GENERAL PRINCIPLES OF TOXICOLOGY

Biotransformation of Xenobiotics

Outline

- Overview of Xenobiotic Biotransformation
- Biotransformation of Xenobiotics
- Cytochrome P450
- Cytochrome P450 Superfamily
- Glucoronidation
- Sulfation
- Conjugation with Glutathione
- Other Reactions
- Reviewing the Key Concepts

Overview

- Increasingly, humans are subjected to exposure to various xenobiotics—drugs, food additives, pollutants, etc.

- Understanding how xenobiotics are handled at the cellular level is important in learning how to cope with the chemical onslaught.

- Knowledge of the metabolism of xenobiotics is basic to a rational understanding of pharmacology and therapeutics, pharmacy, toxicology, management of cancer, and drug addiction.

Biotransformation of Xenobiotics

- Most xenobiotics are subject to metabolism (chemical alteration) in the human body, with the liver being the main organ involved; occasionally, a xenobiotic may be excreted unchanged.

- At least 30 different enzymes catalyze reactions involved in xenobiotic metabolism; however, this Lecture will only cover a selected group of them.

Biotransformation of Xenobiotics

- It is convenient to consider the metabolism of xenobiotics in two phases.

- In phase 1, the major reaction involved is hydroxylation, catalyzed by members of a class of enzymes referred to as monooxygenases or cytochrome P450s.

- Hydroxylation may terminate the action of a drug, though this is not always the case.

Biotransformation of Xenobiotics

- In addition to hydroxylation, these enzymes catalyze a wide range of reactions, including those involving deamination, dehalogenation, desulfuration, epoxidation, peroxygenation, and reduction.

- Reactions involving hydrolysis (eg, catalyzed by esterases) and certain other non-P450-catalyzed reactions also occur in phase 1.

Biotransformation of Xenobiotics

- In phase 2, the hydroxylated or other compounds produced in phase 1 are converted by specific enzymes to various polar metabolites.

- This is done by conjugation with glucuronic acid, sulfate, acetate, glutathione, or certain amino acids, or by methylation.

Biotransformation of Xenobiotics

- The overall purpose of the two phases of metabolism of xenobiotics is to increase their water solubility and thus excretion from the body.

- Very hydrophobic xenobiotics would persist in adipose tissue almost indefinitely if they were not converted to more polar forms.

- In certain cases, phase 1 metabolic reactions convert xenobiotics from inactive to biologically active compounds.

Biotransformation of Xenobiotics

- In these instances, the original xenobiotics are referred to as "protoxicants"

- In other cases, additional phase 1 reactions convert the active compounds to less active or inactive forms prior to conjugation.

- In yet other cases, it is the conjugation reactions themselves that convert the active products of phase 1 reactions to less active or inactive species, which are subsequently excreted in the urine or bile.

Biotransformation of Xenobiotics

- In a very few cases, conjugation may actually increase the biologic activity of a xenobiotic (activation).

- The term "detoxication" is used for many of the reactions involved in the metabolism of xenobiotics.

- However, the term is not always appropriate because some xenobiotics actually increase their biologic activity and toxicity.

Cytochrome P450

- Isoforms of cytochrome P450 hydroxylate A myriad of xenobiotics in phase 1 of their metabolism

- Hydroxylation is the chief reaction involved in phase 1.

- The responsible enzymes are called monooxygenases or cytochrome P450s; the human genome encodes at least 14 families of these enzymes.

Cytochrome P450

- Estimates of the number of distinct cytochrome P450s in human tissues range from approximately 35 to 60.

- The reaction catalyzed by a cytochrome P450 is as follows:

$$RH + O_2 + NADPH + H^+ \rightarrow R-OH + H_2O + NADP$$

Cytochrome P450

- RH above can represent a very wide variety of xenobiotics, including drugs, carcinogens, pesticides, petroleum products, and pollutants.

- In addition, endogenous compounds, such as certain steroids, eicosanoids, fatty acids, and retinoids, are also substrates.

- The substrates are generally lipophilic and are rendered more hydrophilic by hydroxylation.

Cytochrome P450

- Cytochrome P450 is considered the most versatile biocatalyst known.

