Cytochrome P450

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The cytochrome P450 (CYP450) enzymes are a major determinant of the pharmacokinetic behavior of numerous drugs.

CYP450 enzymes are so named because they are bound to membranes within the cell, specifically the endoplasmic reticulum (cyto) and contain a heme pigment (chrome and P) that absorbs light at a wavelength of 450nm when exposed to carbon monoxide. Each cytochrome P450 isoenzyme consists of a single protein chain and one haem group as the binding site for the drug.

The CYP450 enzymes are found predominantly in the liver but also exist in the small intestine (reducing drug bioavailability), brain, lung, adrenal gland, kidney, bone marrow, skin, ovary, testes, and placenta.

Classification

There are about 50 different CYP's found in humans.
There are many different isoforms of cytochrome P450. An isoform is a CYP enzyme variant that derives from one particular gene. These isoenzymes are classified according to similarities of their amino acid sequencing into families (number), subfamilies (letter) and individual genes/specific enzymes (number).

Families: Members of a family must have at least 40% sequence homology. Families are numbered e.g. CYP 1, CYP 2. There are at least 74 CYP families but only about 17 have been described in man.

Subfamilies: Members of a subfamily must have at least 55% sequence homology. About 30 subfamilies are well described in humans. Subfamilies are identified by a letter e.g. CYP2D

Individual genes: There are about 50 important genes in man. Individual genes are identified by a number e.g. CYP2D6

CYP450 and metabolism

Metabolism involves enzymatic conversion of one chemical entity to another within the body. Biotransformation is the metabolism of substances foreign to the body.

Most drugs are highly lipid soluble and nonpolar and are not easily eliminated by the kidney so most lipophilic substances are converted to more polar/hydrophilic products which can then be excreted in the urine. Biotransformation makes drugs water soluble.

Most drugs are metabolized by two groups of enzymes; CYP450 and non-microsomal enzymes (e.g. monoamine oxidase, alcohol dehydrogenase). The cytochrome P450 enzyme system catalyses the metabolism of endogenous and exogenous compounds. Here we are predominantly interested in the catabolism of drugs by the cytochrome P450 system.

Drug metabolism occurs predominantly (but not exclusively) in the liver. Other sites include kidney, lungs, blood, GIT and placenta. Drug metabolism involves two kinds of reactions; Phase 1 and Phase 2. These usually, although not always, occur sequentially.

Phase 1 reaction: Metabolic transformation.
Catabolic reaction that occurs by oxidation, reduction or hydrolysis. The result of this transformation can be:

Active drug converted to inactive metabolite (most common).
Inactive drug converted to active metabolite (prodrug e.g. tramadol).
Active drug converted to active metabolite (prolonging the effect of the drug e.g. morphine).

Phase 1 reactions often introduce a reactive group, such as hydroxyl, into the molecule. This then serves as the point of attack for the conjugating system to attach a substituent e.g. glucuronide. CYP450 is responsible for 70-80% of phase 1 metabolism. Oxidation is the most common phase 1 reaction. Phase 1 oxidative reactions catalyzed by CYP450 include epoxidation, N-dealkylation, O-dealkylation, S-oxidation and hydroxylation. Oxidation can result in both activation and inactivation of a compound.

Drug oxidation by the P450 system requires drug/substrate, P450 enzyme, molecular oxygen, NADPH and a flavoprotein (NADPH P450 reductase). Basically one atom of oxygen is added to the drug to create a hydroxyl group, the other atom of oxygen is converted to water. A typical cytochrome P450 oxidation reaction is as follows, where R is the substrate/drug:

$$\mathrm{NADPH + H^+ + O_2 + RH \Rightarrow NADP^+ + H_2O + R\text{-}OH}$$

Reductive reactions are much less common than oxidation reactions (e.g. warfarin inactivated by conversion of a ketone to a hydroxyl group by CYP 2A6)

Phase 2 reaction: Conjugation reaction
Phase 2 reactions are synthetic/anabolic. Involves conjugation which usually results in an inactive product. A covalent link is formed between a functional group on the substrate and acetate, amino acids, glucoronic acid or glutathione. These polar inactive products can then be excreted in the urine/faeces. Phase 2 metabolism does not require cytochrome P450 enzymes.

