroxytestosterone (6βT), d_3-6β-hydroxytestosterone (d_3-6βT), hydroxytolbutamide (HTB), intranasal (IN), intravenous (IV), lethal dose (LD_{50}), triethylenethiophosphoramide (thioTEPA)

Chemical nomenclature of investigational compound: (2S, 2;S)=2,6-diamino-N-(1-phenylpropan-2-yl) hexamide di-methanesulfonate; molecular weight: 455.59; generic name: lisdexamfetamine dimesylate; CAS numbers: 608137-32-2 (free base); 608137-33-3 (mesylate)

Abstract

The human cytochrome P450 (CYP450) system is implicated in many drug interactions. Lisdexamfetamine dimesylate (NRP104), the proposed generic name for a new agent under investigation for treatment of attention-deficit/hyperactivity disorder (ADHD), was recently analyzed for inhibitory drug-drug interactions with 7 major CYP450 isoforms using pooled human liver microsomes. Probe substrates were used near the K_m concentration values reported in the literature for CYP1A2 (phenacetin), CYP2A6 (coumarin), CYP2B6 (bupropion), CYP2C9 (tolbutamide), CYP2C19 ([*S*]-mephenytoin), CYP2D6 (dextromethorphan), and CYP3A4 (midazolam and testosterone), and lisdexamfetamine was evaluated at concentrations ranging from 0.01 to 100 μM for its ability to inhibit the activity of these 7 CYP450 isoforms. NADPH was added to one set of samples to initiate metabolic reactions, which were then terminated by adding organic solvent, vortexing the samples, and placing them on ice. The relevant substrates were then introduced to both sets of samples so that the percentage of remaining activity could be measured and compared. In addition, these samples were compared with other samples with the same concentrations of lisdexamfetamine but without preincubation. None of the 7 CYP450 isoforms demonstrated any concentration-dependent inhibition. Comparison of results from microsomes preincubated with and without NADPH showed no mechanism-based inhibition. Neither concentration-dependent nor mechanism-based inhibition due to time-dependent inactivation of human CYP450 isoforms was demonstrated for lisdexamfetamine during *in vitro* testing. The evidence suggests that lisdexamfetamine has a low potential for drug-drug interactions or initiation of drug-drug interactions.

Approximately 3.9 million children have been diagnosed with ADHD, making it one of the most common behavioral disorders of childhood (Centers for Disease Control and Prevention, 2005). Epidemiologic studies have estimated the prevalence of ADHD in school-aged children to be 8% to 10% (American Academy of Pediatrics, 2000; Dulcan, 1997). Using efficacious, well-tolerated drug treatments with a decreased likelihood of drug interactions in this vulnerable population is of paramount importance.

Food and Drug Administration approval is being sought for lisdexamfetamine dimesylate (proposed generic name), a medication developed for treatment of attention-deficit/hyperactivity disorder (ADHD) in children (Mickel et al., 2006). Lisdexamfetamine is an inactive prodrug where *d*-amphetamine is covalently bonded to *l*-lysine, an essential amino acid. After ingestion, the pharmacologically active *d*-amphetamine is released when the covalent bond is cleaved during metabolism. The covalent bond linking *l*-lysine to *d*-amphetamine is an amide bond and the structure of lisdexamfetamine resembles a dipeptide. A proteolytic enzyme or enzymes, therefore, are likely responsible for the biotransformation of the prodrug to release *d*-amphetamine. Lisdexamfetamine is stable to hydrolysis in serum and minimal amounts of *d*-amphetamine are released when the drug is administered by parenteral routes (Mickel et al., 2006). When administered at therapeutic doses by the intended oral route lisdexamfetamine releases *d*-amphetamine bioequivalent to equimolar doses of *d*-amphetamine sulfate. The specific enzyme or enzymes that metabolize lisdexamfetamine thus appear to be digestive enzymes that are more abundant in the gastrointestinal tract (Mickel et al., 2006). Lisdexamfetamine was designed to have comparable efficacy and tolerability to that of current

extended-release stimulant formulations used to treat ADHD, but with reduced potential for abuse, diversion and overdose toxicity (Jasinski and Krishnan, 2006).

