TOXICOLOGY AND FORENSIC MEDICINE

Openventio PUBLISHERS

ISSN 2474-8978 Open Journal http://dx.doi.org/10.17140/TFMOJ-2-120

Research

*Corresponding author
David R. Wallace, PhD
Professor
Department of Pharmacology and Physiology
Oklahoma State University Center for Health Sciences
1111 West 17th Street
Tulsa, Oklahoma 74107-1898, USA
Tel. +1-918-561-1407
Fax: +1-918-561-5729
E-mail: david.wallace@okstate.edu

Volume 2 : Issue 2
Article Ref. #: 1000TFMOJ2120

Article History
Received: August 2nd, 2017
Accepted: August 17th, 2017
Published: August 17th, 2017

Citation
Wallace DR, Crosswy KK. Interactions between over-the-counter and illicit drugs utilizing cytochrome p450 metabolism: Potential for exacerbation of pharmacological response. *Toxicol Forensic Med Open J*. 2017; 2(2): 51-62. doi: 10.17140/TFMOJ-2-120

Interactions between Over-the-Counter and Illicit Drugs Utilizing Cytochrome P450 Metabolism: Potential for Exacerbation of Pharmacological Response

David R. Wallace, PhD[1,2*]; Kara K. Crosswy, MS[2]

[1]Department of Pharmacology and Physiology, Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma 74107-1898, USA

[2]Department of Forensic Sciences, Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma 74107-1898, USA

ABSTRACT

Aim: To determine the interaction of over-the-counter (OTC) and illicit psychostimulants at the cytochrome P450 enzyme, CYP2D6. CYP2D6 is responsible for 20% of hepatic Phase I metabolism and is a site of drug-drug interactions, leading to increased drug toxicity.
Materials and Methods: We examined the effects the OTC drugs; 1) the prototype H_2-antagonist cimetidine (CMT) and 2) the opioid agonist cough suppressant dextromethorphan (DEX); as well as two scheduled drugs, methamphetamine (MA) and 3,4-methylenedioxymethamphetamine (MDMA) for their ability to interfere with CYP2D6 activity. Assays with human CYP2D6 determined the inhibitory potential (IC_{50}) of each drug. Kinetic analysis (V_{max} and K_m) was accomplished using rodent hepatic microsomes.
Results: Maximum inhibition of CYP2D6 activity following exposure to CMT+MDMA was significantly reduced 75-85% compared to quinidine (control) values. These data showed inhibitory effects in CYP2D6 activity in each compound tested. Alterations in CYP2D6 activity may result in complex drug-drug interactions leading to elevated plasma levels of drugs and increased risk for toxicity. Assays using rat CYP2D2 demonstrated V_{max} elevations in the CMT group (493%) compared to control (naïve, no treatment) values (19.9±5.1 pmol/mg protein/min). The K_m was increased 218% in CMT compared to controls (3.1±0.5 μM). Collectively, all MA challenged groups exhibited increases in total enzyme [V_{max}; 280-490%] and affinity [K_m; 165-220%] values compared to the control group. The increase in both V_{max} and K_m suggests that the low affinity/high capacity CYP2D2 isoform is upregulated.
Conclusion: Our findings suggest that *in vivo*, MA acts as a CYP2D2-inducer, which will lead to altered secondary drug metabolism, increasing the risk of drug-related toxicity. Coupled with the ability of CMT and DEX to interfere with MA metabolism, a complex drug-drug interaction is possible, leading to increased toxicity. Our findings substantiate the hypothesis that the combination of illicit and OTC drugs could result in complex drug-drug interactions increasing the risk for severe drug-related toxicity.

KEY WORDS: Cimetidine; Methamphetamine; Dextromethorphan; Ecstasy; CYP2D6; Hepatosomes; Methylenedioxymethamphetamine.

ABBREVIATIONS: MA: Methamphetamine; AM: Amphetamine; OTC: Over-the-counter; MDMA: 3,4-Methylenedioxymethamphetamine; CMT: Cimetidine; DEX: Dextromethorphan; CYP: Cytochrome P450; NE: Norepinephrine; DA: Dopamine; 5-HT: Serotonin; MDA: 3,4-Methylenedioxymethamphetamine; AMMC: 3-[2-(*N,N*-diethyl-N-methylamino) ethyl]-7-methoxy-4-methylcoumarin; HHMA: 3,4-Dihydroxymethamphetamine; AHMC: 3-[2-(*N,N*-diethylamino)ethyl]-7-hydroxy-4-methylcoumarin; PCP: Phencyclidine; DXO: Dextrorphan.

TOXICOLOGY AND FORENSIC MEDICINE
Open Journal
Openventio PUBLISHERS
ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

INTRODUCTION

The cytochrome P450 (CYP) enzyme system is a superfamily of hemoproteins that catalyze the Phase I metabolism of numerous xenobiotics and is usually associated with hepatic metabolism.[1,2] CYP enzymes are also found in the small intestine, kidney, lung, and brain.[3] CYP2D6 is the second largest drug metabolizer (20%) and is an important part of the phase I drug metabolism, which transforms a functional group or adds a functional group to the drug to introduce or unmask polar bodies.[4,5] The active catalytic site of CYP2D6 contains acidic amino acid residues (Asp301 and Glu216) and as such will bind substrates which contain basic nitrogen and planar aromatic ring groups.[6] CYP2D6 has been studied extensively due to its genetic polymorphisms and its large number of substrates.[1] Based on genetic profiling, CYP2D6 polymorphisms are separated into the following classifications: 1) poor, 2) normal, and 3) extensive metabolizer. Allelic variants that are prevalent in ethnic populations that are known to be poor metabolizers are the CYP2D6*10 and CYP2D6*17 variants.[7] Ethnic populations which express these alleles, would have reduced function and be 'poor' metabolizers, whereas individuals with the 'normal' alleles will be able to adequately metabolize, and the last group, the 'extensive' metabolizers, will either express more of the active alleles, or will have been 'induced' by a secondary drug leading to a greater number of enzyme molecules.[2,8,9] Most drugs or chemicals exhibit reversible inhibition where there is competition for the catalytic site.[9] Inhibition magnitude is a function of the inhibiting agent concentration and the affinity of the agent for CYP2D6.[2] It is clear that the combination of ethnic/genetic effects coupled with the induction/inhibition ability of various drugs, the potential for drug-drug interactions resulting in an increased incidence of toxicity is significant. Improved understanding of CYP2D6 activity in the presence of illicit drugs will lead to better interpretation and predictions of drug-drug interactions.

Methamphetamine (MA) and 3,4-methylenedioxymethamphetamine (MDMA) are abused psychostimulant drugs often ingested as part of polydrug use. Drug-drug interactions at the level of the hepatic CYP microsomal system (Phase I reactions) can result in unexpected and dangerous side effects. Interactions between drugs may also affect forensic/medical toxicological analysis and interpretation. OTC-related inhibition of MA or MDMA metabolism will result in the accumulation of the illicit drug leading to increased toxicity. Cimetidine (CMT) and MDMA[10-12] are inhibitors of CYP2D6 whereas dextromethorphan (DEX) and MA are substrates for the CYP2D6 isozyme.[2,13,14] Comparing the actions of CMT and DEX at CYP2D6, CMT exhibits 20-fold lower potency at inhibiting compared to DEX (200 μM *vs*. 10 μM).[15] Inhibitors of CYP activity lead to increased drug adverse effects associated with the increase in the drug concentration.[1,2,9] Clemens et al confirmed the increase in adverse effects when MDMA and MA are administered concurrently.[16] The ability of X-ray crystallography to determine the CYP2D6 three-dimensional structure has aided significantly in understanding the mechanism of substrate activity at the CYP2D6 catalytic site.[17] A sample schematic for the metabolism of MA, MDMA, CMT, and DEX (Figure 1) includes the primary metabolite for each of the parent compounds.