Function of CYP450 enzymes

CYP is integral to the metabolism of both endogenous and exogenous compounds.

Physiological function:

- Biosynthesis and degradation of endogenous compounds e.g. steroid hormones, cholesterol and fatty acids.
- Physiological role in the brain where it is involved in the release of peptide hormones from the hypothalamus and pituitary.
- Regulation of vascular tone in the brain by arachidonic acid metabolites.
- CYP2D6 may regulate metabolism and processing of neurotransmitters such as dopamine and serotonin and have a role in determining mental state/personality.
- Vascular autoregulation particularly in the brain.
- Adrenal and gonadal steroidogenesis are influenced by the CYP450 enzymes.
- CYP450 has a role in the control of body fluid volume and composition and hence blood pressure, by its action on arachidonic acid, the metabolites of which have both vasoconstrictive and vasodilatory activity.

Pharmacological function:

- CYP450 in the liver plays a major role in drug metabolism by converting drugs from a hydrophobic state to a more readily excretable hydrophilic form, mainly by an oxidation reaction. Metabolism of exogenous substances (drugs, environmental pollutants, chemicals, anaesthetic agents) may produce metabolites that are toxic or carcinogenic.
- CYP450 enzymes are essential for the production of cholesterol, steroids, prostacyclins and thromboxane A2.
- Detoxification of foreign chemicals/toxins.

CYP450 enzymes

These enzymes differ from one another in amino acid sequence, in sensitivity to inhibitors and inducers, and in the specificity of the reactions that they catalyse. Different members of the family have distinct but often overlapping substrate specificities with some enzymes acting on the same substrates but at different rates. One drug can be metabolized by several different isoenzymes.

There are 74 CYP gene families, but CYP families 1-3 are responsible for the metabolism of drugs in the human liver.

CYP450's in the CYP2C, 2D and 3A subfamilies are most active in metabolizing known clinically used drugs. CYP3A4 and CYP2D6 metabolise most anaesthetic drugs.

The CYP's most important in oxidative metabolism are:

- CYP3A4
- CYP2D6*
- CYP2C9*
- CYP2C19*
- CYP2A6*
- CYP2E1
- CYP1A2

* polymorphic

Enzyme induction can occur with all of these, except CYP2D6.
The following are polymorphic: CYP2D6, CYP2C9, CYP2C19, and CYP2A6.
More than one CYP450 can be involved in the metabolism of a particular drug.

Specific enzymes

CYP3A4: These enzymes account for 30% of liver P450 and 70% of gut P450. CYP3A4 is the most common cytochrome subfamily in the liver. Significant first pass metabolism of midazolam is brought about by mucosal CYP3A4 of the small intestine. Rifampicin is the most potent inducer of CYP3A4. The CYP3A4 enzyme is responsible for the metabolism of several drugs used in anaesthesia: Opioids (fentanyl, sufentanyl, alfentanil), benzodiazepines (midazolam, diazepam) and local anaesthetics (lignocaine). One study showed that the elimination half-life of midazolam was prolonged by 50% and its clearance reduced by 30% by the co-administration of fentanyl at induction, probably as a result of competitive inhibition of CYP3A4 activity.

CYP2D6: This enzyme has been extensively studied. Genetic polymorphisms of this enzyme exist. The enzyme is NOT inducible by pharmacological agents. Drugs that inhibit the enzyme essentially turn the patients taking them into poor metabolisers. This enzyme is responsible for the biotransformation of codeine to morphine. Substrates: Codeine, beta blockers, antidepressants, antipsychotics, neuroleptics, antiarrhythmic. Anaesthetic drugs: Codeine, tramadol, ondansetron, granisetron.

CYP2C9: Substrates include NSAIDS, warfarin.

CYP2C19: Substrates include TCA's, diazepam, clopidogrel.

CYP2E1: Responsible for metabolism of volatiles. CYP2E1 is a major catalyst for the formation of trifluoroacetylated proteins from halothane which have been implicated as target antigens responsible for halothane hepatitis.

