Xenobiotics in tissue and organs

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Abstract. Transport xenobiotis throug tissues and organs was studied. The aim of this paper dynamic xenobiotic transfer and accumulation in the specific tissues and organs were investigated. Some of xenobiotics are suficiently soluble in blood to account for simple solution as a route of distribution. Many xenobiotics are distributed in association which plasma proteins. Cellular components are responsible for the transport of xenobiotics, but such transport represents a minor route. This paper contributes dynamic chemicals circulation definition in the body.

Keywords: Xenobitotic distribution, blood, body, tissue, specific organs, enzymes.

1. Introduction

After entering the blood by absorption or intravenous administration, xenobiotics are available for distribution throughout the body. The inital rate of distribution to organs and tissues is determined by the blood flow to that organ and the rate of diffusion of the chemical into the specific organ or tissue.. Uptake of xenobiotics into organs
or tissues may occurs either by passive diffusion or by special transport processes. Within tissues, binding storage, or biotransformation can occur. After reaching equilibrium
the distribution of a chemical into organs and tissuesis determined largely by affinity, blood flow determines distribution only during the initial phase shortly after uptake.

Body fluids are distributed among three distinct compartments vascular, interstitial and intracellular water. Plasma water plays an important role in the distribution of xenobiotics.Plasma water and interstitial water represent the extracellular water. Human plasma accounts for about 4% of the total body weight and 53% of the total volume of blood. By comparison interstitial tissue fluids account for 13 % of body weight and intracellular fluids for 41% [1]-[3] .

The concentration of a xenobiotic in blood after exposure will depend largely on its apparent volume of distribution. If the xenobiotic is distributed only in plasma, a high concentration will be achieved within the vascular tissue. In contrast, the concentration will be markedly lower if the same quantity of xenobiotic were distributed in a larger pool including interstitial water or intracellular water.

2. Factors of xenobiotics distribution

Factors that affect distribution, apart from binding to blood macromolecules, include route of administration, rate of biotransformation, polarity of the parent xenobiotics or biotransformation products, and rate of excretion by the liver or kidneys. Gastrointenstinal absorption and intraperitoneal administration provide immediate passage of a compund to the liver, whereas dermal or respiratory routes provide at least one passage through the systemic circulation prior to reaching the liver. Most xenobiotics are metabolized products that are more polar and thus more readily excreted than the parent molecules. Therefore, the rate of metabolism is a critical determinant in the distribution of a compund because those compounds that are readily metabolized are usually readily excreted and thus are proportionally less prone to accumulate in certain tissues. The same principle applies to polarity since very polar xenobiotics will be excreted readily. Chemicals may circulate either free or bound to plasma protein or blood cells, the degree of binding and factors influencing the equilibrium with the free form may influence the availability for biotransformation, storage, or excretion[4].

Patterns of xenobiotic distribution reflect certain physiological properties of the organism and physicochemical properties of the xenobiotics. An initial phase of distribution may be distinguished that reflects cardiac outputb and blood flow to organs. Heart, liver, kidney ,brain and other well perfused organs receive most of lipohilic xenobiotics within the first few minutes after absorption. Delivery to the smooth muscles, most visclers, and sceen is slower, consequently, the time to reach a steady state concentration of a xenobiotic in these organs may be

several hours. A second phase of xenobiotic distribution may therefore be distinguished (Fig.1). It is limited by blood flow to an organ or tissue and involves a far larger fraction of the body mass than does the first phase of distribution as shown in Fig.1.