Amphetamine (AMP) was first synthesized as a nasal decongestant to replace ephedrine.[18] Later, AMP was used to treat

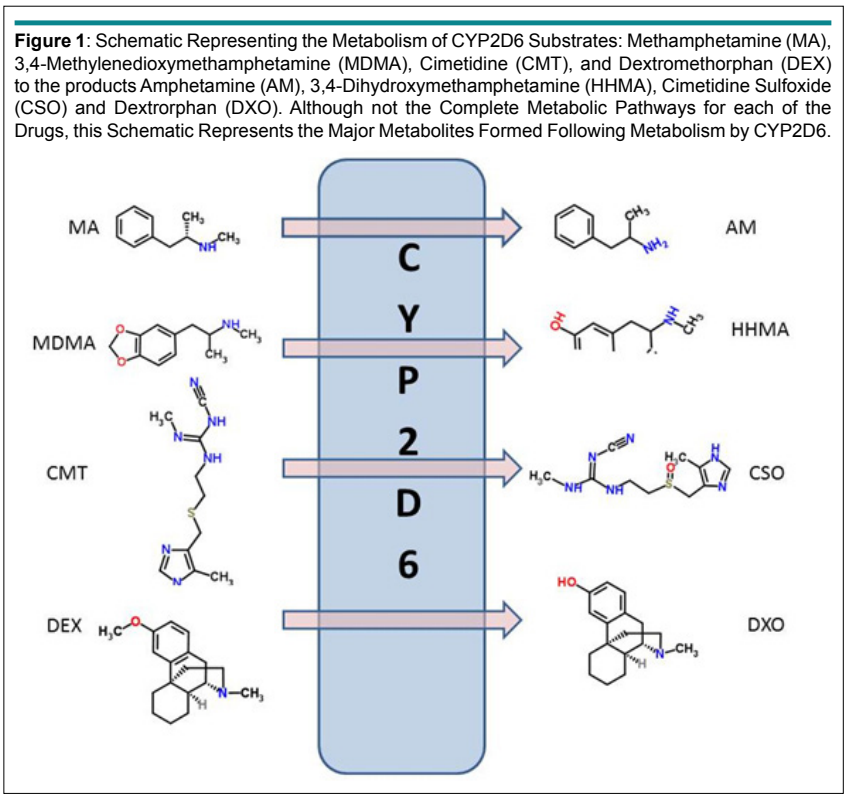


Figure 1: Schematic Representing the Metabolism of CYP2D6 Substrates: Methamphetamine (MA), 3,4-Methylenedioxymethamphetamine (MDMA), Cimetidine (CMT), and Dextromethorphan (DEX) to the products Amphetamine (AM), 3,4-Dihydroxymethamphetamine (HHMA), Cimetidine Sulfoxide (CSO) and Dextrorphan (DXO). Although not the Complete Metabolic Pathways for each of the Drugs, this Schematic Represents the Major Metabolites Formed Following Metabolism by CYP2D6.

ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

narcolepsy and hyperactivity, but its abuse skyrocketed.[19] After World War II, AMP and related drugs like methamphetamine (MA) were available without a prescription thus increasing use and abuse. It was not until the Controlled Substances Act (1986) that the regulation of AMP/MA increased and these agents were classified as "C-II" which means that there are medical uses, but the agents are highly addictive.[19] MA is a derivative of AMP and belongs to the phenethylamine class of psychostimulants.[20] MA is commonly accepted as more addictive and favored by drug addicts due to its increased ability to enter the central nervous system (CNS).[19,21] Of the two racemic forms of MA, the *d*-isomer exhibits greater stimulatory potency.[22] The mechanism associated with AMP and MA addiction involves increased activity of the dopaminergic system where dopamine (DA) release is increased, or DA is displaced from its storage vesicles elevating synaptic DA concentration.[19,20] At higher doses, MA reduced both DA and serotonin (5-HT)synthesis.[19,20] MA itself has low potency for stimulating DA release, but is metabolized to p-hydroxymethamphetamine (~15%) and the active metabolite AMP (4-7%) *via* CYP2D6. AMP is subsequently metabolized to p-hydroxyamphetamine and other inactive metabolites. Currently, MA is an easily obtainable drug, and the ease of production, increased potency, and availability has led to increased MA abuse.

Methylenedioxymethamphetamine (MDMA) was first synthesized as an appetite suppressant, yet never received approval for medical use. It was abused in the 1960s and 1970s for its psychoactive and hallucinogenic properties, and it was classified as "C-I" (no medicinal use, highly addictive).[19] Currently, MDMA is an illicit recreational drug that is very popular at all-night parties or raves. MDMA is an analog of MA formed by methylenedioxy substitution[22] and is structurally similar to mescaline and methylenedioxyamphetamine, MDA.[23] MDMA affects several neurotransmitter systems including norepinephrine (NE), DA, 5-HT, and the neurotransmitter γ-amino butyric acid, GABA.[22] MDMA is a strong and selective 5-HT neurotoxin in numerous animal species, including non-human primates.[24] In rats, MDMA stimulates 5-HT release which is believed to be *via* the reversal of the 5-HT transporter.[22] There are two forms of MDMA: the R-(-)-isomer and the more potent neurotoxin, the S-(+)-isomer. MDMA is O-demethylated to 3,4-dihydroxymethamphetamine [HHMA] *via* CYP2D6[23,25] followed by N-demethylation to the active metabolite MDA *via* CYP1A2 and CYP2D6.[22] MDA potently inhibits the activity of CYP2D6 through irreversible, covalent, binding to the CYP2D6 catalytic site.[10] When taken orally, MDMA is well absorbed with a half-life of 7 hours in humans, with MDA reaching peak plasma concentrations in 4-6 hours.[26] Nearly two-thirds of MDMA is excreted in the urine unchanged.[22,26] There is evidence to support long-term electrophysical abnormalities in MDMA users and suggest that typical recreational doses of MDMA are enough to cause long-term altered cortical activity in humans.[24] MDMA and MA, when taken concurrently, can produce greater adverse effects, dependent on the order of administration. The sequence of administration of MA and MDMA appears to have pharmacological relevance.[16] Collectively, these reports suggest that there could be a synergistic or additive effect of MDMA and MA that may be due to interactions at a common metabolic point, such as CYP2D6.

Cimetidine (CMT) is a commonly used over-the-counter medication for the treatment of acid reflux disease and heartburn. CMT was one of first in the class of histamine H_2 blockers/antagonists that prevent H^+ secretion from the parietal cells into the stomach lumen.[27] CMT can bind to the cytochrome P450 heme iron reactive site, inhibiting all cytochrome-dependent phase I enzyme activity.[28] Due to this interaction at P450 sites, CMT has been associated with many drug-drug interactions involving the inhibition of CYP2D6 and other P450 isozymes.[29-31] When CMT is combined with MA, levels of both MA and AMP were significantly higher in the rat CNS compared to rats that did not receive CMT.[32] CMT is metabolized by P450 enzymes to its major metabolite, an S-oxide.[27,29,33] The half-life of CMT is relatively short (2-4 hours) and is quickly cleared from the body by urinary excretion (70% of unchanged CMT) or the S-oxide form (20%).[34,35] The use of CMT as an acid-reducer is widespread, but the exact mechanism of P450 inhibition is not entirely understood. More work is needed to enhance our understanding of the potential of drug-drug interactions associated with CMT use.

Dextromethorphan (DEX) is an antitussive used in cold and cough medications to relieve non-productive coughs.[36] The abuse of DEX has been recognized since 1975, yet DEX has not been placed on the Controlled Substances Act.[37] DEX does not bind to opioid receptors like classical opioids and it has no analgesic activities; however, it does bind to a site associated with sigma-site ligands and also to the phencyclidine (PCP) N-methyl-D-aspartate (NMDA) glutamate receptor channel site.[38,39] At higher concentrations, recreational users reach dissociative effects similar to PCP or ketamine.[38,40] DEX is a substrate for CYP2D6 and may also interfere with the metabolism of many other drugs like CMT. DEX had been used to determine if a person is a rapid, normal or slow CYP2D6 metabolizer by measuring the rate which the active metabolite dextrorphan (DXO) is formed.[41] Both DEX and DXO have been shown to reduce MA self-administration at doses <30 mg/kg in rats,[42] suggesting that co-administration of DEX and MA may reduce the reinforcing properties associated with MA administration. Quinidine, a prototype substrate for CYP2D6, inhibits the DEXO-demethylation to DXO, thus increasing the DEX/DXO ratio.[36,43-45] After oral administration, DEX undergoes hepatic metabolism (85%) to increase elimination in the urine, but almost 15% of DEX is excreted unchanged.[44,46]

This study examines the interactions between two OTC drugs and two illicit drugs at CYP2D6 and asks: *can OTC drugs interfere with the metabolism of the illicit drugs leading to increased toxicity?* Drugs and Human Performance Fact sheets from the National Highway Traffic Safety Administration[47] also report "potential inhibitors of the CYP2D6 isozyme could decrease the rate of methamphetamine elimination if administered concurrently, while potential inducers could increase the rate of

ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

elimination." Collectively, this work will improve our understanding of potential drug-drug interactions between OTC and illicit drugs and the potential hazards associated with polydrug use/abuse. This work is significant since unforeseen drug interactions may lead to the misinterpretation of toxicology results.

