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Review Article

Xenobiotics: An Essential Precursor for Living System

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ABSTRACT

A xenobiotic is a chemical which is found in an organism but which is not normally produced or expected to be present in it. It can also cover substances which are present in much higher concentrations than are usual. Specifically, drugs such as antibiotics are xenobiotics in humans because the human body does not produce them itself, nor are they part of a normal diet. Natural compounds can also become xenobiotics if they are taken up by another organism, such as the uptake of natural human hormones by fish found downstream of sewage treatment plant outfalls, or the chemical defenses produced by some organisms as protection against predators. Xenobiotic metabolism is the set of metabolic pathways that modify the chemical structure of xenobiotics. In general, drugs are metabolized more slowly in fetal, neonatal and elderly humans and animals than in adults. The body removes xenobiotics by xenobiotic metabolism. This consists of the deactivation and the excretion of xenobiotics and happens mostly in the liver. Excretion routes are urine, feces, breath, and sweat. Hepatic enzymes are responsible for the metabolism of xenobiotics by first activating them (oxidation, reduction, hydrolysis and/or hydration of the xenobiotic) and then conjugating the active secondary metabolite with glucuronic or sulphuric acid, or glutathione followed by excretion in bile or urine. Environmental xenobiotics are xenobiotic substances with a biological activity that are found as pollutants in the natural environment.

Keywords: Xenobiotic metabolism, Metabolic pathways, Oxidation, Reduction, Hydrolysis, Secondary metabolite, Environmental xenobiotics.

INTRODUCTION

However, the term xenobiotics is very often used in the context of pollutants such as dioxins and polychlorinated biphenyls and their effect on the biota,

because xenobiotics are understood as substances foreign to an entire biological system, i.e. artificial substances, which did not exist in nature before their synthesis by humans. The term xenobiotic is derived from the Greek words ξένος (xenos) = foreigner, stranger and βίος (bios, vios) = life, plus the Greek suffix for adjectives -τικός, -ή, -ό (tic). Xenobiotics are any chemical compounds that are found in a living organism, but which are foreign to that organism, in the sense that it does not normally produce the compound or consume it as part of its diet. For example, in humans, most drugs are part of this category, since people don't produce them naturally, or consume them under normal circumstances. Xenobiotics can also be defined as substances that are present in higher-than-normal concentrations, or ones that are entirely artificial and did not exist before they were produced synthetically by humans. Drug metabolism is divided into three Phases. In Phase I, enzymes such as cytochrome P450 oxidases introduce reactive or polar groups into xenobiotics. These modified compounds are then conjugated to polar compounds in Phase II reactions. These reactions are catalysed by transferase enzymes such as glutathione *S*-transferase. Finally, in Phase III, the conjugated xenobiotics may be further processed, before being recognized by efflux transporters and pumped out of cells.

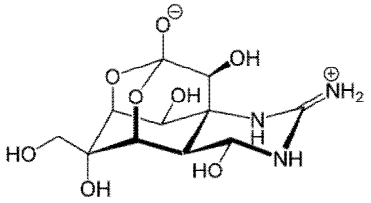
A compound that is normal to one organism may be a xenobiotic to another. A commonly used example of this is the effect experienced by fish that live downstream from the outlet of a sewage treatment plant. Hormones produced by humans may be present, even in treated water and these compounds are foreign as far as fish are concerned[1].

XENOBIOTIC METABOLISM/DRUG METABOLISM

Drug metabolism also known as xenobiotic metabolism is the biochemical modification of pharmaceutical substances or xenobiotics respectively by living organisms, usually through specialized enzymatic systems. Drug metabolism often converts lipophilic chemical compounds into more readily excreted hydrophilic products. The rate of metabolism determines the duration and intensity of a drug's pharmacological action.

The body removes xenobiotics by xenobiotic metabolism. This consists of the deactivation and the excretion of xenobiotics and happens mostly in the liver. Excretion routes are urine, feces, breath and sweat. Hepatic enzymes are responsible for the metabolism of xenobiotics by first activating them (oxidation, reduction, hydrolysis and/or hydration of the xenobiotic) and then conjugating the active secondary metabolite with glucuronic or sulphuric acid or glutathione, followed by excretion in bile or urine. An example of a group of enzymes involved in xenobiotic metabolism is hepatic microsomal cytochrome P450. These enzymes that metabolize xenobiotics are very important for the pharmaceutical industry, because they are responsible for the breakdown of medications.