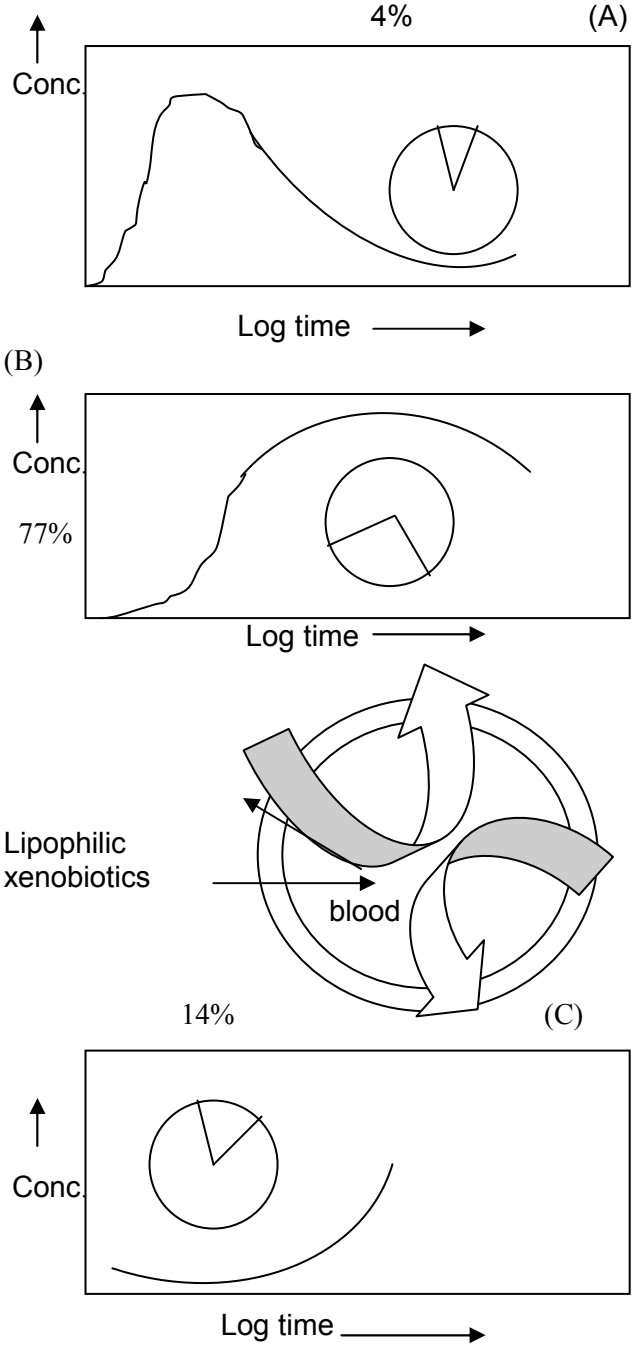

Fig. 1 Input and redistribution of lipohilic xenobiotics with blood

Lipophilic xenobiotics in the blood are first distributed to well perfused organs (A), after some time, they are redistributed to organs with lower blood flow representing a larger fraction of the body weight (B) and (C).

The chemicals circulation either free, or bound to plasma protein or blood cells has shown in Fig. 2.

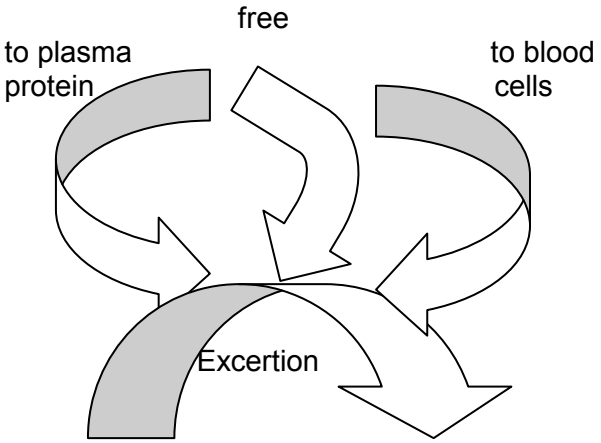

Fig.2 Chemicals circulation

Chemicals accumulatuion in the body can be defined:

$$W\frac{dc_X}{dt} = F_F c_{X,F} + F_P c_{X,P} + F_B c_{X,B} - F_E c_{X,E} \quad (1)$$

where F denotes flow, c denotes concentration, W is weight, index F means free, P is plasma, B is blood, E excertion, and X denotes xenobiotic.

Only a limited number of xenobiotics are suficiently soluble in blood to account for simple solution as a route of distribution, many xenobiotics are distributed in association which plasma proteins is of key importance in transport. Many organic and inorganic compounds of low molecular mass appear to bind to lipoproteins, albumins, and other proteins in plasma and are transported as protein conjugates. This binding is revesible. Cellular components may also be responsible for the transport of xenobiotics, but such transport represents a minor route. The transport of xenobiotics by lymph is usually of little quatitative importance since intestinal blood flow is 500-700 times greather than intestinal lymph flow.

Few studies have been performed on the reversible binding of toxic xenobiotics, but available evidence suggests a significant role for lipoproteins in plasma. Many studies of plasma protein binding to serum albumin is particularly important fpor these chemicals [5]-[7]. These plasma proteins may bind xenobiotics as well as some physiological constituents of the body**.** Examples of plasma proteins, that may bind xenobiotics are albumin, α-,β-lipoproteins, and several metal binding proteins such as transferin. Lipoproteins are important for the transport of lipid soluble endogenous chemicals such

as vitamins, steroid hormones, and cholestero, but they may also bind lipohilic xenobiotics. If a xenobiotic is bound to a protein, it may not reach the site of action, tus protein binding may influence the toxic effects exerted by a xenobiotics.