Animal studies have indicated that in rats, the LD_{50} of the lisdexamfetamine is 5 times larger than that reported for amphetamine sulfate (unpublished observations). In comparison with *d*-amphetamine sulfate, exposure to *d*-amphetamine is decreased and delayed after intravenous (IV) and intranasal (IN) administration and at high oral doses above the therapeutic level of lisdexamfetamine (Mickle et al., 2006).

Although the toxicity and tolerability of lisdexamfetamine appear to be favorable, if drug-drug interactions result in serious AEs, it could limit the usefulness of the compound. One of the most important determinants of potential drug interactions is the human cytochrome P450 (CYP450) system. The CYP450 system is a family of heme protein isozymes responsible for the oxidative metabolism of drugs, environmental contaminants, and xenobiotics (Prough and Spearman, 1987; Yang and Lu, 1987; Peter et al., 1990; Wrighton et al., 1993). CYP450 drug interactions usually result from either enzyme inhibition or enzyme induction (Cupp and Tracy, 1998). Inhibition may be competitive or noncompetitive, as well as reversible or irreversible as observed through damage to the heme or apoprotein, such as with cimetidine and chloramphenicol (Nedelcheva and Gut, 1994). Interactions may also be potentiated by genetic polymorphisms, as some individuals are poor metabolizers through certain CYP450 isozymes, while others are extensive metabolizers (Goldstein et al., 1994; Nedelcheva and Gut, 1994; Cupp and Tracy, 1998). Adverse reactions that may not occur when a drug is administered alone may emerge when the compound is taken in conjunction with other medications that either induce or

inhibit one or more CYP450 isozymes (Cupp and Tracy, 1998). Inhibition may result in elevated serum levels of the unmetabolized agent, which can cause increases in both primary and adverse effects. Conversely, if the primary compound is a prodrug, inhibition may result in delayed or inhibited onset of action and reduced efficacy.

Although CYP450 isozymes can be found in many body tissues, they are most prevalent in the liver (Cupp and Tracy, 1998). The isozymes of the CYP3A subfamily are the most abundant cytochrome enzymes in humans, constituting 30% of liver cytochrome P450 enzymes, and are involved in many clinically important drug interactions (Shimada et al., 1994; Slaughter and Edwards, 1995; Cupp and Tracy, 1998). Because of interspecies differences in substrate specificities for some CYP450 isozymes (Waxman et al., 1988), in vitro human liver microsomes were used in this study to evaluate the likelihood of drug-drug interactions for lisdexamfetamine. The isozymes CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 were included in the investigation.

Methods

Chemicals

The test compound, lisdexamfetamine (NRP104), was supplied by Organichem Corp (Rensselaer, NY), with a certificate of analysis reporting 99.4% purity. A purity of 100% was assumed for preparation of spiking solutions, which was completed on a daily basis so that a 2 μL spike of the appropriate solution into 500 μL of total volume would achieve the desired concentration. Potassium phosphate monobasic, potassium phosphate dibasic, sodium carbonate, NADPH tetrasodium salts, and other reagents were purchased from Sigma Chemical Co (St. Louis, Mo) or equivalent vendors. Methanol, acetonitrile, and water (all HPLC grade) and ethyl acetate, acetone, and other solvents were purchased from Fisher Scientific International, Inc (Hampton, NH), Honeywell Burdick & Jackson (Muskegon, Mich), Mallinckrodt Baker Inc (Phillipsburg, NJ), or equivalent vendors. The following compounds were supplied by CellzDirect Inc (Pittsboro, NC): phenacetin, acetaminophen (APAP), d_4-acetaminophen (d_4-APA), α-naphthoflavone, coumarin, 7-hydroxycoumarin (7OHCMN), 4-methyl-7-hydroxycoumarin, tranylcypromine, bupropion, hydroxybupropion (OHBP), trazodone, triethylenethiophosphoramide (thioTEPA), tolbutamide, hydroxytolbutamide (HTB), d_9-hydroxytolbutamide, sulfaphenazole, (*S*)-mephenytoin, 4′-hydroxymephenytoin (4HMPN), dextromethorphan, dextrorphan (DRR), quinidine, midazolam, 1′-hydroxymidazolam (1OHMDZ), α-hydroxytriazolam, testosterone, 6β-hydroxytestosterone (6βT), d_3-6β-hydroxytestosterone (d_3-6βT), and ketoconazole. All were of the highest purity available. Stock solutions were prepared according to CellzDirect test methods.

