The Potential Role of the Cytochrome P-450 2D6 Pharmacogenetic Polymorphism in Drug Abuse

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INTRODUCTION AND BACKGROUND

The Cytochrome CYP2D6 Genetic Polymorphism

Cytochromes P-450 (P450s) are enzymes involved in the oxidative metabolism of a wide array of endogenous and exogenous molecules including steroids, plant metabolites, prostaglandins, biogenic amines, drugs, and chemical carcinogens. This broad spectrum of reactions is due to multiple P-450 isozymes with differing but overlapping substrate specificities. The mammalian P-450 superfamily consists of at least 12 families and over 400 individual genes (Nelson et al. 1993). Genetic variants of P-450 have been discovered because of atypical clinical responses in individuals who were subsequently shown to have a reduced ability to metabolize a drug. The most widely studied P-450 polymorphism was first revealed with the antihypertensive drug debrisoquin (Mahgoub et al. 1977). This drug is 4-hydroxylated by a P-450 enzyme called cytochrome P-450 2D6 (abbreviated CYP2D6) that is encoded by the CYP2D6 gene.

At least 30 drugs, many of them derived from plant alkaloids, have subsequently been shown to be oxidized by CYP2D6, including tricyclic antidepressants, some neuroleptics, beta blockers, and antiarrhythmic agents such as perhexiline, flecainide, and encainide. Several drugs of abuse including codeine (Chen et al. 1988), hydrocodone (Otton et al. 199 *a*), oxycodone (Otton et al., unpublished observation), dextromethorphan (Schmid et al. 1985), and p-methoxyamphetamine (PMA) (Kitchen et al. 1979) are known to be metabolized by this enzyme. CYP2D6-mediated metabolism of these drugs is known to be a major source of pharmaco-kinetic variation and variation in drug effect.

Functional and Molecular Characterization of CYP2D6

The activity of CYP2D6 is measured using a subclinical dose of debrisoquin or another probe drug such as dextromethorphan. Using dextromethorphan, the amounts of unchanged drug and metabolite excreted in urine over zero to 8 hours are measured and expressed as an O-demethylation ratio (ODMR) (i.e., the ratio of dextromethorphan/dextrorphan or (dextromethorphan + 3-methoxymorphinan)/(dextrorphan + 3- hydroy- morphinan)). The frequency distribution of the logarithm of these ratios is bimodal (figure 1), with 7 to 10 percent of the population comprising the upper mode (called poor metabolizers [PMs]). The frequency shows considerable interethnic variation (Kalow 1991); for example, the frequency among African Americans is about 2 percent and among Chinese less than 1 percent. The remainder of the population, who perform this reaction to varying degrees, are called extensive metabolizers (EMs). ODMR values are constant over time and are not influenced by gender, smoking, and classical P-450 inducers such as phenobarbital and rifampicin (Eichelbaum et al. 1986; Vincent-Viry et al. 1991).

In addition to functional assays for CYP2D6, polymerase chain reaction (PCR) amplification assays have been developed for the detection of specific mutations in the CYP2D6 gene (table 1). Additional genotyping

