General Principles of Pharmacology and Toxicology

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We are not talking about metabolism, but Drug Metabolism

- Metabolism is the process by which your body converts food into energy.
- During this biochemical process, calories — from carbohydrates, fats and proteins — are combined with oxygen to release the energy your body needs to function.
History

Richard Tecwyn Williams (1909-1979)

• 1942, worked on the metabolism on TNT with regard to toxicity in munitions workers; due to the war he assembled teams to work on metabolism of sulfonamides, benzene, aniline, acetanilide, phenacetin, and stilbesterol

• Developed concept of Phase 1 & Phase 2 Reactions.
Drug Metabolism

• Drug metabolism means the necessary changes in the drug molecules which are essential for the easy excretion from the body.

• Metabolism cause the drugs to be more water soluble and / or more ionizable.

• Drug metabolism can result in toxication or detoxication - the activation or deactivation of the chemical. While both occur, the major metabolites of most drugs are detoxication products.

• Chemical modification of drugs in the body is also called biotransformation.
Factors affecting biotransformation

race

age

sex

species (rabbit, monkey, mouse, man)

biotransformation route

other drug administration

food
What are Xenobiotics?

- Xenobiotics are substances foreign to the body.
- Xenobiotics to which humans are exposed include:
  - Environmental pollutants
  - Food additives
  - Cosmetic products
  - Agrochemicals
  - Processed food

and

- Drugs
Metabolism of xenobiotics

• Many xenobiotics are lipophilic chemicals

• In absence of metabolism, xenobiotics would not be efficiently eliminated and would accumulate in the body (causing toxicity?)

• Most xenobiotics are subjected to metabolic pathways that convert these hydrophobic chemicals into more hydrophylic derivatives that are readily eliminated (urine or bile)

Remember pro-drugs! (*Prednisone*, a synthetic cortico-steroid drug, *is converted by the liver into the active drug prednisolone, which is also a steroid*).
Sites of drug metabolism

Liver
Intestine
Kidney
Lungs
Nasal mucosa
Brain
Hepatic microsomal enzymes (oxidation, conjugation)

Extrahepatic microsomal enzymes (oxidation, conjugation)

Hepatic non-microsomal enzymes (acetylation, sulfation, GSH, alcohol/aldehyde dehydrogenase, hydrolysis, ox/red)
Kinetics of metabolism

Two main kinetics are applied:
- Catalized by enzymes
- Michaelis-Menten Kinetics

\[ v = \text{rate of drug metabolism} = \frac{V_{\text{max}} [C]}{K_m + [C]} \]

- \( V_{\text{max}} \) = the maximum metabolism rate capable by enzymes
- \( K_m \) = the Michaelis-Menten constant
- \( C \) = Concentration of drug
Kinetics of metabolism

1) First-order kinetics
In most clinical situations, the concentration of drug is much less than $K_m$, the Michaelis constant, so the equation would be:

$$v = \text{rate of drug metabolism} = \frac{V_{\text{max}} [C]}{K_m + [C]}$$

Therefore, the rate of drug metabolism is directly proportional to the concentration of drug and a constant fraction of drug is metabolized per unit of time.
Kinetics of metabolism

1) Zero-order kinetics:
With few drugs (aspirin, ethanol, phenytoin), the doses are large and $C$ is greater than $K_m$, so the equation would be:

$$v = \text{rate of drug metabolism} = \frac{V_{\text{max}} [C]}{K_m + [C]}$$

$$v = \text{rate of drug metabolism} = \frac{V_{\text{max}} [C]}{[C]} = V_{\text{max}}$$

The enzyme is saturated by a high free drug concentration and the rate of metabolism remains constant (zero-order or non-linear). Therefore a constant amount of drug is metabolized per unit of time.
The phases of drug metabolism

Xenobiotic metabolism consists of 2 main phases:

- Phase I "oxygenases" (CYP or P450, FMO, EH)
- Phase II reactions "transferases" (UGT, GST, NAT, MT)
- Other enzymes (Alcohol dehydrogenases, Aldehyde dehydrogenases, NQO)
Drug molecule

More hydrophilic metabolite → Conjugate

Kidney → Urine

Intestines

Bile

De-conjugation and reuptake (entero-hepatic cycling)

Feces
Phase I reactions

- Phase I reactions of drug metabolism include:
  - Oxidation
  - Reduction
  - Hydrolytic reactions

- Phase I enzymes introduce functional groups (-OH, -COOH, -SH, -O-, NH₂) into the compound which leads to increase the water solubility of drug (a little) and drug inactivation (usually).

- Phase I reactions are catalyzed by superfamilies of:
  - Cytochrome P system (CYPs)
  - Flavin-containing monooxygenase (FMOs)
  - Epoxide hydrolases (EHs)
CPY P450 (CYP)

• CYPs are heme proteins. To date about 40 genes in humans.