DMD Fast Forward. Published on October 11, 2006 as DOI: 10.1124/dmd.106.011973
This article has not been copyedited and formatted. The final version may differ from this version.

Human Liver Microsomes

Human liver microsomes pooled from males and females (N = 15) were provided by CellzDirect for use in this study. The microsomes previously characterized for major CYP450 activity by the In Vitro Drug Development Services Division of CellzDirect were CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4.

Analytical Methods and Incubation Procedure

Human liver microsomal samples were preincubated with lisdexamfetamine, both with and without NADPH, at 37 °C for 15 minutes. An incubation buffer of 0.5 mL, 100 mM potassium phosphate buffer, pH 7.4, was used in all reaction mixtures, all of which met calibration and quality control (QC) standards. All samples were injected onto a Micromass Quattro II LC-MS/MS equipped with an appropriate HPLC column. Quantitation for all of the following was performed with a weighted (1/x) linear least squares regression analysis generated from fortified matrix calibration standards—except for hydroxybupropion (CYP2B6) and 6β-hydroxytestosterone (CYP3A4), for which a weighted ($1/x^2$) linear least squares regression analysis was used.

Microsomal incubations for IC_{50} estimation. The ability of the test compound to inhibit the activity of each of the 7 CYP450 isoforms was evaluated in pooled human liver microsomes. The tested isoforms, their respective marker substrates, and incubation conditions are summarized in Table 1. Human liver microsomes diluted in 100 mM potassium phosphate buffer at pH 7.4 were spiked with the appropriate marker substrate and lisdexamfetamine (0, 0.01, 0.1, 1, 10, and 100 μM), and the triplicate suspensions were allowed to equilibrate. Total incubation volumes were

DMD Fast Forward. Published on October 11, 2006 as DOI: 10.1124/dmd.106.011973
This article has not been copyedited and formatted. The final version may differ from this version.

approximately 0.5 mL. The final concentration of each substrate was near the K_m value reported in the literature (Waxman et al., 1988; Miles et al., 1990; Peter et al., 1990; Veronese et al., 1991; Wrighton et al., 1993; Goldstein et al., 1994; Gorski et al., 1994; Kerry et al., 1994; Kobayashi et al., 1999; Faucette et al., 2000; Hesse et al., 2000). Metabolic reactions were initiated by adding NADPH (1 mM final concentration). Reactions were terminated by adding organic solvent, vortexing, and placing on ice. Samples were then extracted and analyzed as previously described.

Determination of APAP (CYP1A2). The marker metabolite APAP was quantitated from 1 to 1000 ng/mL. Freshly prepared samples containing APAP in a reaction mixture containing 0.1 mg/mL final human liver microsomal protein were treated with ethyl acetate and the internal standard d_4-APAP. All samples were then basified and extracted in ethyl acetate. The organic layer was transferred to a new tube, evaporated to dryness, reconstituted in a mobile phase, and injected onto a Micromass Quattro II LC-MS/MS equipped with an appropriate HPLC column. Peak areas of the m/z 152→110 product ion of APAP were measured against peak areas of the m/z 156→114 product ion of the deuterated internal standard.