Organisms can also evolve to tolerate xenobiotics. An example is the co-evolution of the production of tetrodotoxin in the rough-skinned newt and the evolution of tetrodotoxin resistance in its predator, the common garter snake. In this predator-prey pair, an evolutionary arms race has produced high levels of toxin in the newt and correspondingly high levels of resistance in the snake. This evolutionary response is based on the snake evolving modified forms of the ion channels that the toxin acts upon, so becoming resistant to its effects[2].



Tetrodotoxin

Xenobiotic metabolism (from the Greek xenos "stranger" and biotic "related to living beings") is the set of metabolic pathways that modify the chemical structure of xenobiotics, which are compounds foreign to an organism's normal biochemistry, such as drugs and poisons. These pathways are a form of biotransformation present in all major groups of organisms and are considered to be of ancient origin. These reactions often act to detoxify poisonous compounds; however, in some cases, the intermediates in xenobiotic metabolism can themselves be the cause of toxic effects.

The reactions in these pathways are of particular interest in medicine as part of drug metabolism and as a factor contributing to multidrug resistance in infectious diseases and cancer chemotherapy. The actions of some drugs as substrates or inhibitors of enzymes involved in xenobiotic metabolism are a common reason for hazardous drug interactions. These pathways are also important in environmental science, with the xenobiotic metabolism of microorganisms determining whether a pollutant will be broken down during bioremediation, or persist in the environment. The enzymes of xenobiotic metabolism, particularly the glutathione *S*-transferase are also important in agriculture, since they may produce resistance to pesticides and herbicides[3].

Drug metabolism is divided into three Phases. In Phase I, enzymes such as cytochrome P450 oxidases introduce reactive or polar groups into xenobiotics. These modified compounds are then conjugated to polar compounds in Phase II reactions. These reactions are catalyzed by transferase enzymes such as glutathione *S*-transferase. Finally, in Phase III, the conjugated xenobiotics may be further processed, before being recognized by efflux transporters and pumped out of cells[4,5].

FACTORS AFFECTING DRUG METABOLISM

The duration and intensity of pharmacological action of most lipophilic drugs are determined by the rate they are metabolized to inactive products. The Cytochrome P450 mono-oxygenase system is the most important pathway in this regard. In general, anything that increases the rate of metabolism (e.g. enzyme induction) of a pharmacologically active metabolite will decrease the duration and intensity of the drug action. The opposite is also true (e.g., enzyme inhibition). However, in cases where an enzyme is responsible for metabolizing a pro-drug into a drug, enzyme induction can speed up this conversion and increase drug levels, potentially causing toxicity.

Various physiological and pathological factors can also affect drug metabolism. Physiological factors that can influence drug metabolism include age, individual variation (e.g. pharmacogenetics), enterohepatic circulation, nutrition, intestinal flora, or sex differences.

In general, drugs are metabolized more slowly in fetal, neonatal and elderly humans and animals than in adults.Genetic variation (polymorphism) accounts for some of the variability in the effect of drugs. With N-acetyltransferases (involved in Phase II reactions), individual variation creates a group of people who acetylate slowly (slow acetylators) and those who acetylate quickly, split roughly 50:50 in the population of Canada. This variation may have dramatic

consequences, as the slow acetylators are more prone to dose-dependent toxicity. Cytochrome P450 monooxygenase system enzymes can also vary across individuals, with deficiencies occurring in 1-30% of people, depending on their ethnic background.

Pathological factors can also influence drug metabolism, including liver, kidney, or heart diseases. In-silico modeling and simulation methods allow drug metabolism to be predicted in virtual patient populations prior to performing clinical studies in human subjects[6]. This can be used to identify individuals most at risk from adverse reaction.