• CYPs are named with the root CYP followed by a number showing the family, a letter showing the subfamily and a second number showing CYP isoforms:
  • CYP34A: family 3, subfamily A and gene number 4.

• In humans, 12 CYPs in families 1-3 are primarily responsible for xenobiotic metabolism:
  • CYP 1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5)

CYPs are expressed in liver, GI tract, lung, kidney and CNS.

The most importants: CYP 2C, 2D and 3A

CYP 3A4 is involved in metabolism of ~50% of clinically used drugs.
**RELATIVE HEPATIC CONTENT OF CYP ENZYMES**

- CYP 2C: 17%
- CYP 1A2: 12%
- CYP 3A4-5: 26%
- CYP 2D6: 2%
- CYP 2E1: 7%
- OTHER: 36%

**% DRUGS METABOLIZED BY CYP ENZYMES**

- CYP 3A4-5: 33%
- CYP 2D6: 23%
- CYP 2C9: 14%
- CYP 1A2: 14%
- CYP 2C19: 11%
- CYP 2E: 5%
NADPH\text{+H}^+ 
\rightarrow 2 \text{e}^- 
\text{FAD} 
\rightarrow 2 \text{e}^- 
\text{FMN} 
\rightarrow \text{O}_2 \text{H}^+ 
\rightarrow \text{H}_2\text{O} 
\rightarrow \text{O}_2^- 
\rightarrow \text{R-H} \rightarrow \text{R-OH} 
\rightarrow \text{ER membrane} 
\rightarrow \text{Cytochrome P450} 
\rightarrow \text{Cytochrome P450 reductase}
Take a look!

Comprehensive guide to CYPs:

http://drnelson.utmem.edu/human.P450.table.html
## Participation of the CYP Enzymes in Metabolism of Some Clinically Important Drugs

<table>
<thead>
<tr>
<th>CYP Enzyme</th>
<th>Examples of substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A1</td>
<td>Caffeine, Testosterone, R-Warfarin</td>
</tr>
<tr>
<td>1A2</td>
<td>Acetaminophen, Caffeine, Phenacetin, R-Warfarin</td>
</tr>
<tr>
<td>2A6</td>
<td>17β-Estradiol, Testosterone</td>
</tr>
<tr>
<td>2B6</td>
<td>Cyclophosphamide, Erythromycin, Testosterone</td>
</tr>
<tr>
<td>2C-family</td>
<td>Acetaminophen, Tolbutamide (2C9); Hexobarbital, S-Warfarin (2C9,19); Phenytoin, Testosterone, R-Warfarin, Zidovudine (2C8,9,19);</td>
</tr>
<tr>
<td>2E1</td>
<td>Acetaminophen, Caffeine, Chlorzoxazone, Halothane</td>
</tr>
<tr>
<td>2D6</td>
<td>Acetaminophen, Codeine, Debrisoquine</td>
</tr>
<tr>
<td>3A4</td>
<td>Acetaminophen, Caffeine, Carbamazepine, Codeine, Cortisol, Erythromycin, Cyclophosphamide, S- and R-Warfarin, Phenytoin, Testosterone, Halothane, Zidovudine</td>
</tr>
</tbody>
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Adapted from: S. Rendic Drug Metab Rev 34: 83-448, 2002
Inducers of CYPs

- Certain drugs are capable of increasing the synthesis of one or more CYP isozymes:
  - *Phenobarbital*
  - *Rifampin* (an antituberculosis drug)
  - *carbamazepine*

- Induction results in increased biotransformations of drugs and decrease in plasma concentrations of drugs with concurrent loss of pharmacologic effect.

- Consequences of increased drug metabolism include:
  1) decreased plasma drug concentrations
  2) decreased drug activity if metabolite is inactive
  3) increased drug activity if metabolite is active
  4) decreased therapeutic drug effect
Some of the more important inducers for CYP

- In addition to drugs, natural substances and pollutants can also induce CYP isozymes.

  - polycyclic aromatic hydrocarbons (found as air pollutants) can induce CYP1A. *amitriptyline* and *warfarin* are metabolized by P4501A2. Polycyclic hydrocarbons induce P4501A2, which decreases the therapeutic concentrations of these agents.
Inhibitors of CYPs

• Inhibition of CYP isozyme activity is an important source of drug interactions that leads to serious adverse events (mainly through competition for the same isozyme).

• Inhibition of drug metabolism may lead to:
  • increased plasma levels over time with long-term medications
  • prolonged pharmacological drug effect
  • increased drug-induced toxicities
Inhibitors of CYPs

• Numerous drugs have been shown to inhibit one or more of the CYP-dependent biotransformation pathways of warfarin:

  • Omeprazole is a potent inhibitor of three of the CYP isozymes responsible for warfarin metabolism. If the two drugs are taken together, plasma concentrations of warfarin increase, which leads to greater inhibition of coagulation and risk of hemorrhage and other serious bleeding reactions.

  • Cimetidine blocks the metabolism of theophylline, clozapine, and warfarin.