Determination of 7-hydroxycoumarin (CYP2A6). The marker metabolite 7OHCMN was quantitated from 0.25 to 100 ng/mL. Freshly prepared samples containing 7OHCMN in a reaction mixture containing 0.025 mg/mL final human liver microsomal protein were treated with ethyl acetate and the internal standard 4-methyl-7-hydroxycoumarin. All samples were then acidified. The organic layer was transferred to a new tube, evaporated to dryness, reconstituted in a mobile phase, and injected onto a Micromass Quattro II LC-MS/MS equipped with an

appropriate HPLC column. Peak areas of the m/z 161→133 product ion of 7OHCMN were measured against peak areas of the m/z 175→133 product ion of the internal standard.

Determination of hydroxybupropion (CYP2B6). The marker metabolite OHBP was quantitated from 1 to 1000 ng/mL. Freshly prepared samples containing OHBP in a reaction mixture containing 0.25 mg/mL final human liver microsomal protein were treated with isoamyl alcohol/ethyl acetate (1:100, v:v) and the internal standard trazodone. A saturated sodium chloride solution was added to all samples. The organic layer was transferred to a new tube, evaporated to dryness, reconstituted in a mobile phase, and injected onto a Micromass Quattro II LC-MS/MS equipped with an appropriate HPLC column. Peak areas of the m/z 257→239 product ion of OHBP were measured against peak areas of the m/z 372→176 product ion of the internal standard.

Determination of hydroxytolbutamide (CYP2C9). The marker metabolite HTB was quantitated from 10 to 2000 ng/mL. Freshly prepared samples containing HTB in a reaction mixture containing 0.1 mg/mL final human liver microsomal protein were treated with acetone and the internal standard d_9-hydroxytolbutamide. The samples were acidified and extracted into diethyl ether:ethyl acetate (1:1, v:v). The organic layer was transferred to a new tube, evaporated to dryness, reconstituted in a methanol:ammonium acetate buffer (40:60, v:v), and injected onto a Micromass Quattro II LC-MS/MS equipped with an appropriate HPLC column. Peak areas of the m/z 285→186 product ion of HTB were measured against peak areas of the m/z 294→186 product ion of the internal standard.

Determination of 4′-hydroxymephenytoin (CYP2C19). The marker metabolite 4HMPN was quantitated from 5 to 1000 ng/mL. Freshly prepared samples containing 4HMPN in a reaction mixture containing 0.1 mg/mL final human microsomal protein were treated with acetone. The samples were acidified and extracted into diethyl ether:ethyl acetate (1:1, v:v). The organic layer was transferred to a new tube, evaporated to dryness, reconstituted in a mobile phase, and injected onto a Micromass Quattro II LC-MS/MS equipped with an appropriate HPLC column. Peak areas of the m/z 233→161 product ion of 4HMPN were measured.

Determination of dextrorphan (CYP2D6). The marker metabolite DRR was quantitated from 5 to 1000 ng/mL. Freshly prepared samples containing DRR in a reaction mixture containing 0.1 mg/mL final human liver microsomal protein were treated with acetone. The samples were basified with NaOH and extracted into ethyl acetate. The organic layer was transferred to a new tube, evaporated to dryness, reconstituted in a mobile phase, and injected onto a Micromass Quattro II LC-MS/MS equipped with an appropriate HPLC column. Peak areas of the m/z 258→157 product ion of DRR were measured.

Determination of 1′-hydroxymidazolam (CYP3A4). The marker metabolite 1OHMDZ was quantitated from 0.5 to 200 ng/mL. Freshly prepared samples containing 1OHMDZ in a reaction mixture containing 0.025 mg/mL final human liver microsomal protein were treated with ethyl acetate and the internal standard α-hydroxytriazolam. The organic layer was transferred to a new tube, evaporated to dryness, reconstituted in a mobile phase, and injected onto a Micromass Quattro II LC-MS/MS equipped with an appropriate HPLC column. Peak areas of the m/z

342→168 product ion of 1OHMDZ were measured against peak areas of the m/z 359→331 product ion of the internal standard.