EXPERIMENTAL DESIGN

Chemicals and Drugs

Methamphetamine HCl, 3, 4-methylenedioxymethamphetamine HCl, cimetidine, dextromethorphan-HBr, acetonitrile, dimethyl sulfoxide (DMSO), 3-[2-(N, N-diethyl-N-methylamino) ethyl]-7-methoxy-4-methylcoumarin (AMMC)[48] were purchased from Sigma Chemical Company (St. Louis, MO, USA). The inhibitor screening kit CYP2D6/AMMC was purchased from BD Biosciences (Woburn, MA). The P450 HTS kit include: CYP2D6 (CYP2D6*1 + P450 reductase), non-fluorescent substrate (AMMC), fluorescent metabolite (AMHC), cofactors (1.3 mM NAPD+, 66 mM $MgCl_2$, and 66 mM glucose 6-phosphate), glucose 6-phosphate dehydrogenase (40 Units/ml in 5 mM sodium citrate buffer, pH 7.5), reaction buffer solution (0.5 M potassium phosphate, pH 7.4, filter sterilized), stop solution reagent (0.5 M Tris base reagent, filter sterilized), CYP2D6 positive control inhibitor (quinidine),[28] and NADPH regenerating system. The ability of this kit to perform *in vitro* screening of CYP2D6 substrates and inhibitors and its comparison to other screening modalities have been previously described.[49,50]

Animals

Male Sprague-Dawley rats (6-months old/375-425 grams, Harlan Sprague-Dawley Laboratories, Indianapolis, IN) were randomly assigned to treatment groups; group housed, and allowed access to food (Pro-lab Rat, Mouse, and Hamster Chow #3000) and water ad libitum in a temperature controlled room (23±2°C) and 12-hour light cycle. All animals experienced the same environmental conditions over the course of the experiment. Twenty rats (N=4 for each group) were placed in quarantine upon arrival for one week and then moved to the animal colony and allowed to habituate in their environment for at least one week prior to use. Animals were maintained according to NIH guidelines in our USDA-certified facilities. The protocol for the use of animals in this research was approved by the IACUC of Oklahoma State University Center for Health Sciences.

Inhibition Studies Purified CYP2D6

AMMC, 3-[2-(N, N-diethyl-N-methylamino) ethyl]-7-methoxy-4-methylcoumarin, is a non-fluorescent substrate that is demethylated to the fluorescent metabolite AHMC, 3-[2-(N, N-diethylamino) ethyl]-7-hydroxy-4-methylcoumarin hydrochloride, which was used to screen for interactions with CYP2D6.[51,52] Quinidine, a potent inhibitor of CYP2D6, was used as the control compound to determine intra- and inter- assay variability. Stock solutions (1 mM) of CMT, DEX, MA, and MDMA were each prepared in HPLC-grade acetonitrile before each CYP2D6 assay. Groups were divided by treatment: CMT, DEX, MA, MDMA, CMT+MA, CMT+MDMA, DEX+MA, DEX+MDMA, CMT+DEX+MA, and CMT+DEX+MDMA. Each drug was serially diluted (1:3) from highest concentrations (20 µM) to lowest concentrations (3 nM) and pre-incubated at 37 °C for 10 min in a black/clear bottom 96-well plates. Separate vehicle assays were performed (maximum 2% acetonitrile) and there was no effect on CYP2D6 activity (data not shown), which is similar to reports by the manufacturer. The enzyme-substrate mix [CYP2D6*1-P450 reductase + 10 mM AMMC] was added and plates were incubated at 37 °C for 30 min. The conversion of AMMC to AHMC was terminated by the addition of stop reagent and the amount of fluorescent product, AHMC, was determined using a fluorescent plate reader (Synergy HT with KC4 software; Bio-TEK Instruments, Inc., Winooski, VT, USA). Measurement parameters for determining AHMC fluorescence utilized an excitation/emission filter of 360 nm/460 nm. Background values were subtracted from the treatment wells before statistical analysis.

Inhibition Studies: Rat CYP2D2

Animals and treatment: Rats were randomly assigned to one of the 5 treatment groups: The first group was control (naïve); treatment groups included: vehicle control (VC; 0.9% saline and DMSO, 4%); CMT (10 mg/kg); DEX (10 mg/kg); and the combination of CMT+DEX (10 mg/kg each). Rats received a single daily injection from day 1 till day 7 at the same time each day. All compounds were administered *via* intraperitoneal (IP) injections to minimize first-pass effects. On Day 8, the drug-treated rats (not naïve rats) were challenged with a 5 mg/kg IP injection of MA. Seven hours after MA injection, rats were lightly anesthetized using carbon dioxide gas, and sacrificed by decapitation. The median and the left lateral hepatic lobes were harvested and frozen in liquid nitrogen. Samples were stored at -80 °C until use.

Hepatic microsome preparation: Hepatic microsomal fractions were prepared with slight modifications as previously described.[53] Briefly, frozen (-80 °C) rat livers were thawed and minced in 2-4 mL of homogenizing buffer (0.1 M potassium phosphate, pH 7.4 and 0.25 M sucrose), then brought to 30 mL with additional homogenizing buffer. Homogenization was completed with 10 strokes at 900 rpm using a Teflon pestle/glass homogenizer (Wheaton, USA). Nuclei and mitochondria were removed by centrifugation at 9,000 x*g* for 20 min at 4 °C. The resulting supernatant (S1) was centrifuged at 100,000 x*g* for 60 min. The pellet (P2; containing microsomes) was resuspended in 20 mL of incubation buffer (0.1 M potassium phosphate, pH 7.4, 0.25M sucrose, 1 mM EDTA, and 5% glycerol) and used immediately or stored frozen (-80 °C) until use. Florence et al showed washed microsomes could be stored at -80ºC for up to 30 days without loss of activity.[54] All stored microsomes in the present studies were used before the end of the 30 day period.

ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

Protein Analysis: Commercially available Coomassie-blue-based protein assay (Bio-Rad, Richmond, CA, USA) based on the Bradford method was used to determine total protein concentration.[55] Based on specific CYP2D2 activity, the calculations for pmol/mgprotein/min was determined.

***In vivo* Kinetic Studies:** The *in vivo* kinetic studies used the HTS kit from the *in vitro* studies with modifications. The assay was used to quantify the CYP2D2 (rat) enzyme activity for each of the drug treatment groups following MA challenge by measuring the production of fluorescent AHMC. The activity of CYP2D2 was determined following seven-day exposure to CMT, DEX, and CMT/ DEX, or saline using AMMC as a probe. This probe has been shown to be highly selective for rat CYP2D2, as well as the human CYP2D6 isoform.[48,56] Rat hepatic microsomes were used in place of the purified human CYP2D6 utilized in the *in vitro* assay.

Assays were performed as described above. The enzyme mix for the treatment groups was prepared for each microsomal fraction by adding H_2O, buffer (0.5 M potassium phosphate, pH 7.4, filter sterilized), and enzyme (microsomal fraction), a 79:20:0.75 mix. For the treatment groups, enzyme mix and AMMC (0.5 µM to 3.29 µM) were mixed in the well for a total volume of 100 µL. Varying AMMC concentrations produced a concentration response curve that determined enzyme kinetics. The plate was incubated for 30 min at 37 °C, and the reaction was terminated by the addition of stop reagent. The fluorescence generated by AMMC was quantified as described previously at excitation/emission wavelengths of 360 nm/460 nm. Assays were performed as four assays (N=4) in duplicate.

Statistical Analysis

For the *in vitro* CYP2D6 inhibition assays, the inhibitory potency of quinidine and each test compound was determined by measuring the IC_{50} value for each compound. This data was curve fit using an iterative nonlinear curve fitting program in PRISM 7.01 (GraphPad Software Inc., San Diego, CA, USA). IC_{50} values and the maximum inhibition percentage were then analyzed using the Kruskal-Wallis nonparametric test followed by the Dunn's comparison (using quinidine as the comparison group). A significance level was set at α=0.05.