  Consider erythromycin, ketoconazole, and ritonavir!
Phase I reactions not involving the CYP system

oxidation of catecholamines or histamine

alcohol dehydrogenation (for example, ethanol oxidation)

esterases (for example, metabolism of pravastatin in liver)

hydrolysis (for example, of procaine)
Grapefruit juice ---- Study hours ---- Exam alert!

Grapefruit juice may inhibit drug metabolism. Grapefruit juice inhibits CYP3A4 and, thus, drugs such as *amlodipine*, *clarithromycin*, and *indinavir*, which are metabolized by this system, have greater amounts in the systemic circulation, leading to higher blood levels and the potential to increase therapeutic and/or toxic effects of the drugs.

Have a closer look at your drug of choice!
An Example:

Felodipine is a calcium antagonist for high blood pressure.
Take a look!

Tirona RG, Bailey DG.

Herbal product-drug interactions mediated by induction.

FMOs (Flavin-containing monooxygenases)

Another family of phase I enzymes highly expressed in the liver

There are 6 families of FMOs, with FMO3 most abundant in liver

FMO3 metabolizes:

- nicotine
- $\text{H}_2$-receptor antagonists (Cimetidine and Ranitidine)
- antipsychotics (clozapine)
- antiemetics itopride

FMO3 is not induced or inhibited by any clinically used drugs (new drugs?)
EH (Epoxide Hydrolase)

- Two forms of epoxide hydrolase (EH) hydrolyze epoxides produced by CYPs which leads to deactivation of potentially toxic derivatives:
  - Soluble form (SEH) in the cytosol
  - Microsomal form (mEH) in the membrane

- Carbamazepine is a prodrug converted to carbamazepine-10,11-epoxide by CYP3A4. This metabolite is hydrolyzed by mEH to a dihydriodiol (inactivation). Valproic acid (anticonvulsant) inhibit mEH, so if it is used together with carbamazepine, elevate its active metabolites (interaction).
Phase II reactions

• This phase consists of conjugation reactions.

• If the metabolite from Phase I metabolism is sufficiently polar, it can be excreted by the kidneys. However, many Phase I metabolites are too lipophilic to be retained in the kidney tubules. A subsequent conjugation reaction with an endogenous substrate, such as glucuronic acid, sulfuric acid, acetic acid, or an amino acid, results in polar, usually more water-soluble compounds that are most often therapeutically inactive.

• A notable exception is morphine-6-glucuronide, which is more potent than morphine.
Glucuronidation

\[
\text{UDP-} \alpha\text{-D-glucuronic acid} + \text{ROH or } R_3N \rightarrow \text{UGT} \rightarrow \text{O-glucuronide or N\textsuperscript{+}-glucuronide}
\]
Glucuronidation

- Glucuronidation is the most common and the most important conjugation reaction.

- Neonates are deficient in this conjugating system, making them particularly vulnerable to drugs such as *chloramphenicol*, which is inactivated by the addition of glucuronic acid.
Metabolism of Morphine

Morphine + UDP-glucuronide
→
Morphine-glucuronide + UDP
→
Urine, bile

Glucuronate

Morphine
Sulfation

Examples: ethanol, p-hydroxyacetanilide, 3-hydroxycoumarin
Sulfation may produce active metabolite

Minoxidil → Minoxidil-sulfate
Glutathion conjugation

Glutathione-S-Transferase

Glutathione-S-

Urine

OH

NH

CO

NH

CO
N-Acetylation

- The cytosolic N-acetyltransferases (NATs) are responsible for metabolism of drugs and environmental agents containing an aromatic amine or hydrazine group.

- NATs are among the most polymorphic of all human xenobiotic drug metabolizing enzymes.

Some of drugs subjected to acetylation are listed:

- Acebutolol (Arrhythmias, Hypertension)
- Amantadine (Influenza, Parkinson)
- Benzocaine (local anesthesia)
- Caffeine (neonatal respiratory distress syndrome)
- Clonazepam (Epilepsy)
- Isoniazid (Tuberculosis)
- Sulfonamides (antibacterial agents)
Methylation

• In humans, xenobiotics can undergo O-, N-, and S-methylation.

• Methyl transferases (MTs) are expressed in humans:
  • Catechol-O-methyltransferase (COMT)
  • Phenol-O-methyltransferase (POMT)
  • Thiopurine S-methyltransferase (TPMT)
  • Thiol methyltransferase (TMT)
WWW Resources

http://www.icgeb.trieste.it/p450/
  • Directory of P450 Containing Systems; comprehensive web site regarding all aspects of chemical structure (sequence and 3D) of P450 proteins from all species; steroid ligands; links to related sites including leading researchers on P450

http://www.fda.gov/cder/guidance/

http://www.sigmaaldrich.com/Area_of_Interest/Biochemicals/Enzyme_Explorer.html
  • Site has many commercially available drug metabolizing enzymes and useful links to multiple drug metabolism resources