Determination of 6β-hydroxytestosterone (CYP3A4). The marker metabolite 6βT was quantitated from 5 to 5000 ng/mL. Freshly prepared samples containing 6βT in a reaction mixture containing 0.05 mg/mL final human liver microsomal protein were treated with ethyl acetate and the internal standard d_3-6βT. The organic layer was transferred to a new tube, evaporated to dryness, reconstituted in 1 mM ammonium acetate buffer, pH 2.5:ethanol (1:1, v:v), and injected onto a Micromass Quattro II LC-MS/MS equipped with an appropriate HPLC column. Peak areas of the m/z 305→269 product ion of 6βT were measured against peak areas of the m/z 308→272 product ion of the internal standard.

QC samples and incubations. Analytical QC samples containing high, medium, or low concentrations of the appropriate metabolite were examined within each group of incubation samples. Analytical runs were accepted only if two thirds of all QC samples and at least 50% of the QC samples at each level were ±25% theoretical value. To ensure that the enzymatic reaction was operating within the criteria established during the CellzDirect validation of each individual assay, a series of duplicate QC incubations (QC_i) at either 5 or 6 different substrate concentrations was conducted parallel to each study incubation. The QC_i were used to estimate the K_m value for the substrate/enzyme. Results from the study incubation were considered acceptable if the K_m value estimated from the samples fell between one third and 3 times the mean K_m value established during the CellzDirect validation exercise. In addition, the specificity of experimental conditions was evaluated with specific inhibitors to verify that the experimental

conditions for IC_{50} values were susceptible to inhibition. The specific inhibitors used during these incubations were furafylline (CYP1A2), tranylcypromine (CYP2A6), thio-TEPA (CYP2B6), sulfaphenazole (CYP2C9), ticlopidine (CYP2C19), quinidine (CYP2D6), and ketoconazole (CYP3A4). Results were considered satisfactory if significant inhibition (>50%) was observed.

Mechanism-based inhibition screening. Triplicate samples for mechanism-based inhibition screening were preincubated for 15 minutes at 37 °C with 1 μM lisdexamfetamine with or without NADPH. The percentage of remaining activity of CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 was measured with the relevant substrates—phenacetin, coumarin, bupropion, tolbutamide, (*S*)-mephenytoin, dextromethorphan, midazolam, and testosterone—at single concentrations approximating their respective apparent K_m values (Table 2). In addition, the potential of lisdexamfetamine to cause mechanism-based inhibition of CYP450 in pooled human liver microsomes was evaluated. Metabolite formation for each activity was monitored with the validated LC-MS/MS method described earlier. The percentage of remaining activity of microsomes preincubated with lisdexamfetamine and NADPH was compared with that of microsomes preincubated with lisdexamfetamine without NADPH. These preincubated samples were also compared with samples preincubated with and without the test compound.

Data Analyses

Micromass Masslynx (version 3.4, Manchester, UK) was used to compile and process chromatographic data graphed with Microsoft Excel® 97 (Redmond, Wash). Reaction velocities were calculated with the equation:

$$V\ (\text{nmol/min/mg}) = \text{calculated ng/mL} \times 0.5\ \text{mL (MW ng/nmol)/min/mg protein}$$

K_m and V_{max} values were estimated by nonlinear regression analysis of the data, using the Michaelis-Menten equation and Systat® 6.0.1 (SPSS Inc, Chicago, Ill).

Results

Estimated IC_{50}

Incubation of lisdexamfetamine in microsomal suspensions at concentrations ranging from 0.01 to 100 μM demonstrated no concentration-dependent inhibition for any of the isoenzymes under investigation (Table 3). Since there was no inhibition from 0.01 to 100 μM, IC_{50} values were not determined. Percentage of remaining activity ranged from 82.4% for CYP3A4 (6βT), with lisdexamfetamine at a concentration of 1 μM, to 111% for CYP3A4 (MDZ), with lisdexamfetamine at a concentration of 0.1 μM.