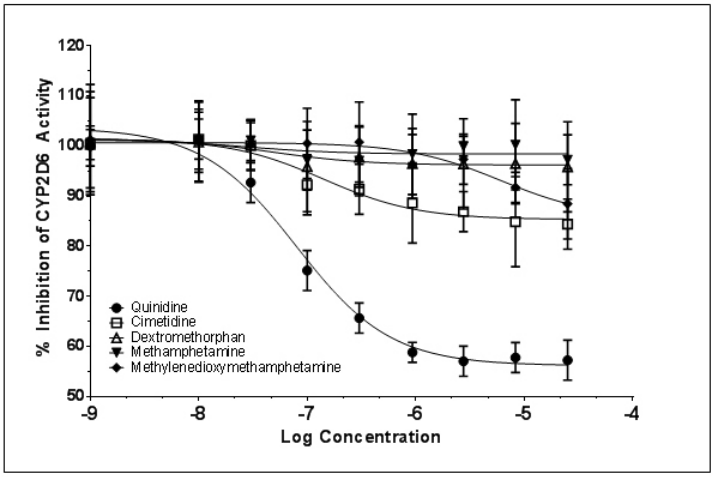
For the *in vivo* enzyme kinetic assay, data was collected similar to above and calculated using a nonlinear fit (rectangular hyperbola) of the data, yielding V_{max} and K_m values. Kinetic data were then analyzed using one-way ANOVA followed by Dunnett's posthoc test to compare each of the treatment groups to the K_m (µM) and V_{max} (pmol of AMHC formed/mg protein/minute) values obtained from naïve rats. The significance level again was set at α=0.05. All data are expressed as the mean±standard error of the mean (SEM).

RESULTS AND DISCUSSION

Inhibition Studies Purified CYP2D6

To determine inhibition of CYP2D6 by quinidine and test compounds the IC_{50} values were calculated for each inhibition curve (Figure 2). Then, the mean log IC_{50} values for each were group were obtained and compared (Figure 3A and 4A). The IC_{50} values for each test compound were then compared to the

Figure 2: Inhibition of CYP2D6 Activity by OTC and Illicit Drugs. Purified Human CYP2D6 was Incubated in the Presence of the Non-Fluorescent 3-[2-(N, N-Diethyl-N-Methylamino) Ethyl]-7-Methoxy-4-Methylcoumarin (AMMC, 10 mM) which was then Demethylated to the Fluorescent Metabolite 3-[2-(N, N-Diethylamino) Ethyl]-7-Hydroxy-4-Methylcoumarin Hydrochloride (AHMC). Enzyme-Substrate Mixtures were incubated with 8 Concentrations of CMT, DEX, MA or MDMA (0.2 nM – 100 µM) for 10 min at 37°C. The Quantity of Fluorescent Product (AHMC) was then Measured with a Fluorescence Excitation/Emission Filter of 360 nm/460 nm. Data was Fit using Nonlinear Regression Analysis and both 1-site and 2-site Models were Compared. Curves were best Fit to the Simpler, 1-site Model and from this Analysis, the IC_{50} and Maximum Inhibition Values could be Determined (expressed in Figures 3 and 4). Each of the Data Points Represent 4 Assays (N=4) Performed in Duplicate, Except for the DEX Group which was an N=3. Data then Expressed as the Mean±SEM.



ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

quinidine IC_{50} value (3.8 nM) which was used as the 'control.' Our values are similar to the values reported elsewhere and in the manufacturer literature (3-11 nM).[50] Figure 2 represents the inhibition curve from each test compound (and quinidine) and the data are expressed as the mean±SEM of 4 assays performed in duplicate for each test compound except for the DEX and CMT+MA groups which are three assays performed in duplicate. Another indicator of effect at CYP2D6 was the measurement of the maximum inhibition elicited by each of the test compounds. The maximum inhibition (35-40%) of quinidine was set as 100% inhibition, and each group was then calculated as a percentage of the quinidine maximum (Figure 3B and 4B). When examining single compound, there was no effect on the log IC_{50} values compared to quinidine ($H_{5,20}$=5.66; p=0.2263, Figure 3A). Comparing the maximum inhibition of the single test compounds to quinidine revealed a significant effect of treatment ($H_{5,20}$=13.69; p=0.0084, Figure 3B) with both the MA and MDMA groups exhibiting significantly less inhibition compared to the quinidine group (p<0.05). When examining the effects of multiple drug exposures, to determine if there may be synergistic, additive or potentiating effects, there was a generalized lowering of log IC_{50} values ($H_{7,21}$=14.42; p=0.0252, Figure 4A). Comparison to quinidine values revealed that only the CMT+DEX+METH log IC_{50} value was significantly (p<0.05) reduced compared to quinidine values. In the combination treatment groups, all groups exhibited significantly reduced inhibition compared to quinidine, yet there were no differences between any of the combination treatment groups (Figure 4B). Collectively these data suggest none of the drugs resulted in the inhibition of CYP2D6 to the same extent as quinidine, the prototype CYP2D6 inhibitor. Although, not potent inhibitors, each of the test compounds did elicit some level of inhibition of CYP2D6 activity. Each test compound (single or combination) displayed a lower affinity for CYP2D6 compared to quinidine (usually 10- to 100-fold lower IC_{50} values compared to the 3-11nM IC_{50} value reported for quinidine). In general, the *in vitro* assays suggest that each of the OTC and illicit drugs can inhibit CYP2D6 to a relatively small extent and that the weak

Figure 3: Effects of Single Drug Exposure on CYP2D6 Activity. The IC_{50} (A) and Maximum Inhibition (B) Values were Determined from the Nonlinear Analysis (Figure 2). The Values for Quinidine (IC_{50} and Maximum Inhibition) were used as the Positive Control to which the other Drug Exposure Effects were Compared. When Examining Single Compound Comparisons (A), there was no Effect ($H_{5,20}$=5.66; p=0.2263) on the log IC_{50} Values Compared to Quinidine Values. Comparing the Maximum Inhibition (B) of the Single Test Compounds to the Maximum CYP2D6 Inhibition by Quinidine, there was a Significant Effect of Treatment ($H_{5,20}$=13.69; p=0.0084) with both the MA and MDMA Groups Exhibiting Significantly Less Inhibition Compared to the Quinidine Group (p<0.05). Data are Expressed as mean±SEM of 4 Assays (3 for DEX) Run in Duplicate. *p<0.05 Compared to Quinidine Value.

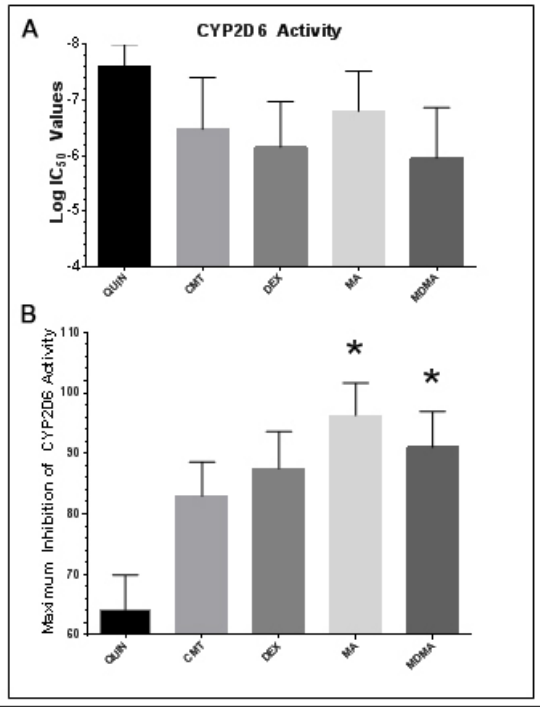
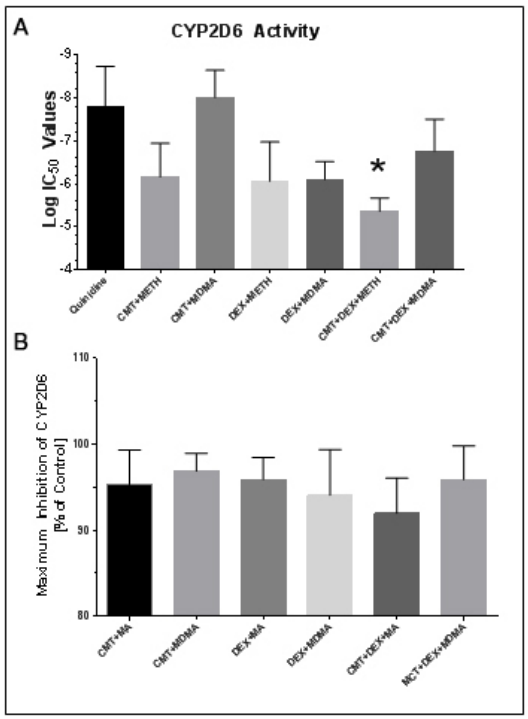