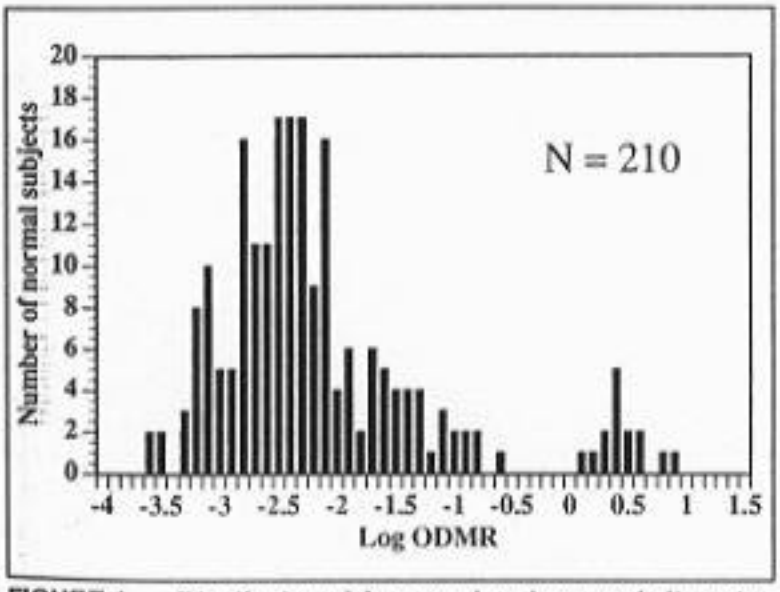

FIGURE 1. *Distribution of dextromethorphan metabolic ratio in normal subjects.*

assays use restriction fragment length polymorphisms (e.g., Xbal haplotypes) or allele-specific endonuclease digestion of PCR products to genotype the CYP2D6 locus. These assays can correctly detect > 95 percent of all mutant PM alleles found in caucasians (Broly et al. 1991). The CYP2D6 gene is part of a highly homologous gene cluster on chromosome 22 that includes CYP2D7 and CYP2D8P (Kimura et al. 1989). CYP2D8P contains several gene-disrupting insertions, deletions, and termination codons, indicating that this is a pseudogene. CYP2D7 is normal except for the presence of an insertion in exon 1 which disrupts the reading frame. The transcript for CYP2D7 has not been isolated, and it is unclear what, if any, the metabolic profile for this gene might be. The genotyping assays using PCR technology have an initial amplification that is specific for CYP2D6 introns in order to exclude the very similar CYP2D7 and CYP2D8P genes.

TABLE 1. *Frequencies of EMs and PMs among caucasians.*

Phenotype	Genotype	Frequency(%)
EM - rapid	wt/wt	54
EM - intermediate	wt/A, B, or D	39
PM - slow	A, B, or D/A, B, or D	7

Multiple CYP2D6 alleles that contain point mutations or codon deletions have been isolated (table 2). These mutant alleles, in addition to the CYP2D6 gene deletion (CYP2D6D), result in absent or impaired enzyme function (Broly and Meyer 1993; Gaedigk et al. 1991; Hanioka et al. 1990; Johansson et al. 1993, 1994; Kagimoto et al. 1990; Meyer 1994; Tyndale et al. 1991*a*). If present in the homozygous state, defective alleles result in a PM phenotype. Heterozygous combinations of mutant and wild type (wt) alleles tend to result in decreased CYP2D6 activity, although no functional means are available to fully distinguish between these heterozygous people and those who are homozygous wt/wt. In addition, CYP2D6 gene duplications that result in ultra-rapid enzyme function have recently been identified ($CYP2L_{2\&12}$) (table 2; Johansson et al. 1993).

TABLE 2. *Summary of CYP2D6 alleles, Xbal haplotypes, and their phenotypic consequences.*

Allele	Metabolic activity	Xbal haplotype (kb)
CYP2D6-wt	normal	29
CYP2D6-L	normal	29
(CYP2D6-L)2	ultra-rapid	42
(CYP2D6-L)12	ultra-rapid	175
CYP2D6-C	slightly decreased	29
CYP2D6-J	slightly decreased	29, 44
CYP2D6-W	slightly decreased	29, 44
CYP2D6-Ch1	slightly decreased	29, 44
CYP2D6-A	absent	29
CYP2D6-B	absent	29, 44, 9 + 16
CYP2D6-D	absent	11.5, 13
CYP2D6-E	absent	29
CYP2D6-F	absent	29
CYP2D6-G	absent	29

KEY: kb = kilobase.

While the majority of alleles in caucasians have now been isolated, the frequency of the CYP2D6 alleles listed in table 2 varies dramatically between ethnic groups. In addition, it is likely that many other alleles remain unidentified in other ethnic groups. Based on an understanding of the variants found in caucasians, one might predict that the unidentified variants might be unable to form a functional enzyme and have altered substrate specificity, selectivity, or turnover of the enzyme. The clinical importance of such variants is generally unknown; however, it is feasible that some ethnoracial differences in drug abuse patterns may be explainable by such differences.