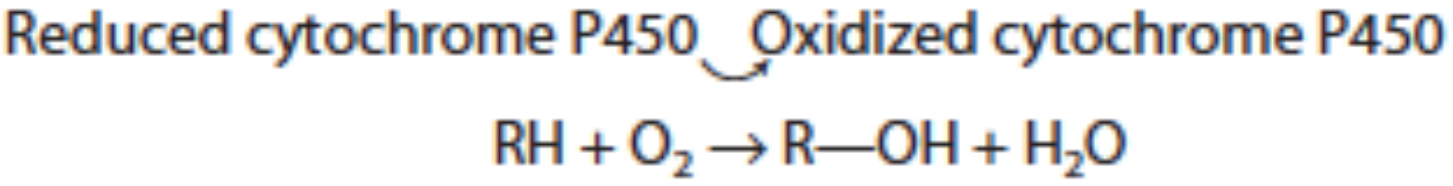
- The actual reaction mechanism is complex and has been briefly described previously.

- It has been shown by the use of $^{18}O_2$ that one atom of oxygen enters R—OH and one atom enters water.

Cytochrome P450

- This dual fate of the oxygen accounts for the former naming of monooxygenases as “mixed function oxidases.”

- The reaction catalyzed by cytochrome P450 can also be represented as follows



Cytochrome P450

- The major monooxygenases in the endoplasmic reticulum are cytochrome P450s.

- Among reasons that this enzyme is important is the fact that approximately 50% of the toxicants humans ingest are metabolized by isoforms of cytochrome P450; these enzymes also act on various carcinogens and pollutants.

Cytochrome P450 Superfamily

- Isoforms of Cytochrome P450 Make Up a Superfamily of Heme-Containing Enzymes

- The following are important points concerning cytochrome P450s.

1. Because of the large number of isoforms (about 150) that have been discovered, it became important to have a systematic nomenclature for isoforms of P450 and for their genes.

Cytochrome P450 Superfamily

- This is now available and in wide use and is based on structural homology.
- The abbreviated root symbol CYP denotes a cytochrome P450.
- This is followed by an Arabic number designating the family; cytochrome P450s are included in the same family if they exhibit 40% or more sequence identity.

Cytochrome P450 Superfamily

- The Arabic number is followed by a capital letter indicating the subfamily, if two or more members exist; P450s are in the same subfamily if they exhibit greater than 55% sequence identity.

- The individual P450s are then arbitrarily assigned Arabic numerals.

- Thus, CYP1A1 denotes a cytochrome P450 that is a member of family 1 and subfamily A and is the first individual member of that subfamily.

Cytochrome P450 Superfamily

- The nomenclature for the genes encoding cytochrome P450s is identical to that described above except that italics are used; thus, the gene encoding CYP1A1 is *CYP1A1.*

2. Like haemoglobin, they are haemoproteins.

3. They are widely distributed across species. Bacteria possess cytochrome P450s.

Cytochrome P450 Superfamily

4. They are present in highest amount in liver and small intestine but are probably present in all tissues.

- In liver and most other tissues, they are present mainly in the membranes of the smooth endoplasmic reticulum, which constitute part of the microsomal fraction when tissue is subjected to subcellular fractionation.

Cytochrome P450 Superfamily

- The mitochondrial cytochrome P450 system differs from the microsomal system in that it uses an NADPH-linked flavoprotein, adrenodoxin reductase, and a nonheme iron-sulfur protein, adrenodoxin.

- In addition, the specific P450 isoforms involved in steroid biosynthesis are generally much more restricted in their substrate specificity.

Cytochrome P450 Superfamily

5. At least six isoforms of cytochrome P450 are present in the endoplasmic reticulum of human liver.

- Each of these has a wide and somewhat overlapping substrate specificities and acting on both xenobiotics and endogenous compounds.

6. NADPH, not NADH, is involved in the reaction mechanism of cytochrome P450.

Cytochrome P450 Superfamily

- The enzyme that uses NADPH to yield the reduced cytochrome P450 is called NADPH-cytochrome P450 reductase.

- Electrons are transferred from NADPH to NADPH cytochrome P450 reductase and then to cytochrome P450.

- This leads to the reductive activation of molecular oxygen, and one atom of oxygen is subsequently inserted into the substrate.

Cytochrome P450 Superfamily

7. Lipids are also components of the cytochrome P450 system.

The preferred lipid is phosphatidylcholine, which is the major lipid found in membranes of the endoplasmic reticulum.