Figure 4: Effects of Combination Drug Exposure on CYP2D6 Activity. The IC_{50} (A) and Maximum Inhibition (B) Values were Determined from the Nonlinear Analysis (Figure 2). The Values for Quinidine (IC_{50} and Maximum Inhibition) were used as the Positive Control to which the other Drug Exposure Effects were Compared. Examining the IC_{50} Values (A), the Combination Drug Exposure had Generally Lower IC_{50} Values Compared to the Quinidine Groups ($H_{7,21}$=14.42; p=0.0252). Comparison to Quinidine Values Revealed that only the CMT+DEX+METH Combination Group had Significantly (p<0.05) Lower log IC_{50} Values Compared to the Quinidine Group. In the Combination Treatment Groups, all Groups Exhibited Significantly Reduced Inhibition Compared to Quinidine (Approximately 35% Inhibition to 5-10%), yet there were no differences between any of the Combination Treatment Groups (B). Data are Expressed as mean±SEM of 4 Assays (3 for CMT+MA) Performed in Duplicate. *p<0.05 Compared to Quinidine Value.



ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

inhibition observed does not exhibit any other characteristics in the combination groups such as synergism, potentiation or additivity.

Each of the drugs tested have reported action at CYP2D6 either as an inhibitor, an inducer, or a substrate. CMT has been reported to have inhibitory effects on CYP2D6 in both humans and rodents.[33,57-59] DEX interacts with a greater number of P450 isozymes, with CYP2D6 being a major contributor to DEX metabolism by O-demethylation.[60] It is clear that OTC drugs such as CMT and DEX can play a significant role in the development of drug-drug interactions leading to increased toxicity by interference with CYP2D6 metabolism. There is an extensive body of work regarding the metabolism of MA and MDMA. CYP2D6 is involved in multiple steps in the metabolism of both MA and MDMA.[61] Earlier reports suggest that the parent compound for MA and its parent compound, amphetamine, act at CYP2D6 with low micromolar affinity.[62] Our findings demonstrate that a potential interaction between OTC and illicit drugs does exist[63] and that the interaction may lead to increased risk of toxicity. Overall, our findings *in vitro* show the calculated IC_{50} values for the test compounds were reduced by a 75-85% decrease compared to IC_{50} values associated with quinidine. The IC_{50} value for quinidine of 3.8 nM was consistent with the value reported by the manufacturer and other researchers.[50,64,65] The efficacy of inhibition or maximum inhibition was lower than the inhibition that was observed with quinidine, suggesting that the test compound only weakly interacted with CYP2D6 compared to quinidine.

Studies using MA and MDMA employed a variety of different methodologies to measure kinetic parameters.[10-12,62,64] Taavitsainen et al reported an IC_{50} value for MA of 414 µM, but this study used DEX as the substrate probe, not AMMC.[64] De la Torre et al reported MDMA's rate of activation is decreased when quinidine is added, suggesting MDMA is metabolized in part by CYP2D6.[66] Studies that include CMT used different methodology such as Western blotting with serum containing anti-CYP2D6[67] and use different factors to determine the kinetic parameters for CMT.[29,68] Early studies examining the effects of CMT on CYP2D6 as well as other P450 isozymes describe the binding actions of CMT to CYP2D6 and that this interaction can reduce the metabolism of a benzodiazepine by up to 45%.[69,70] A report by Madeira et al. showed that CMT and DEX could reciprocally interfere with CYP2D6-mediated metabolism.[29] Literature describing the actions of DEX on CYP2D6 are the most prevalent and use DEX as a probe to determine enzyme activity or phenotyping.[29,41,68] Also DEX has been used to categorize the metabolizer-typing of the patient.[45] Studies on DEX-mediated CYP2D6 inhibition report IC_{50} values for DEX of 1.89 µM to 2.0 µM dependent on drug concentration.[48,65] Based on the DEX studies, it is clear that DEX exerts a robust effect at CYP2D6 and could be an important OTC drug when considering potential drug-drug interactions.

Inhibition Studies with rodent isozyme: CYP2D2

Rats were treated as described above for 7 days (day 1 to day 7) and then challenged on Day 8 with MA. Following challenge (7 hours), rats were sacrificed and hepatic lobes removed to determine the conversion of AMMC to AMHC. The K_m(µM) and V_{max}(pmol of AMHC/mg protein/minute) values are presented in Table 1. Comparison of K_m values across treatments revealed a significant effect of treatment ($F_{4,15}$=4.067; p=0.0199). Although reductions in K_m ranged from 65-121% compared to naïve values, the actual range was from 3-6.8 µM. The saline, CMT, and DEX groups were significantly different from naïve control values (p<0.05). The only group that was not statistically different from naïve was the combination of CMT+DEX. It is unclear as to whether these relatively small changes would result in significant changes in metabolism *via* CYP2D2 (or CYP2D6 in humans). Comparing V_{max} values revealed the more robust changes. Across all treatment groups, it appeared that the CYP2D2 activity had shifted to a higher capacity (280-490%). V_{max} values were significantly ($F_{4,15}$=4.342; p=0.0157) increased compared to naïve values by 2.8- to 4.9-fold. Dunnett's posthoc analysis showed that the only group that was significantly different from naïve was the CMT group (p<0.05).

We further confirmed and extended the *in vitro* findings with *in vitro* studies utilizing adult male Sprague-Dawley rats. Rodent CYP2D2 activity following daily drug administration was measured using a modified assay based on the *in vitro* studies. Our *in vivo* results suggest that both V_{max} and K_m values were elevated in the treatment groups. V_{max} values increased 280-490% and K_m values increased 165-220% in treatment groups compared to V_{max} and K_m values from the control (naïve) group. Collectively, these results suggest that following treatment with CMT, DEX or a combination, the CYP2D2 enzyme in the rat shifts towards a low affinity/high capacity CYP2D2 isoform.

Table 1: Mean K_m & V_{max} Values for each Treatment Groups.

	Naive	Saline	CMT	DEX	CMT/DEX
K_m (µM) Mean±SEM	3.08±0.46	6.81*±0.73	6.72*±1.34	6.40*±0.47	5.09±0.55
V_{max} (pmol/mg protein/min) Mean±SEM	19.9±5.1	65.9±12.0	98.3*±22.1	70.5±15.1	56.4±6.1

*p<0.05 compared to corresponding naïve values.

ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

The rightward shift direction of the kinetic curves would lead to a slowing of metabolism over time, and as a result, increasing the concentration of other drugs/compounds which require CYP2D6 for their metabolism. All test compounds reduced the activity of CYP2D6, i.e., weak inhibition; therefore further studies are relevant.