The Rat Models of the Polymorphism

Rats have five CYP2D genes, CYP2D1 through CYP2D5, although CYP2D1 is the predominant enzyme expressed in rat liver. In the female Dark Agouti (DA) rat, the CYP2D1 gene is not expressed (Matsunaga et al. 1989) and these animals exhibit impaired oxidation of human CYP2D6 substrates (e.g., debrisoquin (Al-Dabbagh et al. 1981), dextromethorphan (Zysset et al. 1988)) compared to Sprague-Dawley (SD) rats. However, unlike the human deficiency, the deficiency in the CYP2D1 is due to a regulatory difference, not a defective gene for the enzyme. Nevertheless, the DA female rat may

be used as an animal counterpart of the human PM, and the SD female rat as the EM counter-part. Although females of these two rat strains are useful animal models, the rat CYP2D1 is not functionally identical to human CYP2D6. For example, quinidine is a very potent and long-lasting inhibitor of the activity of CYP2D6, both in vitro and in vivo, and its diastereoisomer quinine is 200 times less potent (Otton et al. 1984). However, quinine is the more potent inhibitor of rat CYP2D1 (Kobayashi et al. 1989).

CYP2D Enzyme in Brain

Catalytic, pharmacological, immunological, and molecular criteria have been used to identify cytochrome CYP2D in mammalian brain (Fonne-Pfister et al. 1987; Niznik et al. 1990; Tyndale et al. 1991*b*). The initial observation of CYP2D in dog brain was made during screening of central and peripheral tissues with tritiated GBR-12935, which labels the dopamine transporter protein and the so-called piperazine acceptor site or mazindol-insensitive site in brain tissue (Niznik et al. 1990). High concentrations of the piperazine acceptor site were found in liver microsomes. The similarity between amphetamine derivatives that inhibited both GBR-12935 striatal binding and hepatic CYP2D6 activity prompted further studies that demonstrated correlations between the inhibitor profile at the piperazine acceptor site purified from dog striata and the inhibition constant (K_i) for human hepatic CYP2D6 ($r = 0.85$). Immunoprecipitation and Western blotting experiments confirmed the presence of CYP2D in dog striata.

Subsequent studies focused on the catalytic activity of CYP2D in canine striata (Tyndale et al. 1991*b*). One of the classic CYP2D6 substrates, sparteine, was used as the marker for CYP2D activity in dog striata. High ($r \geq 0.95$) correlations were observed between inhibition of sparteine oxidation (K_i values) in canine striata and in human hepatic microsomes, and in human CYP2D6 expressed in HepG2 cells ($r = 0.93$). (-)-Cocaine was found to have particularly high inhibitory potency ($K_i = 74$ nanomolars (nM) for canine striatal CYP2D), and a high degree of overlap was found between compounds binding to the dopamine transporter and striatal CYP2D. The distribution of CYP2D in dissected regions of dog brain demonstrated a fortyfold range in activity, with the highest level being found in supraorbital cortex and parietal cortex, and the lowest in the cerebellum.

An uneven distribution of CYP2D activity was also observed in preliminary studies of dissected monkey *(C. Aethiops)* brain (Tyndale et al., unpublished data). Subcellular preparations of the nucleus accumbens, amygdala, and parietal cortex oxidized sparteine at rates of 300 to 600 picomoles per milligram of protein per hour (pmol/mg protein/hour). (By contrast, the rate in monkey hepatic microsomes was 62,000 pmol/mg protein/hour). The rate in striatum, hippocampus, and temporal and olfactory cortex was approximately 75 pmol/mg protein/hour. The spinal cord, frontal cortex, midbrain, medulla, and cerebellum had negligible activity. CYP2D activity in monkey brain displayed the stereoselective inhibition by quinidine and quinine that is characteristic of monkey liver CYP2D and human liver CYP2D6. Regional variation in the distribution of CYP2D messenger ribonucleic acid (mRNA) in rat and human brain has also been observed (Tyndale et al., unpublished observations). There is molecular evidence for CYP2D6 mRNA in human caudate (Tyndale et al. 1991*b*). The polymerase chain reaction was used to amplify a complementary deoxyribonucleic acid (cDNA) fragment (513 base pains) from a human caudate lambda-gt11 library. One hundred percent nucleotide identity to the CYP2D6 mRNA was found.