Mechanism-Based Inhibition

Comparison of microsomes preincubated for 15 minutes with lisdexamfetamine 1 μM and NADPH with microsomes preincubated with lisdexamfetamine without NADPH indicated no mechanism-based inhibition (Table 4). Whereas results from non-preincubated samples indicate that the mean percentage of remaining activity ranges from 82.4% for CYP3A4-6βT to 102% for CYP2C19, samples preincubated for 15 minutes with NADPH had a mean percentage of remaining activity ranging from 78.8% for CYP1A2 to 102% for CYP3A4-6βT. Samples preincubated without NADPH had a mean percentage of remaining activity ranging from 62.3% for CYP3A4-6βT to 97.1% for CYP3A4-MDZ.

Discussion

This is the first investigation of the CYP450 interaction with lisdexamfetamine. Incubation of lisdexamfetamine with human liver microsomes resulted in no concentration-dependent or mechanism-based inhibition of any of the isoenzymes under investigation—CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4.

Stimulant medications remain the gold-standard treatment for ADHD. However, some concerns about their potential for abuse, diversion and overdose toxicity still remain. If lisdexamfetamine is shown to have tolerability and efficacy profiles similar to those of the commonly used extended-release stimulants, it could be an important therapeutic option for ADHD.

Metabolism of amphetamine primarily involves oxidation of the benzene ring at the 4 position to from 4-hydroxy-amphetamine and on the α or β carbons to form alpha-hydroxy-amphetamine or norephedrine, respectively. Both 4-hydroxy-amphetamine and norephedrine are active and are further metabolized to form 4-hydroxy-norephedrine. Alpha-hydroxy-amphetamine is deaminated to form phenylacetone, which is further metabolized to benzoic acid and hippuric acid. The enzymes involved in amphetamine metabolism have not been fully defined; however, CYP2D6 is responsible for formation of 4-hydroxy-amphetamine (Bach et al., 1999). Inhibition of CYP2D6 by amphetamine and inhibition of CYP1A2, 2D6 and 3A4 by one or more of its metabolites has been observed with human microsomal preparations. Amphetamine is also known to inhibit monoamine oxidase. Hydrolysis of lisdexamfetamine results in exposure to *d*-amphetamine and therefore lisdexamfetamine could be predicted to ultimately have a similar

potential for drug-drug interactions as amphetamine. *In vitro* results in this study, however, indicate that any interactions would likely be due to amphetamine and its metabolites rather than the intact lisdexamfetamine prodrug.

Potential drug-drug interactions are a serious concern with any compound. Problems with CYP450 inhibition can result in increased serum levels of unmetabolized drug, possibly leading to AEs or even toxic effects. Failure to detect any significant inhibition of the CYP450 system by lisdexamfetamine is therefore a significant finding. No significant inhibition of the CYP450 system by lisdexamfetamine suggests that this drug could be a safe therapeutic option for ADHD.

Results from this study demonstrate that CYP450 inhibition does not occur to any significant degree with lisdexamfetamine. There is good reason to believe that the results of this in vitro study will correlate well with in vivo effects. Therefore, the data presented here for lisdexamfetamine suggest that this agent has low potential for interactions with other drugs.

Acknowledgment

Editorial assistance provided by NeoHealth, Inc.

References

American Academy of Pediatrics (2000) Clinical practice guideline: diagnosis and evaluation of the child with attention-deficit/hyperactivity disorder. *Pediatrics* **105**: 1158-1170.

Bach MV, Coutts RT, Baker GB (1999) Involvement of CYP2D6 in the in vitro metabolism of amphetamine, two N-alkylamphetamines and their 4-methoxylated derivatives. *Xenobiotica* **29**:719-732.

Centers for Disease Control and Prevention (2005) National Center for Health Statistics. *FASTATS—Attention Deficit Hyperactivity Disorder.* Available at: http://www.cdc.gov/nchs/fastats/adhd.htm. Accessed December 9, 2005.

Cupp MJ and Tracy TS (1998) Cytochrome P450: New nomenclature and clinical implications. *Am Fam Phys* **57**:107-116.

Dulcan M (1997) Practice parameters for the assessment and treatment of children, adolescents, and adults with attention-deficit/hyperactivity disorder. American Academy of Child and Adolescent Psychiatry. *J Am Acad Child Adolesc Psychiatry* **36** (suppl 10): 85S-121S.