BIOTRANSFORMATION OF XENOBIOTICS

That the exact compounds an organism is exposed to will be largely unpredictable, and may differ widely over time, is a major characteristic of xenobiotic toxic stress[7]. The major challenge faced by xenobiotic detoxification systems is that they must be able to remove the almost-limitless number of xenobiotic compounds from the complex mixture of chemicals involved in normal metabolism. The solution that has evolved to address this problem is an elegant combination of physical barriers and low-specificity enzymatic systems.

All organisms use cell membranes as hydrophobic permeability barriers to control access to their internal environment. Polar compounds cannot diffuse across these cell membranes and the uptake of useful molecules is mediated through transport proteins that specifically select substrates from the extracellular mixture. This selective uptake means that most hydrophilic molecules cannot enter cells, since they are not recognized by any specific transporters[8]. In contrast, the diffusion of hydrophobic compounds across these barriers cannot be controlled and organisms, therefore, cannot exclude lipid-soluble xenobiotics using membrane barriers.

However, the existence of a permeability barrier means that organisms were able to evolve detoxification systems that exploit the hydrophobicity common to membrane-permeable xenobiotics. These systems therefore solve the specificity problem by possessing such broad substrate specificities that they metabolize almost any non-polar compound.[7] Useful metabolites are excluded since they are polar, and in general contain one or more charged groups. The detoxification of the reactive by-products of normal metabolism cannot be achieved by the systems outlined above, because these species are derived from normal cellular constituents and usually share their polar characteristics. However, since these compounds are few in number, specific enzymes can recognize and remove them. Examples of these specific detoxification systems are the glyoxalase system, which removes the reactive aldehyde methylglyoxal and the various antioxidant systems that eliminate reactive oxygen species[9,10].

PHASES OF DETOXIFICATION

The metabolism of xenobiotics is often divided into three Phases: modification, conjugation and excretion. These reactions act in concert to detoxify xenobiotics and remove them from cells.

PHASE I–MODIFICATION

In Phase I, a variety of enzymes acts to introduce reactive and polar groups into their substrates. One of the most common modifications is hydroxylation catalyzed by the cytochrome P-450-dependent mixed-function oxidase system. These enzyme

complexes act to incorporate an atom of oxygen into nonactivated hydrocarbons, which can result in either the introduction of hydroxyl groups or *N*-, *O*- and *S*-dealkylation of substrates[11]. The reaction mechanism of the P-450 oxidases proceeds through the reduction of cytochrome-bound oxygen and the generation of a highly-reactive oxyferryl species, according to the following scheme[12].

$$NADPH+H^{+}+RH \longrightarrow ADP^{+}+H_2O+ROH$$

Phase I reactions (also termed nonsynthetic reactions) may occur by oxidation, reduction, hydrolysis, cyclization, decyclization and addition of oxygen or removal of hydrogen, carried out by mixed function oxidases, often in the liver. These oxidative reactions typically involve a cytochrome P450 mono-oxygenase (often abbreviated CYP), NADPH and oxygen. The classes of pharmaceutical drugs that utilize this method for their metabolism include phenothiazines, paracetamol and steroids. If the metabolites of Phase I reactions are sufficiently polar, they may be readily excreted at this point. However, many Phase I products are not eliminated rapidly and undergo a subsequent reaction in which an endogenous substrate combines with the newly incorporated functional group to form a highly polar conjugate.

A common Phase I oxidation involves conversion of a C-H bond to a C-OH. This reaction sometimes converts a pharmacologically inactive compound (a pro-drug) to a pharmacologically active one. By the same token, Phase I can turn a nontoxic molecule into a poisonous one (toxification). Simple hydrolysis in the stomach is normally an innocuous reaction, however there are exceptions. For example, Phase I metabolism converts acetonitrile to $HOCH_2CN$, which rapidly dissociates into formaldehyde and hydrogen cyanide, both of which are toxic.

Phase I metabolism of drug candidates can be simulated in the laboratory using non-enzyme catalysts[13]. This example of a biomimetic reaction tends to give products that often contain the Phase I metabolites. As an example, the major metabolite of the pharmaceutical trimebutine, desmethyl trimebutine (nor-trimebutine), can be efficiently produced by *in-vitro* oxidation of the commercially available drug. Hydroxylation of an *N*-methyl group leads to expulsion of a molecule of formaldehyde, while oxidation of the *O*-methyl groups takes place to a lesser extent.