Relevance of CYP2D6 to Drug Abuse

A number of drugs of abuse are known substrates (e.g., codeine, hydrocodone, p-methoxyamphetamine, amphetamine) or inhibitors (e.g., (-)-cocaine, pentazocine) of CYP2D6. For some of these drugs, the pharmacokinetic differences due to the polymorphism will be so profound that they are likely to exceed pharmacodynamic sources of variation in response. For other drugs (e.g., hydrocodone to hydromorphone, codeine to morphine, oxycodone to oxymorphone), CYP2D6 may not contribute importantly to the overall clearance of the drug, but may catalyze the formation of highly active metabolites.

The consequences of absent or inhibited CYP2D6 for any particular drug depend on the relative activity of the parent drug and its various metabolites. In some cases, the pharmacology of the metabolite is qualitatively similar to the parent drug (e.g., hydrocodone to hydromorphone, codeine to morphine); in other cases, the pharmacology is different (e.g., dextro-methorphan to dextrorphan); more usually, it is not properly understood (e.g., PMA to 4-hydroxyamphetamine). In addition, CYP2D6 occurs within the central nervous system (CNS) (Fonne-Pfister et al. 1987; Tyndale et al. 1991*b*). Its role in the brain is unknown, but potential formation of active drug metabolites at their site of action makes the presence

of CYP2D6 here of great functional significance. A computer simulation study of interregional differences in CNS drug metabolism has provided a model that could account, in part, for large intersubject variability in the pharmacodynamic effects of psychoactive drugs (Britto and Wedlund 1992).

The authors hypothesize the following:

- The genetically determined activity of CYP2D6 that results in EMs (90 percent) and PMs (10 percent) of some drugs of abuse is both an important risk factor and protective factor in drug abuse and toxicity from drugs of abuse.

- Certain inhibitors of CYP2D6 or the PM state itself result in unexpected toxicity from drugs of abuse (e.g., PMA) or may have utility in the treatment of drug dependence (e.g., preventing activation of a pro-drug such as codeine or oxycodone).

- CYP2D6 may play an important neuroregulatory role in the brain, and may modulate brain functions important in drug-reinforced behavior or neurotoxicity.

CYP2D6-deficient individuals (PMs) should have a much decreased probability of abusing a drug converted to an active metabolite capable of maintaining drug-taking behavior (e.g., codeine, oxycodone, hydrocodone, dextromethorphan). In EMs, the probability is increased and is propor-tionate to the individual's genotype (homozygous versus heterozygous) and absolute CYP2D6 activity. Conversely, PMs should experience greater risk of abuse and of toxicity to a drug that is inactivated by CYP2D6 (e.g., PMA, methamphetamine); EMs should have a lesser risk.

Amphetamines and Phenethylamines. Deficiency in the p-hydroxylation of amphetamine was one of the observations that led to the discovery of the CYP2D6 polymorphism (Dring et al. 1970; Smith 1986). A single oral administration of the radiolabelled enantiomers of amphetamine to three volunteers with subsequent analysis of urine indicated that about 5 percent of (+)-amphetamine was converted to p-hydroxyamphetamine in two subjects but to a much less extent in the third subject, who was later found to have CYP2D6 deficiency (Smith 1986). The main excretion product was unchanged amphetamine (although the extent of excretion is known to be pH dependent), and the major metabolites were products of side

chain deamination (i.e., benzoic and hippuric acids). Total recovery of the radiolabelled dose in urine after 4 days was around 90 percent (Dring et al. 1970).