8. Most isoforms of cytochrome P450 are inducible.

Cytochrome P450 Superfamily

- For instance, the administration of phenobarbital or of many other drugs causes hypertrophy of the smooth endoplasmic reticulum.

- Hypertrophy is usually accompanied by a three- to fourfold increase in the amount of cytochrome P450 within 4–5 days.

- The mechanism of induction in most cases involves increased transcription of mRNA for cytochrome P450.

Cytochrome P450 Superfamily

- However, certain cases of induction involve stabilization of mRNA, enzyme stabilization, or other mechanisms (eg, an effect on translation).

- Induction of cytochrome P450 has important clinical implications, since it is a biochemical mechanism of drug interaction.

- A drug interaction has occurred when the effects of one drug are altered by prior, concurrent, or later administration of another.

Induction: Example 1

- As an illustration, consider the situation when a patient is taking the anticoagulant warfarin to prevent blood clotting.
- This drug is metabolized by CYP2C9.
- Concomitantly, the patient is started on phenobarbital (an inducer of this P450) to treat a certain type of epilepsy, but the dose of warfarin is not changed.

Induction: Example 1

- After 5 days or so, the level of CYP2C9 in the patient's liver will be elevated three- to fourfold.
- This in turn means that warfarin will be metabolized much more quickly than before, and its dosage will have become inadequate.
- Therefore, the dose must be increased if warfarin is to be therapeutically effective.

Induction: Example 1

- To pursue this example further, a problem could arise later on if the phenobarbital is discontinued but the increased dosage of warfarin stays the same.

- The patient will be at risk of bleeding, since the high dose of warfarin will be even more active than before, because the level of CYP2C9 will decline once phenobarbital has been stopped.

Induction: Example 2

- Another example of enzyme induction involves CYP2E1, which is induced by consumption of ethanol.

- This is a matter for concern, because CYP2E1 metabolizes certain widely used solvents and also components found in tobacco smoke, many of which are established carcinogens.

- Thus, if the activity of CYP2E1 is elevated by induction, this may increase the risk of carcinogenicity developing from exposure to such compounds.

Cytochrome P450 Superfamily

9. Certain isoforms of cytochrome P450 (eg, CYP1A1) are particularly involved in the metabolism of polycyclic aromatic hydrocarbons (PAHs) and related molecules.

- This enzyme is important in the metabolism of PAHs and in carcinogenesis produced by these agents.

- For example, in the lung it may be involved in the conversion of inactive PAHs (procarcinogens), inhaled by smoking, to active carcinogens by hydroxylation reactions.

Cytochrome P450 Superfamily

- Smokers have higher levels of this enzyme in some of their cells and tissues than do nonsmokers.

- Elevated level thus may potentially alter the quantities of metabolites of PAHs (some of which could be harmful).

Cytochrome P450 Superfamily

10. Certain cytochrome P450s exist in polymorphic forms (genetic isoforms), some of which exhibit low catalytic activity.

- These observations are one important explanation for the variations in xenobiotic responses noted among many individuals.

- One P450 exhibiting polymorphism is CYP2D6.

Cytochrome P450 Superfamily

- Certain polymorphisms of CYP2D6 cause poor metabolism of many xenobiotics so they accumulate in the body, with consequences.

- Another interesting polymorphism is that of CYP2A6, which is involved in the metabolism of nicotine to conitine.

- Three CYP2A6 alleles have been identified: a wild type and two null or inactive alleles.

Cytochrome P450 Superfamily

- Individuals with the null alleles, who have impaired metabolism of nicotine, are apparently protected against becoming tobacco- dependent smokers.

- These individuals smoke less, presumably because their blood and brain concentrations of nicotine remain elevated longer than those of individuals with the wild-type allele.

- It has been speculated that inhibiting CYP2A6 may be a novel way to help prevent and to treat smoking.

Conjugation Reaction

- Conjugation reactions prepare xenobiotics for excretion in phase 2 of their metabolism.
- In phase 1 reactions, xenobiotics are generally converted to more polar, hydroxylated derivatives.
- In phase 2 reactions, these derivatives are conjugated with molecules such as glucuronic acid, sulfate, or glutathione.