Faucette SR, Hawke RL, Lecluyse EL, Shord SS, Yan B, Laethem RM and Lindley CM (2000) Validation of bupropion hydroxylation as a selective marker of human cytochrome P450 2B6 catalytic activity. *Drug Metab Dispos* **28**:1222-1230.

Goldstein JA, Faletto MB, Romkes-Sparks M, Kitareewan S, Raucy JL, Lasker JM and Ghanayem BI (1994) Evidence that CYP2C19 is the major (*S*)-mephenytoin 4'-hydroxylase in humans. *Biochemistry* **33**:1743-1752.

Gorski JC, Hall SD, Jones DR, VandenBranden M and Wrighton SA (1994) Regioselective biotransformation of midazolam by member of the human cytochrome P450 3A (CYP3A) subfamily. *Biochem Pharmacol* **47**:1643-1653.

Hesse LM, Venkatakrishnan K, Court MH, von Moltke LL, Duan SX, Shader RI and Greenblatt DJ (2000) CYP2B6 mediates the in vitro hydroxylation of bupropion: Potential drug interactions with other antidepressants. *Drug Metab Dispos* **28**:1176-1183.

Jasinski DR and Krishnan S (2006) Abuse liability of intravenous L-lysine-*d*-amphetamine (NRP104). Abstracts, 68th Annual Scientific Meeting of the College on Problems of Drug Dependence, Scottsdale, AZ.

Kerry NL, Somogyi AA, Bochner F and Mikus G (1994) The role of CYP2D6 in primary and secondary oxidative metabolism of dextromethorphan: In vitro studies using human liver microsomes. *Br J Clin Pharmacol* **38**:243-248.

Kobayashi K, Nakajima M, Oshima K, Shimada N, Yokoi T and Chiba K (1999) Involvement of CYP2E1 as a low-affinity enzyme in phenacetin *O*-deethylation in human liver microsomes. *Drug Metab Dispos* **27**:860-865.

Mickle T, Krishnan S, Bishop B, Lauderback C, Moncrief JS, Oberlender RO, and Piccariello T (2006) Abuse-resistant amphetamine compounds. US Patent 7,105,486.

Miles JS, McLaren AW, Forrester LM, Glancey MJ, Lang MA and Wolf CR (1990) Identification of the human liver cytochrome P450 responsible for coumarin 7-hydroxylase activity. *Biochem J* **267**:365-371.

Nedelcheva V and Gut I (1994) P450 in the rat and man: Methods of investigation, substrate specificities and relevance to cancer. *Xenobiotica* **24**:1151-1175.

Peter R, Bocker R, Beaune PH, Iwasaki M, Guengerich FP and Yang CS (1990) Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P450-IIE1. *Chem Res Toxicol* **3**:566-573.

Prough RA and Spearman ME (1987) Endogenous and dietary chemicals: Metabolism and interaction with cytochrome P450, in *Mammalian Cytochromes P450* (Guengerich FP ed) vol 2 pp 153-172, CRC Press, Boca Raton, Fla.

Shimada T, Yamazaki H, Mimura M, Inui Y and Guengerich FP (1994) Interindividual variations in human liver cytochrome P450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: Studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270**:414-423.

Slaughter RL and Edwards DJ (1995) Recent advances: The cytochrome P450 enzymes. *Ann Pharmacother* **29**:619-624.

Veronese ME, Mackenzie PI, Doecke CJ, McManus ME, Miners JO and Birkett DJ (1991) Tolbutamide and phenytoin hydroxylations by cDNA-expressed human liver cytochrome P450 2C9. *Biochem Biophys Res Commun* **175**:1112-1118.

Waxman DJ, Attisano C, Guengerich FP and Lapenson DP (1988) Human liver microsomal steroid metabolism: Identification of the major microsomal steroid hormone 6β-hydroxylase cytochrome P450 enzyme. *Arch Biochem Biophys* **263**:424-436.