The hallucinogen PMA is O-demethylated by CYP2D6 to form 4-hydroxyamphetamine (Kitchen et al. 1979). A PM was observed to excrete 4.4 percent of a 5 milligram (mg) oral dose of PMA as 4-hydroxyamphetamine, compared with 50 to 65 percent excretion of this metabolite (free and conjugated) in EMs. Shortly after it made its appearance in Ontario in the early 1970s, PMA was associated with a number of deaths (Sellers et al. 1979). A cardinal feature of these deaths was cardiovascular excitation and hyperthermia; it had been noted earlier to produce marked and sustained elevation in blood pressure in some people.

Urinary excretion data of human subjects indicate that 4-hydroxylation of methamphetamine is much more extensive than that of amphetamine; the metabolic ratio (total hydroxymethamphetamine/methamphetamine) in urine averaged about 15 with individual variations of approximately fiftyfold (Shimosato 1988), suggesting considerable importance of CYP2D6 polymorphism in the fate of methamphetamine. On the other hand, there seems to be no information on the further metabolism of p-hydroxymethamphetamine as is available for p-hydroxyamphetamine.

p-Hydroxyamphetamine has been used as a drug (paredrine) in ophthalmology. Like amphetamine, it is known to release norepinephrine from postgan-glionic sympathetic nerve endings; unlike amphetamine, its use is associated with few if any CNS effects, presumably because its relative water solubility slows its passage through the blood-brain barrier (Burde and Thompson 1991). As with amphetamine, the releasing action of p-hydroxyamphetamine is thought to be a consequence of its inhibition of the reuptake mechanism and of monoamine oxidase activity, leading to a postsynaptic accumulation of not only dopamine but also of 5-hydroxy-tryptamine (Arai et al. 1990; Cho et al. 1975).

p-Hydroxyamphetamine is metabolized to pharmacologically active secondary products. Its biotransformation by dopamine-beta-hydroxylase leads to p-hydroxynorephedrine, a false neurotransmitter (Coutts and Baker 1989; Dougan et al. 1986; Smith 1986) with similar biochemical activities as p-hydroxyamphetamine (Arai et al. 1990). p-Hydroxy amphetamine is also metabolized by a neuronal

P450 to alpha-methyl-dopamine and further to alpha-methyl-noradrenaline, a CNS-active hypotensive agent that also may function as a false transmitter (Hoffman et al. 1979). Dougan and colleagues (1986) described the stereoselective neuronal accumulation of hydroxyamphetamine and hydroxynorephedrine in rat striatum and found them to have half-lives of 1.5 and 2.5 days, respectively, at this location.

d-Amphetamine hydroxylation has been observed in whole brain and in striatal preparations in both microsomal and mitochondrial membranes (Liccione and Maines 1989); manganese pretreatment increased amphetamine hydroxylation in both membranes. After administration of amphetamine, p-hydroxyamphetamine (also called alpha-methyl-p-tyramine), p-hydroxynorephedrine (also called alpha-methyl-p-octopamine), alpha-methyl-dopamine, and alpha-methyl-noradrenaline have been found in brain (studies mostly done in rat brain). Formation, or lack of formation, of these metabolites may be catalyzed by CYP2D, with the p-hydroxylation of amphetamine as the first step.

Suzuki and colleagues (1986, 1987) have tested the effects of altering methamphetamine p-hydroxylation in rats; it appeared that inhibition enhanced the methamphetamine-induced stereotyped behavior. One might conjecture that an equivalent effect could occur in humans with inborn CYP2D6 deficiency if a toxic dose of methamphetamine were applied. Unfortunately, studies of animals do not necessarily help; p-hydroxyamphetamine is the main metabolite of amphetamine in rats but not in human liver (see above), and most studies on brain metabolism have not been conducted in human tissue.

Matsuda and colleagues (1989) have shown that centrally acting amphet-amine metabolites may have different effects on different parts of the brain when applied locally or systemically. Thus the locus of formation of a metabolite within the brain could be critical, and metabolite formation in the liver might be of limited relevance.