Glucuronidation

- UDP-glucuronic acid is the glucuronyl donor, and a variety of glucuronosyl transferases, present in both the endoplasmic reticulum and cytosol, are the catalysts.

- Molecules such as 2-acetylaminofluorene (a carcinogen), benzoic acid, phenol, and many steroids are excreted as glucuronides.

- The glucuronide may be attached to oxygen, nitrogen, or sulfur groups of the substrates. Glucuronidation is probably the most frequent conjugation reaction.

Sulfation

- Some alcohols, arylamines, and phenols are sulfated.

- The sulfate donor in these and other biologic sulfation reactions (eg, sulfation of steroids, glycosaminoglycans, glycolipids, and glycoproteins) is adenosine 3-phosphate-5-phosphosulfate (PAPS); this compound is called “active sulfate.”

Conjugation with Glutathione

- Glutathione (γ-glutamyl-cysteinylglycine) is a tripeptide.

- Glutathione is commonly abbreviated GSH (because of the sulfhydryl group of its cysteine.

- A number of potentially toxic electrophilic xenobiotics are conjugated to the nucleophilic GSH in reactions that can be represented as follows:

$$R + GSH \rightarrow R—S—G$$

Conjugation with Glutathione

- The enzymes catalyzing these reactions are called glutathione S-transferases and are present in high amounts in liver cytosol.

- A variety of glutathione S-transferases are present in human tissue.

- If the potentially toxic xenobiotics were not conjugated to GSH, they would be free to combine covalently with DNA, RNA, or cell protein and could thus lead to serious cell damage.

Conjugation with Glutathione

- GSH is therefore an important defense mechanism against certain toxicants and carcinogens.

- Glutathione conjugates are subjected to further metabolism before excretion.

- The glutamyl and glycinyl groups belonging to glutathione are removed by specific enzymes, and an acetyl group (donated by acetyl-CoA) is added to the amino group of the remaining cysteinyl moiety.

Conjugation with Glutathione

- The resulting compound is a mercapturic acid, a conjugate of L-acetylcysteine, which is then excreted in the urine.

- Glutathione has other important functions in human cells apart from its role in xenobiotic metabolism.

- E.g. decomposition of potentially toxic hydrogen peroxide and an important intracellular reductant.

Other Reactions

- The two most important other reactions are acetylation and methylation.

1. Acetylation—These reactions are catalyzed by acetyltransferases present in the cytosol of various tissues, particularly liver.

- Acetyl-CoA (active acetate) is an acetyl donor in some of those reactions.

Other Reactions

- Polymorphic types of acetyltransferases exist, resulting in individuals who are classified as slow or fast acetylators, and influence the rate of clearance of drugs such as isoniazid from blood.

- Slow acetylators are more subject to certain toxic effects of isoniazid because the drug persists longer in these individuals.

2. Methylation—A few xenobiotics are subject to methylation by methyltransferases, employing S-adenosylmethionine as the methyl donor.

Factors Affecting Biotransformation

- The activities of xenobiotic-metabolizing enzymes are affected by age, sex, & other factors

- The activities of these enzymes may differ substantially among species.

- Thus, for example, the possible toxicity or carcinogenicity of xenobiotics cannot be extrapolated freely from one species to another.