In vivo studies showed that pre-treatment with CMT, DEX or CMT+DEX for 7 days followed by an MA challenge on day 8 resulted in CYP2D2 activity that exhibited a lower affinity and higher capacity. This data implies that MA may be an inducer *via* CYP2D2 if elevations in V_{max} represent an increase in the quantity of CYP2D2 protein. Conversely, V_{max} elevation may be due to changes in the catalytic activity of the existing enzymes. Dostalek et al suggest that MA may be an inducer of DEX metabolism *via* CYP2D2; therefore the co-administration of MA with DEX may result in decreased drug plasma levels thus a decrease in drug effects.[71,72] Conversely, reports have shown that co-administration of DEX and MA reduces the reinforcing properties of MA, *via* inhibition of DA neurons in the brain.[73] This effect could lead to increased MA administration to achieve the same "high," which will bring plasma levels closer to toxicity. Similar results were reported by Glick et al and these findings also extended DEX and DXO effects to include reductions in both morphine and nicotine self-administration as well as MA.[42] Studies on MDMA metabolism are more recent, but tend to support previous findings with other MA analogs. Although CYP2D6 is the major route of metabolism, other P450 isozymes contribute to the overall metabolism of MDMA.[11,12,74,75] A recent study examined the potential drug-drug interactions between MDMA and caffeine.[76] Although, the authors report that there are no drug-drug interactions which significantly alter MDMA metabolism, the authors acknowledge the importance of the investigation into drug-drug interactions. DEX effects can be extended to the central nervous system where reductions in self-administration and neuroprotection are believed to be through non-NMDA glutamate receptor-mediated functions.[42,77,78] The K_m value in the control (naïve) group was consistent with the suggested K_m value reported by the manufacturer (BD Biosciences). This suggests that the results from both assays are relevant to each other and can be compared. Most studies that measure enzyme activity of CYP2D6 or CYP2D2 use DEX as the substrate,[12] but this study uses the reduction of AMMC to AHMC to determine the enzyme activity. A few studies report the kinetic parameters for MA, CMT, and DEX, but most use different methods or *in vitro* instead of *in vivo*.[29,61] Lin et al report V_{max} and K_m values for MA, but the values are for both isomers of MA and both types of reactions (4-hydroxylation and N-demethylation) whereas this study did not differentiate between the two isomers or the two types of reactions.[61] Madeira et al report V_{max} and K_m values for CMT but the study is done *in vitro* instead of *in vivo* and with DEX as the probe.[29]

CONCLUSION

Poly-drug use is a growing concern due to the potential for drug-drug interactions and increased risk for severe drug-related toxicity. The current study addressed this question by using the combination of the two scheduled drugs, MA (C-II) and MDMA (C-I), and two common and inexpensive OTC drugs, CMT and DEX. CYP2D6 is an important area of research due to the large role it plays in metabolism, its genetic polymorphisms among humans, and its large number of substrates. There has been some concern regarding the impact of hepatic CYP2D6 interactions since there are other sites that are involved in the metabolism of drugs, such as the intestine, kidney, and brain. The clinically relevant drug, Selegiline, has been used in the treatment of Parkinson's disease and is metabolized to MA by CYP2D6 and as such may be subject to genetic variations. Benetton et al. report that any P450 phenotyping variations would be insignificant for the metabolism of Selegiline to MA, and this was further extended to include the metabolism of MA and MDMA.[66,79] Regardless, there are variations in CYP2D6 activity and not just in hepatic P450 systems. An understudied area is a role that P450 enzymes have in the brain in the metabolism of centrally-acting drugs.[80,81] The pharmacogenetic impact on the metabolism of MDMA is more pronounced with allele-dependency dictating the rate and extent of metabolism.[82] Although, the mechanisms are not clear, there still exists the change for drug-drug interactions leading to toxicity. Since MA and MDMA are popular and easily obtainable, drug- drug interactions are probable. This study determined that the inhibitor potency of all test compounds and quinidine were relatively the same. It was determined that some maximum test compounds inhibition decreased significantly compared to maximum quinidine and CMT/ MDMA inhibition. This suggests that all the test compounds inhibited CYP2D6 activity; one or all of the drugs may not be metabolized as quickly resulting in toxicity of those drugs. The quinidine IC_{50} value was consistent with reported values. This indicates that the CYP2D6 was performed in accordance with the manufacturer's specifications. The V_{max} value in the CMT treated group increased significantly compared to naïve. The K_m values in the CMT and saline treated group increased significantly compared to naïve. Both kinetic parameters showed there was an increase after the MA challenge but no effects due to the OTC drugs. This suggests that the low affinity/high capacity CYP2D2 isoform was upregulated meaning that more CYP2D2 was present, suggesting that MA is an inducer *via* CYP2D2. Understanding that MA can induce CYP2D2 and CYP2D6 is important considering the co-administration of other drugs such as DEX, CMT, or MDMA. This information is vital since many other drugs can be used recreationally to achieve a 'high' or to combine drugs to potentiate or prolong the high associated with an illicit drug. Increasing our understanding of these drug-drug relationships will aid in our interpretation of forensic findings as well as provide a better foundation for understanding toxicological relationships between various drugs.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest involved with this manuscript.

ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

REFERENCES

1. Badyal DK, Dadhich AP. Educational forum cytochrome P450 and drug interactions. *Indian J Pharmacol*. 2001; 33: 248-259.

2. Brown C. Overview of drug interactions modulated by cytochrome P450. *US Pharm*. 2001: HS26-HS45.

3. Nishimura M, Yaguti H, Yoshitsugu H, Naito S, Satoh T. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high sensitivity real time reverse transcription PCR. *Yakugaku Zasshi*. 2003; 123(5): 369-375. doi: 10.1248/yakushi.123.369

4. Drummer O, Odell M. Pharmacokinetics and duration of action. In: Bentliff G, McAllister L, eds. *The Forensic Pharmacology of Drugs of Abuse*. 1st ed. Abingdon, UK: Taylor & Francis; 2001: 27-29.

5. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther*. 2013; 138(1): 103-141. doi: 10.1016/j.pharmthera.2012.12.007

6. Raunio H, Kuusisto M, Juvonen RO, Pentikäinen OT. Modeling of interactions between xenobiotics and cytochrome P450 (CYP) enzymes. *Front Pharmacol*. 2015; 6(123): 1-14. doi: 10.3389/fphar.2015.00123

7. Shen H, He MM, Liu H, et al. Comparative metabolic capabilities and inhibitory profiles of CYP2D6.1, CYP2D6.10, and CYP2D6.17. *Drug Metab Dispos*. 2007; 35(8): 1292-1300. doi: 10.1124/dmd.107.015354

8. Qu Q, Qu J, Han L, et al. Inhibitory effects of phytochemicals on metabolic capabilities of CYP2D6*1 and CYP2D6*10 using cell-based models in vitro. *Nat Publ Gr*. 2014; 35(10): 685-696. doi: 10.1038/aps.2013.202

9. Cupp M, Tracy T. Cytochrome P450: New nomenclature and clinical implications. *Am Fam Physician*. 1998; 57(1): 107-116.

10. Van LM, Heydari A, Yang J, et al. The impact of experimental design on assessing mechanism-based inactivation of CYP2D6 by MDMA (Ecstasy). *J Psychopharmacol*. 2006; 20(6): 834-841. doi: 10.1177/0269881106062902

11. Van LM, Hargreaves JA, Lennard MS, Tucker GT, Rostami-Hodjegan A. Inactivation of CYP2D6 by methylenedioxymethamphetamine in different recombinant expression systems. *Eur J Pharm Sci*. 2007; 32(1): 8-16. doi: 10.1016/j.ejps.2007.05.002

12. Van LM, Swales J, Hammond C, Wilson C, Hargreaves JA, Rostami-Hodjegan A. Kinetics of the time-dependent inactivation of CYP2D6 in cryopreserved human hepatocytes by methylenedioxymethamphetamine (MDMA). *Eur J Pharm Sci*. 2007; 31(1): 53-61. doi: 10.1016/j.ejps.2007.02.005

13. Brown C. Overview of Drug–Drug Interactions with SSRIs. *US Pharm*. 2008: HS3-HS19.

14. Cupp MJ, Tracy TS. Cytochrome P450: New nomenclature and clinical implications. *Am Fam Physician*. 1998; 57(1): 107-116.

15. Martinez C, Albet C, Agundez JA, et al. Comparative in vitro and in vivo inhibition of cytochrome P450 CYP1A2, CYP2D6, and CYP3A by H2-receptor antagonists. *Clin Pharmacol Ther*. 1999; 65(4): 369-376. doi: 10.1016/S0009-9236(99)70129-3

16. Clemens KJ, Cornish JL, Li KM, Hunt GE, McGregor IS. MDMA ('Ecstasy') and methamphetamine combined: Order of administration influences hyperthermic and long-term adverse effects in female rats. *Neuropharmacology*. 2005; 49(2005): 195-207. doi: 10.1016/j.neuropharm.2005.03.002

17. Maréchal J-D, Kemp C, Roberts G, Paine M, Wolf C, Sutcliffe M. Insights into drug metabolism by cytochromes P450 from modelling studies of CYP2D6-drug interactions. *Br J Pharmacol*. 2008; 153: 82-89. doi: 10.1038/sj.bjp.0707570

18. Drummer O, Odell M. Stimulants. In: Bentliff G, McAllister L, eds. *The Forensic Pharmacology of Drugs of Abuse*. 1st ed. Abingdon, UK: Taylor & Francis; 2001: 49-102.