Dextromethorphan. Dextromethorphan has the opposite steric configuration to codeine and morphine, is devoid of analgesic effects, but is as potent an antitussive as codeine. It is the most commonly used antitussive worldwide. Systematic studies concerning its abuse liability are few. In the earliest report (Isbell and Fraser 1953), no abuse liability was found with oral doses up to 100 mg. However, there are many case reports of dextromethorphan abuse and endemic use principally among young people (McElwee and Veltri 1990).

Monkeys and rats can be trained (with difficulty) to self-administer dextromethorphan, but do so more easily for its active metabolite dextrorphan. They also recognize dextrophan in particular as a discriminative stimuli, and generalize from and to phencyclidine (PCP) (Holtzman 1980). Interest in dextrorphan continues because of its similarity to PCP in mechanism of action and behavioral pharmacology, the potential role of N-methyl-D-aspartate (NMDA) antagonists in neuroprotection, and the evolving CNS pharmacology of dextromethorphan and dextrorphan (Musacchio et al. 1988; Tortella et al. 1989). The elucidation of the pharmacogenetic control of dextromethorphan kinetics, metabolism, and effects as a representative of the class could be important (Schadel et al. 1995).

In human volunteers the kinetics of dextromethorphan in EM and PM subjects are very different (Schadel et al. 1995). Hence, in a clinical practice the pharmacologic effects and consequences should be very different in the EM and PM phenotypes.

Prescription Opiates. Historically, methadone has evolved as the only widely accepted and used pharmacologial adjunct for the treatment of opiate abuse. Because methadone has strong reinforcing properties and produces physical dependence, it has generally been reserved for the management of heroin abuse and dependence. From a public health perspective, the misuse and abuse of opiate-containing prescription medications is a poorly understood and largely unaddressed treatment issue. Prescription analgesics including opioids (e.g., codeine, oxycodone, and hydrocodone) alone and in combination are extensively used worldwide and are always within the major classes of drugs prescribed in all countries. While most users have a legitimate need for these medications, there is also substantial evidence of misuse, abuse, and dependence since the frequency of use appears to exceed the frequency of acute and chronic pain in the population.

Canada has the dubious distinction of having the world's largest per capita consumption of precursor opiate-containing compounds (e.g., codeine, oxycodone, and hydrocodone) (Korcok 1979). A drug utilization review of opiate use in Canada from 1978 to 1989 shows continued increase in the use of prescribed codeine combination products. The defined daily dose (DDD)/1000 inhabitants/day for prescribed codeine-acetaminophen products has increased from 3.3 DDD/1000 inhabitants/day in 1982 to 8.1 DDD/1000 inhabitants/day in 1989. Oxycodone-acetaminophen containing products have also been increasing from 0.04 DDD/1000 inhabitants/day in 1978 to 0.21

DDD/1000 inhabitants/day in 1989 (Seto, unpublished data). Intercontinental Medical Statistics Canada reports that acetaminophen with codeine was the most frequently dispensed prescription product in Canada from 1989 to 1992 and accounted for 56 percent of all new prescriptions in 1992 (Theirriault 1992). The situation is similar in the United States, with codeine being the second most frequently dispensed chemical entity according to a 1987 drug utilization review (Tomita et al. 1988). Acetaminophen with codeine ranked as the fifth and fourth leading prescription product dispensed in 1985 and 1986 respectively (Baum et al. 1987). In 1992 and 1993, as determined by chart review and index drug report by patients, 423 patients have presented to the Clinical Research and Treatment Institute of the Addiction Research Foundation with codeine (60 percent), oxycodone (35 percent), or hydrocodone (5 percent) as the primary problem substance (authors' unpublished data). This data likely underestimate the extent of the problem because reseachers have only recently been systematically focusing on these drugs. The clinical impression is that at least half of all polydrug users have lifetime prescription opiate drug abuse or dependence.

In Vitro Oxidation of Drugs of Abuse by CYP2D6

Identification of Substrates/Inhibitors of Human Hepatic CYP2D6.