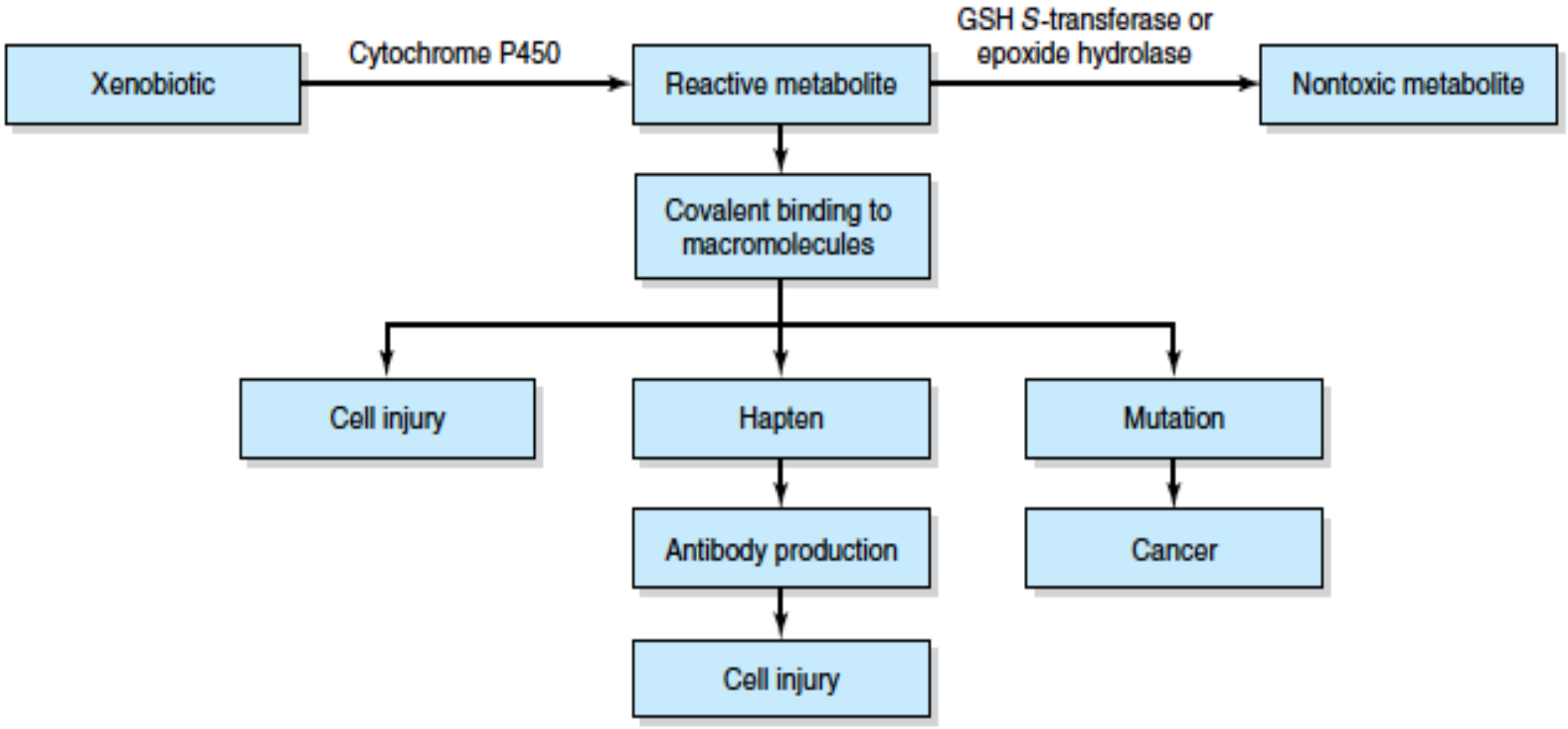
Factors Affecting Biotransformation

- There are significant differences in enzyme activities among individuals, many of which appear to be due to genetic factors.
- The activities of some of these enzymes vary according to age and sex.
- Intake of various xenobiotics can cause enzyme induction.

Factors Affecting Biotransformation

- It is thus important to know whether or not an individual has been exposed to these inducing agents in evaluating biochemical responses to xenobiotics.

- Metabolites of certain xenobiotics can inhibit or stimulate the activities of xenobiotic-metabolizing enzymes.



Possible Effects of Xenobiotics

Reviewing the Key Points

- Xenobiotics are metabolized in two phases.
- The major reaction of phase 1 is hydroxylation catalyzed by a variety of monooxygenases, also known as the cytochrome P450s.
- In phase 2, the hydroxylated species are conjugated with a variety of hydrophilic compounds such as glucuronic acid, sulfate, or glutathione.

Reviewing the Key Points

- The combined operation of these two phases renders lipophilic compounds into water soluble compounds that can be eliminated from the body.

- Cytochrome P450s catalyze reactions that introduce one atom of oxygen derived from molecular oxygen into the substrate, yielding a hydroxylated product.

- NADPH and NADPH-cytochrome P450 reductase are involved in the complex reaction mechanism

Reviewing the Key Points

- All cytochrome P450s have a wide substrate specificity, acting on many exogenous and endogenous substrates.

- Cytochrome P450s are generally located in the endoplasmic reticulum of cells and are particularly enriched in liver.

- Many cytochrome P450s are inducible, with important implications in phenomena such as drug interaction.

End