The extent of binding to plasma proteins varies considerably among xenobiotics. Some will not be boundd with other xenobiotics, more than 90% of the doze administered may be present bound to plasma proteins. These ligand –protein interactions are reversible and provide remarkably efficient means of transporting xenobiotics to various tissues. The xenobiotic protein interaction may be described simply:

$$X_{FREE} + [\text{free sites}] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X_{BOUND} \qquad (2)$$

where X_{FREE} and X_{BOUND} are free and bound xenobiotic molecules, respectively and k_1 and k_2 are the rate constants for association and dissociation. k_1 governs the rate of binding to the protein, and k_2 dictates the rate of xenobiotic release at a site of action or storage. The ratio
k_1/k_2 isa identical with equilibrium constat K Among a group of binding sites on proteins, those with the lowest K values for a given xenobiotic will bind it most tightly.

In contrast to the covalent binding to proteins observed with many xenobiotics or their electrophilic metabolites.The interaction of xenobiotics with plasma proteins is most often noncovalent and reversible. Noncovalent binding is of primary importance with respect to distribution because of the opportunities to dissociate after transport. Binding of xenobiotics to plasma proteins may be due to several types of interactions summarized below.

The degree of ionic binding varies with the chemical nature of each compound and the net charge. Dissociation of ionic bonds usuaslly occurs readily, but some transition metals exibit high association constants , low dissociation values, and exchange is slow Ionic interaction may also contribute to the binding of alkaloids with ionizable nitrogen groups and other ionizable xenobiotics.

Hydrogen bonding is performed as a rule, only the most electronegative atoms form stable hydrogen bonds. Protein side chains containing hydroxyl, amino, carboxyl, imidazole, and carbamoil groups may form hydrogen bonds, as can the nitrogen and oxygen atoms of peptide bonds.

The binding force of van der Waals bonds depends critically on the proximity of interacting atoms and diminishes rapidily with distance. However, when these forces are summed over a large number of interacting atoms that fit together spatially, they can play a significant role in determining the specificity of xenobiotic- protein interactions.

3. Hydrophobic interactions

When two nonpolar groups come together they exclude the water between them, and the mutual repulsion of water results in ahydrophobic interaction. Hydrophobic interaction may be considered as a special; case of van der Waals forces.

Consequence of the bindinmg to plasma proteins are reduced availability of the free xenobiotic in the cells and delayed excretion. The plasma protein bound xenobiotic cannot cross capillary walls due to its high molecular mass. The fraction of dose bound is thus not available for delivery to the extra vascular space or for filtration by the kidney. It is generally acceptedthat the fraction of xenobiotics that is bound may not exert toxic effects. Some plasma proteins that can bind endogenous chemicals and xenobiotics together with examples of bonded xenobiotics are listed below.

α- Lipoproteins	Vitamins, A,K,D Steroid hormones Dieldrin
Albumin	Salicylate, Tetracyclines Phenols Vitamin C

The binding of xenobiotics to plasma proteins is, however most often reversible. The amount of combined xenobiotic is in equilibrium with the amount of free xenobiotic, thus binding usually slows excretion or delivery to cellular sites of action. Toxic consequences of the reversible binding of a xenobiotic to plasma proteins may arise after saturation of the binding capacities pf plasma proteins and by displacement of the bound xenobiotic by another chemical with higher affinity, which increases the free fraction of the formely bound xenobiotic. This will result in an increased equilibrium concentration of the xenobiotic in plasma and the target organ, with potentially harmful consequences.

4. Hold up xenobiotics in organs and tissues

The concentration of a xenobiotic in a tissue may cause toxic effects to that particular tissue. Some xenobiotics actually attain their highest concentration at the site of toxic action. The other xenobiotics may

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be concentrated in tissues without harmful consequences. Some tissues have a high capacity to accommodate certain xenobiotics and may release them only slowly. Absorbed xenobiotics may be concentrated in specific organs or tissues. The compartment or tissue in which a chemical is concentrated can also be considered a storage depot for this xenobiotic. If a chemical is stored in a depot and thus removed from the site of action, for example, polychlorinated biphenyls in fat or lead in bone, no manifestation of toxicity may be observed immediately, although a potential for adverse effects exists.

For example, lead stored in bone does not cause a toxic response but has the potential for mobilization and thus for migration into soft tissues. Toxic effects may appear after mobilization.