The authors have been conducting studies to identify drugs of abuse that interact with CYP2D6 enzyme in human liver microsomes. The results provide drug candidates for further studies aimed at determining whether they are CYP2D6 substrates (e.g., PMA, 3, 4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA)) or inhibitors of CYP2D6 (e.g., fluoxetine, (-)-cocaine). In table 3, competitive inhibitors of CYP2D6-catalyzed formation dextrorphan from dextrometh-orphan are identified with their K_i value. The lower the K_i value, the more potent the inhibitor (higher affinity for CYP2D6).

These in vitro results identify compounds that interact with hepatic CYP2D6, and compounds from this list become the candidates for the authors' investigations of the biological importance of this enzyme activity.

TABLE 3. *A selected list of drugs tested for an interaction with human hepatic CYP2D6.*

	K_i (M)
Phenethylamines	
(+)-Amphetamine[1]	28
(-)-Amphetamine	no interaction
(+)-Methamphetamine[1]	25
4-Methoxyamphetamine (PMA)[1]	18
3,4-Methylenedioxyamphetamine (MDA)[1]	3
Opioids	
Hydrocodone[1]	55
Oxycodone[1]	55
Morphine	150
Nalorphine	35
Methadone	3
Stimulants	
(-)-Cocaine[2]	0.74
Miscellaneous	
Nicotine	no interaction
Ketamine	no interaction
Caffeine	no interaction

KEY: 1 = identified by further studies in vitro as a CYP2D6 substrate; 2 = identified by further studies in vitro not to be a CYP2D6 substrate.

Findings that have stemmed from this screening include the following:

1. The establishment that CYP2D6 converts hydrocodone to its active metabolite hydromorphone in vivo. This drug was subsequently selected as the prototypical pro-drug opiate used to study the role of CYP2D6 activity variants in opiate plasma kinetics and in responses related to abuse (Otton et al. 1993*a*).
2. A possible clinical consequence of the potent (K_i = 0.18 micromolar (M)) inhibition of CYP2D6 by fluoxetine and its major metabolite norfluoxetine. The authors described a case report of a profound increase in the dose of oxycodone required for analgesia after the initiation of fluoxetine therapy. This led

to the observation that oxycodone is converted to its active metabolite oxymorphone via CYP2D6, and that therapeutic doses of fluoxetine inhibit CYP2D6 activity in vivo (Otton et al. 1993*b*). The kinetic profile of fluoxetine and its normetabolite favor its use as a long-acting CYP2D6 inhibitor; hence the drug might be used to inhibit the activation of codeine, hydrocodone, or oxycodone (also called producing a phenocopy because the urine metabolites look the same as in PMs) in abusers and thereby reduce the reinforcing properties of the medication.

3. In vitro inhibition of CYP2D6 activity by methadone (K_i = 3 M) predicted drug interactions in vivo. This finding was confirmed in 42 abusers of oral opiates undergoing treatment with methadone (Wu et al. 1993*a*).

4. The O-demethylation of the hallucinogen PMA to 4-hydroxyamphetamine is catalyzed in vitro by CYP2D6 (Wu et al. 1994). This confirms earlier data from three subjects (one a PM) that this reaction was catalyzed by CYP2D6 (Kitchen et al. 1979). Methamphetamine's major metabolite, 4-hydroxyamphetamine, is formed via CYP2D6 (as determined by incubations of methamphetamine with microsomes prepared from the yeast transformed with an expression plasmid containing full-length human CYP2D6 cDNA (a gift of Dr. S.W. Ellis and Dr. M.S. Lennard, Sheffield, U.K.).

5. Because of (-)-cocaine's extremely high affinity for hepatic CYP2D6 (K_i = 0.07 M), this drug is not metabolized by CYP2D6. The authors incubated (-)-cocaine with cloned human CYP2D6 enzyme expressed in yeast. Using a gas chromatography/mass spectrometry assay, no detectable ecgonine, ecgonine methyl ester, ecgonidine, ecgonidine methyl ester, norecgonidine methyl ester, norecgonine methyl ester, benzoylecgonine, o-m-p-hydroxycocaine, or norcocaine was formed during the incubations (Otton et al., unpublished observations).