CYP1A2 metabolises ropivacaine

Polymorphisms, enzyme induction and enzyme inhibition

Within the human population, there are major sources of interindividual variation in P450 enzymes. Drug-drug interactions can occur when one drug alters the metabolism of another drug by inhibition or induction of the CYP450 enzymes. Metabolic enzymes are under genetic control and so may vary between different populations or individuals.

Changes in activity of CYP450 can therefore result from:

- Genetic polymorphism
- Enzyme inhibition
- Enzyme induction
- Physiological/environmental factors (vitamin deficiencies, pregnancy, fasting)

These factors are of major importance in therapeutics.

Genetic polymorphism

In different people and different populations, **activity** of CYP450 oxidases differs. Because of this different people may respond differently to the same drug. Genetic variation in a population is termed polymorphism when both gene variants exist with a frequency of at least 1%.

Polymorphisms are relevant if:

1. The metabolising enzyme is responsible for 50% or more of the clearance of the drug.
2. The drug has a steep dose-response curve and a narrow therapeutic window.
3. Drug activity depends on an active metabolite formed by a polymorphic enzyme.

Individuals can be classed as having poor, intermediate, extensive (normal) or ultra-rapid CYP450 activity.

A specific gene encodes each CYP450 enzyme. Every person inherits one genetic allele from each parent. Alleles are 'wild type' or 'variant'. 'Wild type' occurs most commonly in the general population. Variant alleles encode an enzyme with reduced or no activity. The frequency of variant alleles varies significantly and depends on race and ethnic background.

Poor metaboliser: 2 copies of variant alleles (lack both copies of the functional allele).

Intermediate metaboliser: Heterozygous for one functional (wild type) and one deficient (variant) allele or two partially defective alleles that cause reduced enzyme activity.

Extensive (normal) metaboliser: 2 copies of wild type alleles (functional allele). Majority of population.

Ultra-rapid metaboliser: Multiple copies of wild type alleles (3 or more functional alleles) resulting in excess enzyme activity.

Genetic variations in CYP450 metabolism should be considered when patients exhibit unusual sensitivity or resistance to drug effects at normal doses.

Testing is available to categorise CYP2D6 metabolism as poor, intermediate, extensive and ultra-rapid. This can be helpful in evaluating the efficacy and dosing of many medications used in breast cancer treatment and psychiatry. Although testing may be beneficial in anaesthesia, associated adjustments in dosing and clear recommendations have not been fully developed and have not become standard practice.

Examples of polymorphisms

CYP2D6 is polymorphic. Codeine's analgesic activity is as a result of its conversion to morphine by CYP2D6. In CYP2D6 poor metabolisers codeine will be ineffective as an analgesic. Ultra-rapid metabolisers will produce more morphine from a standard codeine dose and put the patient at risk of an opioid overdose. This has been implicated in infant toxicity from breastfeeding in mothers taking codeine analgesia. Other drugs metabolized by CYP2D6 e.g. antidepressants and neuroleptics, in poor metabolisers, will predispose the patient to drug toxicity. B-blocker removal can be impaired in 2D6 poor metabolisers.

Another polymorphism with significant consequences is deficient activity of CYP2C9. These patients will be ineffective in clearing (S)-warfarin…so could be fully anticoagulated on just 0.5mg warfarin a day.

Clopidogrel is a prodrug that requires biotransformation to an active metabolite by cytochrome p450 enzymes for its antiplatelet effect. Patients with reduced CYP2C19 activity have significantly lower levels of the active metabolite of clopidogrel, diminished platelet inhibition and therefore a higher rate of adverse cardiovascular events including stent thrombosis. CYP2C19 function is absent in 30% of Chinese people and 15% Caucasian. Genotyping is recommended by the AHA and ACC for those at high cardiovascular risk being treated with clopidogrel.

One beneficial CYP450 phenotype can be seen in patients deficient in CYP2C19…they have higher cure rates for peptic ulcer disease treated with omeprazole due to sustained high plasma levels achieved.

Does polymorphism affect drug design? Designing and making drugs costs a fortune. The interaction between CYP450 and newly designed drugs is so important to pharmaceutical companies that predominant degradation of a drug by one of the polymorphic CYP's is often enough to stop any further research/development on that drug!

CYP polymorphisms may be implicated in an individuals' susceptibility to disease e.g. lung cancer and Parkinson's disease.