19. Levine B. *Principles of Forensic Toxicology*. 4th ed. Washington, DC, USA: AACC Press; 2013.

20. Logan B. Methamphetamine-effects on human performance and behavior. *Forensic Sci Rev*. 2002; 14(1-2): 133-151.

21. Shoblock JR, Sullivan EB, Maisonneuve IM, Glick SD. Neurochemical and behavioral differences between d-methamphetamine and d-amphetamine in rats. *Psychopharmacology (Berl)*. 2003; 165: 359-369. doi: 10.1007/s00213-002-1288-7

22. Logan B, Couper F. 3,4-Methylenedioxymethamphetamine — effects on human performance and behavior. *Forensic Sci Rev*. 2003; 15(1): 11-28.

23. Oesterheld JR, Armstrong SC, Cozza KL. Med-Psych Drug-Drug Interactions Update Ecstasy: Pharmacodynamic and Pharmacokinetic Interactions. *Psychosomatics*. 2004; 45(1): 84-87. doi: 10.1176/appi.psy.45.1.84

24. Casco C, Forcella MC, Beretta G, Grieco A, Campana G. Long-term effects of MDMA (Ecstasy) on the human central nervous system revealed by visual evoked potentials. *Addict Biol*. 2005; 10(2): 187-195. doi: 10.1080/13556210500123340

25. Escobedo I, O'Shea E, Orio L, et al. A comparative study on the acute and long-term effects of MDMA and 3,4-dihydroxy-

ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

methamphetamine (HHMA) on brain monoamine levels after i.p. or striatal administration in mice. *Br J Pharmacol*. 2009; 144(2): 231-241. doi: 10.1038/sj.bjp.0706071

26. De Letter EA, Belpaire FM, Clauwaert KM, Lambert WE, Van Bocxlaer JF, Piette MH. Post-mortem redistribution of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") in the rabbit. Part II: Post-mortem infusion in trachea or stomach. *Int J Legal Med*. 2002; 116(4): 225-232. doi: 10.1007/s00414-002-0293-z

27. Jantratid E, Prakongpan S, Dressman J, et al. Biowaiver monographs for immediate release solid oral dosage forms: Cimetidine. *J Pharm Sci*. 2006; 95(5): 974-984. doi: 10.1002/jps.20614

28. Liska DJ. The detoxification enzyme systems. *Altern Med Rev*. 1998; 3(3): 187-198.

29. Madeira M, Levine M, Chang TKH, Mirfazaelian A, Bellward GD. The effect of cimetidine on dextromethorphan O -demethylase activity of human liver microsomes and recombinant Cyp2D6. *Drug Metab Dispos*. 2004; 32(4): 460-467. doi: 10.1124/dmd.32.4.460

30. Park E, Cho H, Lee Y. Effect of Cimetidine and Phenobarbital on metabolite kinetics of Omeprazole in rats. *Arch Pharm Res*. 2005; 28(10): 1196-11202.

31. Draper AJ, Madan A, Parkinson A. Inhibition of coumarin 7-hydroxylase activity in human liver microsomes. *Arch Biochem Biophys*. 1997; 341(1): 47-61. doi: 10.1006/abbi.1997.9964

32. Suzuki T, Chiang H-JF, Misawa M. Effects of quinidine and cimetidine on the methamphetamine level in the rat brain. *Jpn J Pharmacol*. 1987; 43: 103-106. doi: 10.1254/jjp.43.103

33. Levine M, Bellward GD. Effect of cimetidine on hepatic cytochrome P450: Evidence for formation of a metabolite-intermediate complex. *Drug Metab Dispos*. 1995; 23(12): 1407-1411.

34. Larsson R, Erlanson P, Bodemar G, Norlander B, Fransson L, Strouth L. Pharmacokinetics of cimetidine and its sulphoxide metabolite during haemodialysis. *Eur J Clin Pharmacol*. 1982; 21(4): 325-330. doi: 10.1007/BF00637621

35. Ogbru O. Cimetidine, Tagamet HB. http://www.medicinenet.com/cimetidine/article.html. Accessed June 30, 2017.

36. Manap RA, Wright CE, Gregory A, et al. The antitussive effect of dextromethorphan in relation to CYP2D6 activity. *Br J Clin Pharmacol*. 1999; 48: 382-387. doi: 10.1046/j.1365-2125.1999.00029.x

37. CESAR. Dextromethorphan (DXM). Dextromethorphan (DXM). http://www.cesar.umd.edu/cesar/drugs/dxm.asp. Accessed June 30, 2017.

38. Nicholson K, Hayes B, Balster R. Evaluation of the reinforcing properties and phencyclidine-like discriminative stimulus effects of dextromethorphan and dextrorphan in rats and rhesus monkeys. *Psychopharmacology (Berl)*. 1999; 146(1): 49-59.

39. Ehret GB, Daali Y, Chabert J, et al. Influence of CYP2D6 activity on pre-emptive analgesia by the N-Methyl-D-Aspartate antagonist dextromethorphan in a randomized controlled trial of acute pain. *Pain Physician*. 2013; 16: 45-56.

40. Lotrich F, Rosen J, Pollock B. Dextromethorphan-induced delirium and possible methadone interaction. *Am J Geriatr Pharmacother*. 2005; 3(1): 17-20.

41. Frank D, Jaehde U, Fuhr U. Evaluation of probe drugs and pharmacokinetic metrics for CYP2D6 phenotyping. *Eur J Clin Pharmacol*. 2007; 63(4): 321-333. doi: 10.1007/s00228-006-0250-8

42. Glick SD, Maisonneuve IM, Dickinson HA, Kitchen BA. Comparative effects of dextromethorphan and dextrorphan on morphine, methamphetamine, and nicotine self-administration in rats. *Eur J Pharmacol*. 2001; 422(1-3): 87-90. doi: 10.1016/S0014-2999(01)01066-4

43. Witherow LE, Houston JB. Sigmoidal kinetics of CYP3A substrates: An approach for scaling dextromethorphan metabolism in hepatic microsomes and isolated hepatocytes to predict in vivo clearance in rat. *J Pharmacol Exp Ther*. 1999; 290(1): 58-65.

44. Min DI, Ku Y-M, Vichiendilokkul A, Fleckenstein LL. A urine metabolic ratio of dextromethorphan and 3-methoxymorphinan as a probe for CYP3A activity and prediction of cyclosporine clearance in healthy volunteers. *Pharmacotherapy1*. 1999; 19(6): 753-759. doi: 10.1592/phco.19.9.753.31536

45. Kerryi NL, Somogyit AA, Bochner F, Mikus G, Somogyi AAA. The role of CYP2D6 in primary and secondary oxidative metabolism of dextromethorphan: In vitro studies using human liver microsomes. *Br J clin Pharmac*. 1994; 38(3): 243-248. doi: 10.1111/j.1365-2125.1994.tb04348.x

46. Magarey J. Dextromethorphan. INCHEM. http://www.inchem.org/documents/pims/pharm/pim179.htm. Published 1996. Accessed June 30, 2017.

47. Couper FJ, Logan BK. *Drugs and Human Performance Fact Sheets – Methamphetamine and Amphetamines*. 2014. https://www.nhtsa.gov/sites/nhtsa.dot.gov/files/809725-drugshuman-performfs.pdf. Accessed August 1, 2017.

ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

48. Chauret N, Dobbs B, Lackman RL, et al. The use of 3- [2- (N , N-Diethyl-N-Methylammonium) Ethyl] -7-Methoxy-4- methylcoumarin (AMMC) as a specific Cyp2D6 probe in Human liver microsomes. *Drug Metab Dispos*. 2001; 29(9): 1196-1200.