In Vitro Comparison of Monkey Hepatic CYP2D6-Like Activity With Human CYP2D6. The monkey would be a valuable model for assessing the role of CYP2D6 enzyme activity in drug reinforcement and response. Catalytic, immunologic, and electrophoretic investigations indicated that the enzymes were indistinguishable (Otton et al. 1992). However, the conclusion after more extensive examination of the inhibitor specificity of the two enzymes was that

they were functionally homologous, but not identical (Wu et al. 1993*b*). These studies also predicted that in monkeys an established CYP2D inhibitor, budipine, will more readily produce PM phenocopies than quinidine.

Dextromethorphan Disposition in Rats and Monkeys

Kinetics of Dextromethorphan and Metabolites in Rat Plasma and Brain. Because of dextrorphan's higher affinity for NMDA/PCP sites than dextromethorphan, the abuse characteristics of dextromethorphan are likely due to its CYP2D1-mediated conversion to dextrorphan. Time course studies of this and other metabolites after an intraperitoneal (IP) dose of dextromethorphan indicate that conjugated dextrorphan is the predominant metabolite in plasma, and that only free dextrorphan is present in the brain (Wu et al. 1995). Brain dextrorphan levels were correlated with free dextrorphan in plasma ($r = 0.84$), but not with conjugated plasma metabolite. Similar studies of the disposition of dextrometh-orphan and metabolites following different routes of administration suggest that each will be associated with a different pharmacology (Wu et al. 1995).

FUTURE RESEARCH GOALS AND DIRECTION

Areas which would be important for future research include the following:

1. Identify and characterize more drugs of abuse, particularly phenethylamines and related compounds, that are substrates or inhibitors of the genetically polymorphic human drug metabolizing enzyme cytochrome P-450 2D6 (CYP2D6).

2. Define the localization, catalytic specificity, and regulation of CYP2D forms in rat, monkey, and human brain.

3. Using animal models, determine the importance of CYP2D deficiency or high catalytic activity to the toxicity and behavioral consequences of amphetamines, as models of active drugs of abuse with metabolites of different pharmacologic activity.
4. Determine and compare the clinical consequences of CYP2D6 genotype to the metabolism, kinetics, pharmacologic effects, and abuse liability of methamphetamine, d-amphetamine, and dextromethorphan.

CONCLUSIONS

The authors expect these studies will identify some drugs of abuse for which the EM state, the PM state, or CYP2D6 inhibitors can modify the risk of abuse; explain why some drugs of abuse are so attractive or so toxic to some individuals; explain why some patterns of drug abuse are endemic and rarely epidemic (e.g., PMA, dextromethorphan, smoked methamphetamine, phenethylamine designer drugs); identify the location of CYP2D in the brain and determine its role and importance for variation in drug response to drugs of abuse; justify the incorporation of CYP2D6 screening as part of regulatory or scheduling requirements; and establish animal models for the study of cytochrome P-450s as risk and protective factors in drug abuse.

This work can also result in needed new therapeutic strategies for the treatment of drug abuse. Such issues are particularly important for management of dependence on prescription opiates, which is the third largest drug dependence problem in North America (after nicotine and alcohol) and the least studied and understood.

With respect to treatment, depending on the particular biotransformation pattern and the activity of the metabolites, several therapeutic approaches are evident. For example, EMs (93 percent of the caucasian population) could receive an inhibitor as part of a therapeutic regimen. Such inhibition will alter the kinetics, toxicity, drug-reinforcing properties, and physical dependence liability of some drugs of abuse (e.g., hydrocodone, oxycodone, codeine) and make the drug less pharmacologically attractive.

From a broader scientific perspective, the authors' studies of the drug- metabolizing activity of the brain will contribute to psychopharmacological approaches to the treatment of mental disorders and to researchers' understanding of brain function involving endogenous neurotransmitters, exogenous drugs, neurosteroids, and neuroactive steroids.

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