Oxidation

- Cytochrome P450 mono-oxygenase system
- Flavin-containing mono-oxygenase system
- Alcohol dehydrogenase and aldehyde dehydrogenase
- Monoamine oxidase
- Co-oxidation by peroxidases

Reduction

- NADPH-cytochrome P450 reductase

Cytochrome P450 reductase, also known as NADPH:ferrihemoproteinoxidoreductase, NADPH:hemoproteinoxidoreductase, NADPH:P450 oxidoreductase, P450 reductase, POR, CPR, CYPOR, is a membrane-bound enzyme required for electron transfer to cytochrome P450 in the microsome of the eukaryotic cell from a FAD- and FMN-containing enzyme NADPH:cytochrome P450 reductase The general scheme of electron flow in the POR/P450 system is: NADPH → FAD → FMN → P450 → O_2

- Reduced (ferrous) cytochrome P450

During reduction reactions, a chemical can enter *futile cycling*, in which it gains a free-radical electron, then promptly loses it to oxygen (to form a superoxide anion).

Hydrolysis

- Esterases and Amidase
- Epoxide hydrolase

PHASE II – CONJUGATION

In subsequent Phase II reactions, these activated xenobiotic metabolites are conjugated with charged species such as glutathione (GSH), sulfate, glycine, or glucuronic acid. Sites on drugs where conjugation reactions occur include carboxyl (-COOH), hydroxyl (-OH), amino ($-NH_2$), and sulfahydryl (-SH) groups. Products of conjugation reactions have increased molecular weight and tend to be less active than their substrates, unlike Phase I reactions which often produce active metabolites. The addition of large anionic groups (such as GSH) detoxifies reactive electrophiles and produces more polar metabolites that cannot diffuse across membranes, and may, therefore, be actively transported.These reactions are catalyzed by a large group of broad-specificity transferase, which in combination can metabolize almost any hydrophobic compound that contains nucleophilic or electrophilic groups[13]. One of the most important classes of this group is that of the glutathione *S*-transferase (GSTs).

PHASE III – FURTHER MODIFICATION AND EXCRETION

After Phase II reactions, the xenobiotic conjugates may be further metabolized. A common example is the processing of glutathione conjugates to Acetyl cysteine (mercapturic acid) conjugates[15]. Here, the γ-glutamate and glycine residues in the glutathione molecule are removed by Gamma-glutamyltranspeptidase and dipeptidases. In the final step, the cystine residue in the conjugate is acetylated.

Conjugates and their metabolites can be excreted from cells in Phase III of their metabolism, with the anionic groups acting as affinity tags for a variety of membrane transporters of the multidrug resistance protein (MRP) family[16]. These proteins are members of the family of ATP-binding cassette transporters and can catalyse the ATP-dependent transport of a huge variety of hydrophobic anionsand thus act to remove Phase II products to the extracellular medium, where they may be further metabolized or excreted[17,18].

ENVIRONMENTAL XENOBIOTIC

Environmental xenobiotic are xenobiotic substances with a biological activity that are found as pollutants in the natural environment.

Pharmaceuticals

Pharmaceutical drug is a chemical used for the alteration, diagnosis, prevention and treatment of disease, health conditions or structure/function of the human body. Pharmaceutically active compounds (PhACs) are those pharmaceuticals that have by one route or another entered the environment as the parent compound or as pharmacologically active metabolites. Drugs are developed with the intention of having a beneficial biological effect on the organism to which they are administered, but many such compounds all too often pass into the environment where they may exert an unwanted biological effect[19].

For many years PhACs have been all but ignored as environmental researchers concentrated on the well-known environmentally dangerous chemicals that were/are largely used in agriculture and industry. But with increasing technology to help in the separation and identification of multiple compounds in a mixture, PhACs and their effects have received increasing

attention[20]. PhACs have not (until relatively recently) been seen as potentially toxic because regulations associated with pharmaceuticals are typically overseen by human health organizations which have limited experience with environmental issues[21].