Since the xenobiotic in storage depots is in equilibrium with the free xenobiotic in plasma, mobilization is constant, which results in constant exposure of the target organ to low concentrations of the xenobiotic. Some storage depots for specific chemicals in mammalian organisms are are:

Lead	bone
Fluoride	bone, teeth
Cadmium	kidney
Iron	transferring, a protein in blood
Polychlorinated pesticides Such as DDT	fat
Arsenic	skin

Liver, kidney, fat, bone, and plasma protein may servew as storage depots for absorbed xenobiotics. Both liver and kidney have a high storage capacity for xenobiotics and are major storage sites for a multitude of chemicals. Accumulation of circulating xenobiotics from the blood by active transport systems and binding to certain tissue constituents are major mechanisms involved in renal and hepatic storage.

Several proteins rich in thiol groups present in liver and kidney have a high affinity for xenobiotics[8]. Ligandin, a binding protein is a glutathione S-transferase and thus participates in xenobiotic, binerds organic acids, some azo dyes, and coricosteroids. Metallothionein, a cysteine rich protein present in liver and kidney, serves as a binding and storage protein for several metals including cadmium and zinc. Its biosynthesis is increased after exposure to metals, this may results in storage of a considerable percentage of a cumulative metal dose as metallothionein complex in liver and kidney.

Highly lipophilic chemicals rapidly penetrate membranes and are thus efficiently taken up by tissues. Because of their lipophilicity and in case of inefficient biotransformation, they are stored in the most lipophilic environment in the organism, fat. Most xenobiotics seem to accumulate by physical dissolution of neutral fats, which may constitute between 20 -50% of the body weight in human males. Large amount of lipophilic xenobiotics may therefore be present in fat for xenobiotics not undergoing biotransformation determination of their concentration in body fat is a good measure of exposure.

Usually, xenobiotics stored in fat do not induce toxic responses because the xenobiotic is not readily available at the target site for toxic action. However, during rapid mobilization of fat due to disease or starvation, a sudden increase in the plasma concentration and, thus the toxic effects in taerget organs may occur. For example, signs of organochlorine pesticide intoxication have been observed after starvation in animals pretreated with persistent organochlorine pesticides.

Certain plasma proteins have a high affinity for xenobiotics, binding of a chemical to plasma proteins may constituent both a transport and a storage form. Globulin such as transferrin, involved in iron transport, and ceruloplasmin, cooper, and α-, β-lipoproteins, lipophilic xenobiotics and endogenous chemicals, may be involved in binding.

Storage in tissue may greatly alter the rate of excretion of a xenobiotic. Only xenobiotics present in plasma are available for distribution, present in plasma are available for distribution,biotransformation , and excretion. However, excretion or biotransformation changes the plasma concentration of a xenobiotic, and some of the stored chemical is released into plasma from the site of storage. Because of this mechanism, the rate of excretion of a xenobiotic stored in tissues may be very small.

Xenobiotic metabolism is catalyzed by many different enzymes. For solely operational purposes, the enzymes of biotransformation are separated into two phases. In the first phase reactions, which involve oxidation, reduction, and hydrolysis, a polar group is added to the xenobiotic or is exposed by the enzymes of biotransformation. The second phase reactions are biosynthetic and link the metabolite formed by the first phase reactions to a polar endogenous molecule to produce a conjugate. Various endogenous molecules with high polarity and thus even higher solubility are utilized for conjugation, the conjugates

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formed are often ionized at physiological pH and thus highly water soluble. Moreover, the moieties used for Conjugation are often recognized by specific active transport processes, which assist in their translocation across plasma membranes and thus further enhance the rate of excretion.

The enzymes of biotransformation are localized mainly in the liver. A significant fraction of the blood from the splanchnic area, which also contains xenobiotics absorbed from the intestine, enters the liver. Therefore, the liver has developed the capacity to enzymatically modify most of these chemicals before storage release, or excretion. However, other tissues also contain enzymes that can catalyze biotransformation reactions. The contribution of extrahepatic organs to the biotransformation of a chemical depends on many factors, including chemical structure, dose, and route of administration. Biotransformation of a chemical in extrahepatic tissue may have toxic effects on this specific tissue and thus have important toxicological consequences.

In cells, the first phase enzymes are present mainly in the endoplasmic reticulum, a myriad of lipoprotein membranes extending from the mitochondria and the nucleus to the plasma membranes of the cell. The microsomal fraction obtained by ultyracentrifugation of tissue homogenates, which is endiched in vesicles from the endoplasmic reticulum, is often used to study the enzymatic biotransformation of xenobiotics in vitro.