49. Riley RJ, Grime K. Metabolic screening in vitro: Metabolic stability, CYP inhibition and induction. *Drug Discov Today Technol*. 2004; 1(4): 365-372. doi: 10.1016/j.ddtec.2004.10.008

50. Crespi CL, Miller VP, Penman BW. Microtiter plate assays for inhibition of human, drug-metabolizing cytochromes P450. *Anal Biochem*. 1997; 248(1957): 188-190. doi: 10.1006/abio.1997.2145

51. Palamanda JR, Favreau L, Lin C, Nomeir AA. Validation of a rapid microtiter plate assay to conduct cytochrome P450 2D6 enzyme inhibition studies. *Drug Discov Today*. 1998; 3(10): 466-470. doi: 10.1016/S1359-6446(98)01248-3

52. Turpeinen M, Korhonen LE, Tolonen A, et al. Cytochrome P450 (CYP) inhibition screening: Comparison of three tests. *Eur J Pharm Sci*. 2006; 29(2): 130-138. doi: 10.1016/j.ejps.2006.06.005

53. Nelson AC, Huang W, Moody DE. Variables in human liver microsome preparation: Impact on the kinetics of L-acetylmethadol (LAAM) N-demethylation and dextromethorphan O-demethylation. *Drug Metab Dispos*. 2001; 29(3): 319-325.

54. Florence VM, Di Stefano EW, Sum CY, Cho AK. The metabolism of (R)-(-)-amphetamine by rabbit liver microsomes. *Drug Metab Dispos*. 1982; 10(4): 312-315.

55. Bradford MM. A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72: 248-254.

56. Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: Identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. *Drug Metab Dispos*. 2002; 30(7): 845-852. doi: 10.1124/dmd.30.7.845

57. Pelkonen O, Puurunen J. Effect of cimetidine on microsomal drug metabolism in man. *Biochem Pharmacol*. 1980; 29(22): 3075-3080. doi: 10.1016/0006-2952(80)90448-7

58. Levine M, Law EY, Bandiera SM, Chang TK, Bellward GD. In vivo cimetidine inhibits hepatic CYP2C6 and CYP2C11 but not CYP1A1 in adult male rats. *J Pharmacol Exp Ther*. 1998; 284(2): 493-499.

59. Klotz U, Reimann IW. Drug Interactions through binding to cytochrome P450: The experience with H2-receptor blocking agents. *Pharm Res*. 1984; 1(2): 59-62.doi: 10.1023/A:1016347229923

60. Schmider J, Greenblatt DJ, Fogelman SM, Von Moltke LL, Shader RI. Metabolism of dextromethorphan in vitro: Involvement of cytochromes P450 2D6 and 3A3/4, with a possible role of 2E1. *Biopharm Drug Dispos*. 1997; 18(3): 227-240. doi: 10.1002/(SICI)1099-081X(199704)

61. Lin LY, Di Stefano EW, Schmitz DA, et al. Oxidation of methamphetamine and methylenedioxymethamphetamine by CYP2D6. *Drug Metab Dispos*. 1997; 25(9): 1059-1064.

62. Wu D, Victoria Otton S, Inaba T, Kalow W, Sellers EM. Interactions of amphetamine analogs with human liver CYP2D6. *Biochem Pharmacol*. 1997; 53(11): 1605-1612. doi: 10.1016/S0006-2952(97)00014-2

63. Sorkin E, Darvey D. Review of cimetidine drug interactions. *Drug Intell Clin Pharmacol*. 1983; 17(2): 110-120.

64. Taavitsainen P, Kiukaanniemi K, Pelkonen O. In vitro inhibition screening of human hepatic P450 enzymes by five angiotensin-II receptor antagonists. *Eur J Clin Pharmacol*. 2000; 56(2): 135-140. doi: 10.1007/s002280050731

65. Yamamoto T, Suzuki A, Kohno Y. Application of microtiter plate assay to evaluate inhibitory EŠects of various compounds on nine cytochrome P450 isoforms and to estimate their inhibition patterns. *Drug Metab Pharmacokinet*. 2002; 17(5): 437-448. doi: 10.2133/dmpk.17.437

66. de la Torre R, Yubero-Lahoz S, Pardo-Lozano R, Farré M. MDMA, methamphetamine, and CYP2D6 pharmacogenetics: What is clinically relevant? *Front Genet*. 2012; 3(NOV): 1-8. doi: 10.3389/fgene.2012.00235

67. Orishiki M, Matsuo Y, Nishioka M, Ichikawa Y. In vivo administration of H2 blockers, cimetidine and ranitidine, reduced the contents of the cytochrome P450IID (CYP2D) subfamily and their activities in rat liver microsomes. *Int J Biochem*. 1994; 26(6): 751-758. doi: 10.1016/0020-711X(94)90104-X

68. Martinez C, Albet C, Agundez JA, et al. The genetic polymorphisms of drug-metabolizing enzymes are a common determinant of interindividual differences in the therapeutic effect and toxicity of many drugs. *Clin Pharmacol Ther*. 1999; 65(4): 369-376. doi: 10.1016/S0009-9236(99)70129-3

69. Rendić S. Drug interactions of H2-receptor antagonists involving cytochrome P450 (CYPs) enzymes: From the laboratory to the clinic. *Croat Med J*. 1999; 40(3): 357-367.

70. Ruffalo R, Thompson J, Segal J. Diazepam-cimetidine drug interaction: A clinically significant effect. *South Med J*. 1981; 74(9): 1075-1078.

ISSN 2474-8978 http://dx.doi.org/10.17140/TFMOJ-2-120

71. Dostalek M, Jurica J, Pistovcakova J, et al. Effect of methamphetamine on cytochrome P450 activity. *Xenobiotica*. 2007; 37(12): 1355-1366. doi: 10.1080/00498250701652877

72. Dostalek M, Hadasova E, Hanesova M, et al. Effect of methamphetamine on the pharmacokinetics of dextromethorphan and midazolam in rats. *Eur J Drug Metab Pharmacokinet*. 2005; 30(3): 195-201. doi: 10.1007/BF03190620

73. Yang PP, Huang EYK, Yeh GC, Tao PL. Co-administration of dextromethorphan with methamphetamine attenuates methamphetamine-induced rewarding and behavioral sensitization. *J Biomed Sci*. 2006; 13(5): 695-702. doi: 10.1007/s11373-006-9096-4

74. Kreth KP, Kovar KA, Schwab M, Zanger UM. Identification of the human cytochromes P450 involved in the oxidative Metabolism of "Ecstasy" - related designer drugs. *Biochem Pharmacol*. 2000; 59: 1563-1571. doi: 10.1016/S0006-2952(00)00284-7

75. Meyer MR, Peters FT, Maurer HH. The role of human Hepatic cytochrome P450 isozymes in the metabolism of racemic 3, 4-methylenedioxy- methamphetamine and its enantiomers. *Pharmacology*. 2008; 36(11): 2345-2354. doi: 10.1124/dmd.108.021543

76. Downey C, Daly F, O'Boyle KM. An in vitro approach to assessing a potential drug interaction between MDMA (ecstasy) and caffeine. *Toxicol In Vitro*. 2014; 28(2): 231-239. doi: 10.1016/j.tiv.2013.10.021

77. Shin EJ, Nah SY, Kim WK, et al. The dextromethorphan analog dimemorfan attenuates kainate-induced seizures via sigma1 receptor activation: comparison with the effects of dextromethorphan. *Br J Pharmacol*. 2005; 144(7): 908-918. doi: 10.1038/sj.bjp.0705998

78. Shin EJ, Nah SY, Chae JS, et al. Dextromethorphan attenuates trimethyltin-induced neurotoxicity via σ1 receptor activation in rats. *Neurochem Int*. 2007; 50(6): 791-799. doi: 10.1016/j.neuint.2007.01.008

79. Benetton SA, Fang C, Yang Y-O, et al. P450 Phenotyping of the metabolism of selegiline to desmethylselegiline and methamphetamine. *Drug Metab Pharmacokinet*. 2007; 22(2): 78-87. doi: 10.2133/dmpk.22.78

80. Cheng J, Zhen Y, Miksys S, et al. Potential role of CYP2D6 in the central nervous system. *Xenobiotica*. 2013; 43(11): 973-984. doi: 10.3109/00498254.2013.791410

81. Haduch A, Bromek E, Daniel WA. Role of brain cytochrome P450 (CYP2D) in the metabolism of monoaminergic neurotransmitters. *Pharmacol Rep*. 2013; 65(6): 1519-1528.

82. Ramamoorthy Y, Yu A, Suh N, Haining RL, Tyndale RF, Sellers EM. Reduced (±)-3,4-methylenedioxymethamphetamine ("Ecstasy") metabolism with cytochrome P450 2D6 inhibitors and pharmacogenetic variants in vitro. *Biochem Pharmacol*. 2002; 63(12): 2111-2119. doi: 10.1016/S0006-2952(02)01028-6