Nearly all categories of pharmaceuticals including pain killers (analgesics and anti-inflammatory), antibiotics (antibacterial), anticonvulsant drugs, β-blockers, blood lipid regulators, X-ray contrast media, cytostatic drugs (Chemotherapy), oral contraceptives, and veterinary pharmaceuticals among many others have been found in the environment[22].

CONCLUSION

Drugs such as antibiotics are xenobiotics in human because the human body does not produce them itself, nor are they part of a normal diet. The body removes xenobiotics by xenobiotic metabolism. Xenobiotic metabolism is the set of metabolic pathways that modify the chemical structure of xenobiotics. The body removes xenobiotics by xenobiotic metabolism. This consists of the deactivation and the excretion of xenobiotics, and happens mostly in the liver. Excretion routes are urine, feces, breath, and sweat. Hepatic enzymes are responsible for the metabolism of xenobiotics by first activating them (oxidation, reduction, hydrolysis and/or hydration of the xenobiotic), and then conjugating the active secondary metabolite with glucuronic or sulphuric acid, or glutathione, followed by excretion in bile or urine. Environmental xenobiotics are xenobiotic substances with a biological activity that are found as pollutants in the natural environment.

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REFERENCES

1. Brodie ED, Ridenhour BJ, Brodie ED (2002). "The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts". *Evolution* **56** (10): 2067–82.
2. Geffeney S, Brodie ED, Ruben PC, Brodie ED (2002). "Mechanisms of adaptation in a predator-prey arms race: TTX-resistant sodium channels". *Science* **297** (5585): 1336–9.
3. Boyland E, Chasseaud LF (1969). "The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis". *Adv. Enzymol. Relat. Areas Mol. Biol.* **32**: 173–219.
4. Guengerich FP (2001). "Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity". *Chem. Res. Toxicol.* **14** (6): 611–50.
5. Schlichting I, Berendzen J, Chu K, Stock AM, Maves SA, Benson DE, Sweet RM, Ringe D, Petsko GA, Sligar SG (2000). "The catalytic pathway of cytochrome p450cam at atomic resolution". *Science* **287** (5458): 1615–22.
6. Rostami-Hodjegan A, Tucker GT (2007). "Simulation and prediction of in vivo drug metabolism in human populations from *in-vitro* data". *Nat Rev Drug Discov* **6** (2): 140–8.
7. Jakoby WB, Ziegler DM (1990). "The enzymes of detoxication". *J. Biol. Chem.* **265** (34): 20715–8.
8. Mizuno N, Niwa T, Yotsumoto Y, Sugiyama Y (2003). "Impact of drug transporter studies on drug discovery and development". *Pharmacol. Rev.* **55** (3): 425–61.
9. Thornalley PJ (1990). "The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life". *Biochem. J.* **269** (1): 1–11.