The presence of the first phse enzymes within membranes has important implications since lipophilic chemicals will distribute preferentially into lipid membranes, thus high concentrations of lipophilic xenobiotics are present at this site of biotransformation. In

Oposite to the first phase enzymes, the second phase enzymes are often soluble, nonmembrane associated, and present in the cytoplasm of the cell. They are found in the supernatant, cytosol obtained by ultracentrifugation of homogenized tissues. The subcelluar localizations of enzymes responsible for biotransformation are listed in Table 1.

Table 1. Enzymes classification

Enzymes-phase I	
Cytochrome P450	microsomal
Flavin-dependent monooxygenaze	microsomal
Prostaglandin synthase	microsomal
Epoxide hydrolase	microsomal/cytosolic
Enzyme phase II	
UDP-glucuronyltransferases	microsomal
sulfotransferases	cytosolic
N-acetiltransferases	cytosolic
Glutathione S-transferase	microsomal/cytosolic

The general purpose of biotransformation reactions is detoxification, because xenobiotics should be transformed to metabolites, that are more readily excreted. However, depending on the structure of the chemical and the enzyme catalysing the biotransformation reaction, metabolites with high er potential for toxicity than the parent compound are often formed. This process is termed bioactivation and is the basis for the toxicity and carcinogenicity of many xenobiotics with a low chemical reactivity. Interaction of the toxic metabolite initiates events that may ultimately results in cell death, cancer, teratogenicity, organ failure, or other manifestations of toxicity. Formation of reactive and more toxic metabolites is more frequently associated with the first phase reactions. The second phase reaction may also be involved in bioactivation, as well as combinations of phase I and phase II.

5. Reaction catalyzed by enzymes

Cytochrome P450, a carbon monoxide binding hemoprotein in microsomes, is the most important enzyme system involved in the first phase reactions. Microsomal monooxigenases are the cytohrome P450 enzymes and the mixed function amine oxidase of vlavin dependent monooxygenase. Both enzyme system add a hydroxyl moiety to the xenobiotic. Denatured cytochrome P450, like other proteins shows an absorbance maximum at 420nm.

Cytochrome P450 represent a copupled enzyme system composed of the heme-containing cytochrome P450 and the nicotin amide, adenine dinucleotide phosphate(H) (NADPH) containing cytochrome 450 reductase. This flovoprotein has a preference for NADPH as its cofactor and transfers either one or two electrons from NADPH to cytochrome 450 . The phospholipid matrix is crucial for enzymatic activity since is fascilitates the interaction between both enzymes. Individual enzymes are regulated in their expression by a variety of factors such as treatment with xenobiotics, species, organ, sex, diet. In mammals, two general classes of cytochrome P450 exist: six families involved steroid metabolism and bile acid biosynthesis, and four families containing

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numerous individual cytochromes P450 mainly responsible for xenobiotic biotransformation.

Reaction catalyzed by cytochrome P450:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADPH + \quad (3)$$

where RH is substrate.

Cytochrome 450 enzymes are monooxigenases. These enzymes utilize one of the atoms of molecular oxygen and incorporate it into the xenobiotic in the noted sstoichiometry.

The second oxygen atom is reduced to water with consumption of NADPH as reducing cofactor. There are the
likely mechanisms of electron transfer and xenobiotic oxidation.

In the first step of catalytic cycle, the xenobiotic combines with the oxidized form of cytochrome P450 (Fe^{3+})
Followed by one electron reduction by NADPH cytochrome P450 reductase to form areduced ((Fe^{2+}) cytochrome P450 substrate complex. This complex then combines with molecular oxygen, and anoter electron from NADPH is accepted. In the last step of the catalytic cycle, the oxidized substrate dissociates and regenerates to oxidized form of cytochrome P450.

10. Conclusions

In this paper xenobiotic storage in tissues and organs was investigated. The capacity xenobiotic accumulation function was derived.

Fundamentals xenobiotic transfer rate relations and metabolite formation were examined. Individual enzymes regulation in their expession by a variety of factors treatment with xenobiotics was examined.

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Notation

c - component concentration, mole/cm^3
F-flow
k-specific constant rate, s^{-1}
NADPH- nicotinamide adenine dinucleotide phosphate (H)
P-plazma
P450- cytochrome microsomal enzyme
UDP- glucuronyl transferases
X-xenobiotic
x - component composition, mol/mol
W - weight

Subscript
B-blood
E-enzyme
F-free
P– plazma
X-xenobiotic

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