Enzyme induction and enzyme inhibition

Knowledge of the CYP450 system and its substrates is a key factor in the prevention of important drug-drug interactions, either due to enzyme induction or inhibition. Drugs interact with the CYP450 system in several ways. A drug may be metabolized by only one enzyme or by multiple enzymes (warfarin CYP1A2, 2D6, 3A4). Drugs that cause metabolic drug interactions are referred to as either inducers or inhibitors.

Enzyme induction

An important cause of drug interactions and adverse drug reactions.

Certain drugs induce the enzymes responsible for their metabolism (rifampicin, phenobarbitone). These enzymes also metabolise other drugs. The clearance of the 2nd drug will therefore be increased by an enzyme inducer. This increased clearance means that a greater dose of the 2nd drug will be required to have the same therapeutic effect as that seen without enzyme induction.

Inducers increase CYP450 activity by increasing enzyme synthesis (DNA transcription) so there is usually a delay before enzyme activity increases. Most of the CYP450's can be induced except 2D6! Over 200 drugs can cause enzyme induction.

Result of enzyme induction:

Decreases concentration of the substrate drug. Increased dose required for same therapeutic effect.

Prodrug: Increased production of active drug metabolite. Patient at risk of overdose.

A drug can be metabolized by the same enzyme that it induces. In other words, the inducing agent is normally itself a substrate for the induced enzyme so this can result in slowly developing tolerance. Pharmacokinetic tolerance is less marked than pharmacodynamic tolerance (opioids) but is clinically important when starting treatment with carbamazepine. It is an enzyme inducer but is started at a low dose to avoid toxicity as enzymes are not yet induced, and then gradually increased over a few weeks during which time it induces its own metabolism and its half-life gradually decreases over time.

Rifampicin is the most notable enzyme inducer. Three days of rifampicin treatment will decrease the effectiveness of warfarin.

CYP3A4 metabolises many substrates and is induced by rifampicin, carbamazepine, phenytoin and dexamethasone. This will increase the metabolism of opioids, benzodiazepines and local anaesthetics.

If the active metabolite of a drug is toxic e.g. NAPQI metabolite of paracetamol, then enzyme induction will increase the risk of toxic side effects. The risk of serious hepatic injury following paracetamol overdose is increased in patients whose CYP450 system has been induced, e.g. by the

chronic use of alcohol.

Enzyme inhibition
Inhibitors block the metabolic activity of CYP450 enzymes.

The extent to which an inhibitor affects metabolism of a drug depends on factors like the dose of the inhibitor and the ability of the inhibitor to bind to the enzyme. A drug can be metabolised by and inhibit the same enzyme e.g. erythromycin, or it can be metabolised by one enzyme and inhibit another. Inhibitory effects usually occur immediately.

Inhibition take place by a variety of mechanisms:

- Drug may compete for active site but is itself not a substrate.
- Non-competitive inhibitors bind to the enzyme.
- Mechanism based inhibitors require oxidation by a P450 enzyme, oxidation products bind to haem.
- Direct irreversible inactivation

Result of inhibition of CYP450:
Increase the concentration of the substrate drug.

Decreased activity of prodrug metabolized by enzyme e.g. Clopidogrel is a prodrug, requiring metabolism by CYP450 into its active form to prevent arterial thrombosis. Omeprazole (PPI) inhibits the metabolism of clopidogrel and so reduces the efficacy of the drug in preventing arterial thrombosis. Other PPI's e.g. lansoprazole, have a much lower affinity for the CYP enzyme and so do not affect the efficacy of clopidogrel.
Propofol interferes with the metabolism of alfentanil and sufentanil by inhibiting CYP2B1 and CYP1A1 and so may potentially alter the metabolism of co-administered alfentanil/sufentanil.

Severe toxicity can result if enzyme inhibiting drugs are added to the following medications: Atypical antipsychotics, benzodiazepines, statins, warfarin.

Common inhibitors: Cimetidine, azole antifungals (ketoconazole), HIV protease inhibitors, calcium channel blockers, erythromycin, SSRI antidepressants. Cimetidine, an H2 receptor antagonist, inhibits many different CYP's and is now rarely used because of its inhibitory effect on CYP450.