10. Sies H (1997). "Oxidative stress: oxidants and antioxidants". *Exp. Physiol.* **82** (2): 291–5.
11. Guengerich FP (2001). "Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity". *Chem. Res. Toxicol.* **14** (6): 611–50.
12. Schlichting I, Berendzen J, Chu K, Stock AM, Maves SA, Benson DE, Sweet RM, Ringe D, Petsko GA, Sligar SG (2000). "The catalytic pathway of cytochrome p450cam at atomic resolution". *Science* **287** (5458): 1615–22.
13. Akagah B, Lormier AT, Fournet A, Figadère B (2008). "Oxidation of antiparasitic 2-substituted quinolines using metalloporphyrin catalysts: scale-up of a biomimetic reaction for metabolite production of drug candidates". *Org. Biomol. Chem.* **6** (24): 4494–7.
14. Liston HL, Markowitz JS, DeVane CL (2001). "Drug glucuronidation in clinical psychopharmacology". *J. Clin. Psychopharmacol.* **21** (5): 500–15.
15. Boyland E, Chasseaud LF (1969). "The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis". *Adv. Enzymol. Relat. Areas Mol. Biol.* **32**: 173–219.
16. Homolya L, Váradi A, Sarkadi B (2003). "Multidrug resistance-associated proteins: Export pumps for conjugates with glutathione, glucuronate or sulfate". *Biofactors* **17** (1-4): 103–14.
17. König J, Nies AT, Cui Y, Leier I, Keppler D (1999). "Conjugate export pumps of the multidrug resistance protein (MRP) family: localization, substrate specificity, and MRP2-mediated drug resistance". *Biochim. Biophys. Acta* **1461** (2): 377–94.
18. Commandeur JN, Stijntjes GJ, Vermeulen NP (1995). "Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics". *Pharmacol. Rev.* **47** (2): 271–330.
19. Halling-Sorensen *et al*. (1998). Occurrence, fate and effects of pharmaceutical substances in the environment-A Review. *Chemosphere* **36**: 357-393.
20. Daughton and Ternes. (1999). Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environmental Health Perspectives* **117**: 907-938.
21. Jones *et al*. (2001). Human pharmaceuticals in the aquatic environment. *Environmental Technology* **22**: 1383-1394.
22. Heberer. (2002). Occurrence, fate and removal of pharmaceutical residues in the aquatic environment-A review of recent research data. *Toxicology Letters* **131**: 5-17.

Table 1. Metabolism of xenobiotics

Mechanism	Involved enzyme[14]	Co-factor	Location[14]
Methylation	Methyl transferase	S-adenosyl-L-methionine	Liver. Kidney, lungs, CNS
Sulphation	Sulfotransferases	3'-phosphoadenosine-5'-phosphosulfate	Liver, kidney, intestine
Acetylation	N-acetyltransferases bile acid-CoA:amino acid N-acyltransferase	acetyl coenzyme A	liver, lung, spleen, gastric mucosa, RBCs, ymphocytes
Glucuronidation	UDP-glucuronosyltransferase	UDP-glucuronic acid	liver, kidney, intestine, lung, skin, prostate, brain
Glutathione conjugation	glutathione S-transferase	glutathione	liver, kidney
Glycine conjugation	acetyl Co-enzyme	glycine	liver, kidney

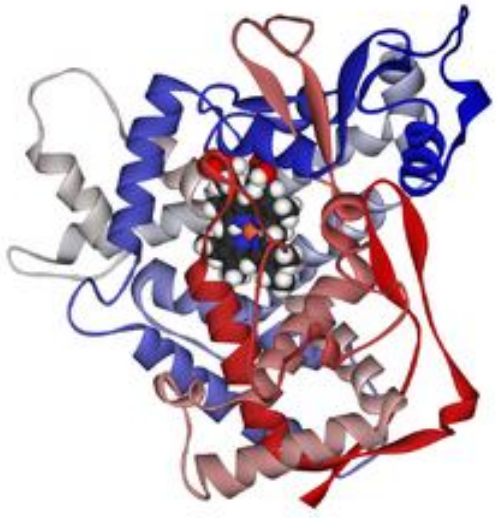

Figure.1. Cytochrome P450 oxidases are important enzymes in xenobiotic metabolism.

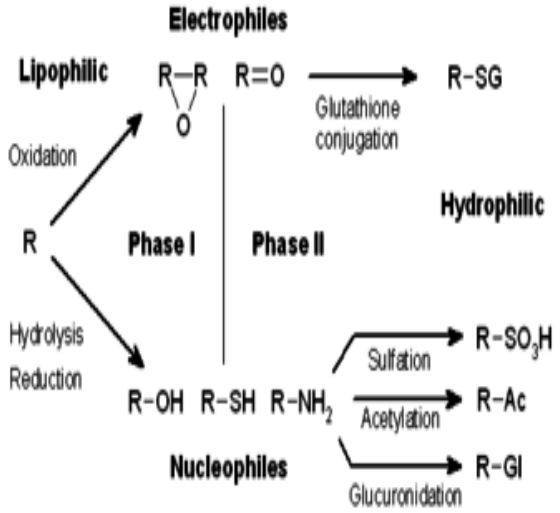

Figure.2. Phases I and II of the metabolism of a lipophilic xenobiotic