Wrighton SA, Stevens JC, Becker GW and VandenBranden M (1993) Isolation and characterization of human liver cytochrome P450 2C19: Correlation between 2C19 and *S*-mephenytoin 4'-hydroxylation. *Arch Biochem Biophys* **306**:240-245.

Yang CS and Lu AYH (1987) The diversity of substrates for cytochrome P450, in *Mammalian Cytochromes P450* (Guengerich FP ed) vol 2 pp 1-18, CRC Press, Boca Raton, Fla.

Supported by New River Pharmaceuticals, Inc and Shire Development, Inc.

Address correspondence to: Suma Krishnan, MS, 1861 Pratt Drive, Suite 1090, Blacksburg, VA 24060. E-mail: skrishnan@nrpharma.com

DMD Fast Forward. Published on October 11, 2006 as DOI: 10.1124/dmd.106.011973
This article has not been copyedited and formatted. The final version may differ from this version.

Tables

Table 1. Isoforms Tested, Marker Substrates, and Incubation Conditions for IC_{50} Estimation

Isoform Monitored	Marker Substrate	Substrate Concentration (μM)	Incubation Time (min)	Protein Concentration (mg/mL)	Metabolite Formed
CYP1A2	Phenacetin	50	30	0.1	Acetaminophen
CYP2A6	Coumarin	1	5	0.025	7-Hydroxycoumarin
CYP2B6	Bupropion	125	20	0.25	Hydroxybupropion
CYP2C9	Tolbutamide	140	20	0.1	Hydroxytolbutamide
CYP2C19	(*S*)-Mephenytoin	50	30	0.1	4′-Hydroxymephenytoin
CYP2D6	Dextromethorphan	7.5	30	0.1	Dextrorphan
CYP3A4	Midazolam	5	4	0.025	1′-Hydroxymidazolam
CYP3A4	Testosterone	50	7	0.05	6β-Hydroxytestosterone

Table 2. Quality Control K_m Estimates for Each Acceptable Incubation

Isoform	Marker Substrate	Range of Acceptance* (μM)	Estimated K_m (μM)
CYP1A2	Phenacetin	11 - 100	35
CYP2A6	Coumarin	0.40 - 3.6	1.2
CYP2B6	Bupropion	43 - 390	180
CYP2C9	Tolbutamide	56 - 500	140
CYP2C19	(*S*)-Mephenytoin	13 - 120	32
CYP2D6	Dextromethorphan	2.3 - 21	7.4
CYP3A4	Midazolam	0.59 - 5.3	1.8
CYP3A4	Testosterone	21 - 190	120

DMD Fast Forward. Published on October 11, 2006 as DOI: 10.1124/dmd.106.011973
This article has not been copyedited and formatted. The final version may differ from this version.

Table 3. Percentage of Remaining Activity for All Isoforms Tested in Lisdexamfetamine IC_{50} Studies

μM Lisdexamfetamine	Isoform							
	CYP1A2	CYP2A6	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP3A (MDZ)	CYP3A4 (6βT)
0.01	99.6	102	101	96.6	95.2	98.4	107	97.0
0.1	99.7	96.7	99.3	93.6	98.4	98.3	111	94.5
1	97.8	101	101	91.4	102	101	95.4	82.4
10	95.1	98.2	88.9	90.6	99.6	97.8	98.6	94.6
100	94.2	92.5	90.8	89.0	87.3	90.0	92.1	101

Table 4. Effect of Lisdexamfetamine Preincubation on Level of Inhibition of CYP450 Marker Metabolite Formation

Isoform	Lisdexamfetamine (1 μM) Mean % Remaining Activity		
	Without Preincubation	15 min Preincubation	
		NADPH	Without NADPH
CYP1A2	97.8	77.2	88.2
CYP2A6	101	78.8	90.3
CYP2B6	101	80.7	83.2
CYP2C9	91.4	86.1	80.6
CYP2C19	102	87.1	81.6
CYP2D6	101	95.5	88
CYP3A4-MDZ	95.4	100	97.1
CYP3A4-6βT	82.4	102	62.3

NADPH indicates nicotinamide adenine dinucleotide phosphate.