Drugs can be intentionally combined to take advantage of CYP450 inhibition: Ritonavir, a protease inhibitor and potent CYP3A4 inhibitor, is added to lopinavir to boost serum levels of the later in HIV.

Detailed information of metabolizing enzymes and of drug interactions for common drugs is increasingly available in texts or electronically.

Environmental / Physiological
Dietary chemicals can affect the concentration of CYP enzymes by a variety of mechanisms such as changes in the rate of gene transcription or degradation of mRNA. Cigarette smoke is an enzyme inducer. Fasting has been shown to induce CYP2E1 enzyme. In general vitamin deficiencies lower CYP450 activity.

Table 1. Significant Cytochrome P450 Enzymes and Their Inhibitors, Inducers, and Substrates

Enzyme	*Potent inhibitors**	*Potent inducers†*	*Substrates*
CYP1A2	Amiodarone (Cordarone), cimetidine (Tagamet), ciprofloxacin (Cipro), fluvoxamine (Luvox‡)	Carbamazepine (Tegretol), phenobarbital, rifampin (Rifadin), tobacco	Caffeine, clozapine (Clozaril), theophylline
CYP2C9	Amiodarone, fluconazole (Diflucan), fluoxetine (Prozac), metronidazole (Flagyl), ritonavir (Norvir), trimethoprim/sulfamethoxazole (Bactrim, Septra)	Carbamazepine, phenobarbital, phenytoin (Dilantin), rifampin	Carvedilol (Coreg), celecoxib (Celebrex), glipizide (Glucotrol), ibuprofen (Motrin), irbesartan (Avapro), losartan (Cozaar)
CYP2C19	Fluvoxamine, isoniazid (INH), ritonavir	Carbamazepine, phenytoin, rifampin	Omeprazole (Prilosec), phenobarbital, phenytoin
CYP2D6	Amiodarone, cimetidine, diphenhydramine (Benadryl), fluoxetine, paroxetine (Paxil), quinidine, ritonavir, terbinafine (Lamisil)	No significant inducers	Amitriptyline, carvedilol, codeine, donepezil (Aricept), haloperidol (Haldol), metoprolol (Lopressor), paroxetine, risperidone (Risperdal), tramadol (Ultram)
CYP3A4 and CYP3A5	Clarithromycin (Biaxin), diltiazem (Cardizem), erythromycin, grapefruit juice, itraconazole (Sporanox), ketoconazole (Nizoral), nefazodone (Serzone‡), ritonavir, telithromycin (Ketek), verapamil (Calan)	Carbamazepine, *Hypericum perforatum* (St. John's wort), phenobarbital, phenytoin, rifampin	Alprazolam (Xanax), amlodipine (Norvasc), atorvastatin (Lipitor), cyclosporine (Sandimmune), diazepam (Valium), estradiol (Estrace), simvastatin (Zocor), sildenafil (Viagra), verapamil, zolpidem (Ambien)

CYP=cytochrome P.

**—These will slow down substrate drug metabolism and increase drug effect.*
†—These will speed up substrate drug metabolism and decrease drug effect.
‡—Brand not available in the United States.

Am Fam Physician 2007

Cancer and CYP450

There is some speculation about the role of CYP proteins and polymorphisms as causes of cancer, some may activate pro-carcinogens to carcinogens and many are involved in the removal of carcinogens from the body. For cancers that are hormone sensitive, CYPs involved in steroid metabolism may play a role in suppression/promotion of malignancies through such metabolism. CYP2D6 has been implicated in mediating carcinogenesis by activating procarcinogens in tobacco smoke leading to lung cancer. Genetic polymorphisms of CYP2E1 may play a role in the development of hepatic cancer.

CYP and age

There is variable expression of CYP enzymes in different people, different race groups and at different ages. CYP 1A2 is not expressed in neonates making them particularly susceptible to toxicity from drugs such as caffeine. Fetuses and neonates have low activity of CYP2D6 and can functionally be considered 2D6 poor metabolisers. CYP3A4 activity is also low in term and preterm neonates, increases in early childhood and then gradually decreases to adult levels around puberty. In the elderly, an age-related 20% reduction in metabolism of CYP2D6 substrates has been observed but the same has not been seen for the CYP3A family.

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