Pharmacokinetics and Metabolic Drug Interactions

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Abstract: Pharmacokinetics and drug metabolism play an important role as determinants of *in vivo* drug action. The CYP450 enzyme family plays a determinant role in the biotransformation of a vast number of structurally diverse drugs. Many drug interactions are a result of the inhibition or induction of CYP enzymes. The non-compartmental pharmacokinetic analysis is the most used method for analyzing data from a drug interaction study. Compartmental analysis can be also useful and sometimes more informative than non-compartmental analysis. Many efforts to reduce polypharmacy are important, and pharmacokinetic tools used to study the mechanism of drug-drug interactions may help in a better management of pharmacotherapy including the avoidance of clinically relevant drug interactions.

Keywords: Pharmacokinetics, Metabolism, Drug interactions.

1. INTRODUCTION

The development of novel therapeutical agents should provide a delicate balance between the chemistry, pharmacology and pharmacokinetics of the drug. Due to ethical constraints, relevant pharmacokinetic and metabolism studies must be carried out extensively in laboratory animals or *in vitro* systems before first drug administration in humans. The complete safety profile of a new drug will be defined only after it has been approved and is in use on the market. In clinical practice, it is not possible to prevent co-prescription of different drugs, with clinical significant interactions.

The biological response of the human body to an exogenous compound e.g. a drug, is dependent on a complex network of factors, as illustrated in Fig. (**1**) [1].

Drug-drug interactions occur when one therapeutic agent either alters the concentration (pharmacokinetic interactions) or the biological effect of another agent (pharmacodynamic interactions). Pharmacokinetic drug-drug interactions can occur at the level of absorption, distribution, or clearance of the affected agent. Many drugs are eliminated by metabolism. The microsomal reactions that have been studied the most involve cytochrome P (CYP) 450 family of enzymes, of which a few are responsible for the majority of metabolic reactions involving drugs. These include the isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 [2].

Enzyme inhibition refers to the decrease in metabolic enzyme activity due to the presence of an inhibitor. Drug metabolism by CYP450 can be inhibited by any of the following three mechanisms: competitive inhibition, noncompetitive inhibition and uncompetitive inhibition. Inhibition of enzyme activity may result in higher concentrations and/or prolonged half-life of the substrate drug, which enhances the potential for toxic side effects. The clinical significance of a specific drug-drug interaction depends on the degree of accumulation of the substrate and the therapeutic window of the substrate [3].

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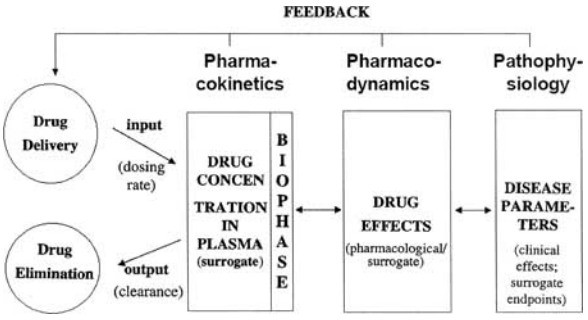

Fig. (1). Schematic illustration of the complex interrelationships of factors that influence drug response [1].

Enzyme induction is associated with an increase in enzyme activity. For drugs that are substrates of the isoenzyme induced, the effect is to lower the concentration of these substrates. The clinical consequence of the presence of an inducing agent and the resultant decrease in concentration of the substrate may mean a loss of efficacy.

Several of the drug metabolizing enzymes are polymorphic, having more than one variant of the gene. Although the CYP isozymes generally have similar functional properties, each one is different and has a distinct role. This polymorphism forms a basis for interindividual differences in the efficacy of drug treatment, side effects of drugs and the toxic and carcinogenic action of xenobiotics. The variability associated with the CYP450 enzymes in each individual result in a marked difference in response when the same drug and the dose are administered to different individuals. Genetic polymorphism of CYP450 enzymes characterizes the general population into three groups: extensive metabolizers, poor metabolizers and ultra extensive metabolizers [4].

The quantitative study of the time course of drug absorption, distribution, metabolism and excretion (ADME) allows the calculation of several important pharmacokinetic parameters such as area under the curve (AUC), bioavailability, clearance and apparent volume of distribution. Pharmacokinetic data analysis using mathematical models is known as compartmental pharmacokinetics. The rate transfer

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between compartments and the rate of elimination are assumed to be following first-order kinetics. However, non-compartmental analysis can be used to determine pharmacokinetic parameters without fitting the pharmacokinetic data to any specific compartmental model, assuming the data follow linear pharmacokinetics. Non-compartmental methods are based on the theory of statistical moments and parameters as the mean residence time, apparent volume of distribution, etc. The basic equations cannot be applied to all drugs. In some situations, complex mathematical models are required to express the pharmacokinetic profiles [5].

In addition to the above aspects of the pharmacokinetics of the parent compound, the pharmacokinetics of a metabolite is also characterized by its formation. The most common sites of biotransformation of the parent drug into metabolite occur in liver, gut, plasma, kidneys and lungs. If the metabolite is formed pre-systemically in the gut, the pharmacokinetics of the metabolite is not only governed by its rate of formation but also by its rate of absorption into the systemic circulation. Many drugs that undergo extensive first-pass metabolism in the gut are generally metabolized by phase I enzymes (specifically CYP450 enzymes) [6].

When a drug-drug interaction occurs, the pharmacokinetics of the inhibited or induced drug is altered. In some instances, there may be dual interactions where both drugs may be inhibited or induced. When the drug is biotransformed into one or more active metabolites, they are also responsible for the pharmacodynamics and therapeutic effect. A differentiation between gut and hepatic metabolism is also of importance in bioequivalence assessment. Pharmacokinetic and pharmacodynamic methods are powerful tools to describe and understand drug action in the intact organism. Integration of the methods can be used to verify that plasma pharmacokinetics is a suitable surrogate for tissue pharmacodynamics [7].

All the above reasons and much more, suggest that the pharmacokinetics is a useful method to study the influence of drug-drug interaction on the values of the pharmacokinetic parameters, which in turn will modify the drug plasma levels with the risk of clinically significant consequences. On the other hand, pharmacokinetic analysis can elucidate the mechanisms of drug-drug interactions, which will clarify important aspects of human pharmacology.

2. DRUG METABOLISM

2.1. Types of Drug Metabolism

Drug metabolism, also known as drug biotransformation, has the objective of making xenobiotics more hydrophilic so they can be efficiently eliminated by the kidney. To increase hydrophilicity, a polar group is added or unmasked. Often the metabolite is inactive and the chemical change alters the shape and charge of the drug so it can no longer bind to its receptor and/or exerts its effect on the receptor's function. In some cases the metabolite retains its pharmacologic effects, it is an active metabolite. In other cases, the parent drug is pharmacologically inactive and requires metabolism for a pharmacologic effect; this type of drug is a prodrug.

There are two major categories of metabolism reactions called Phase I and Phase II [8, 9]. Phase I reactions refers to a set of reactions that result in relatively small chemical changes that make compounds more hydrophilic and also provide a functional group that is used to complete Phase II reactions. Phase I reactions are concerned with addition or unmasking of functional, polar moiety, the chemical processes being the oxidation and/or reduction, or hydrolysis. Phase I metabolism can occur during drug absorption, either in the gut wall or in the liver, before the drug reaches the systemic circulation [5]. The presystemic clearance, or first-pass metabolism, determines the fraction of the oral dose that will reach the systemic circulation, i.e. the fraction of the drug that is bioavailable.

The majority of Phase I reactions are mediated by a large family of cytochrome P450 enzymes. Functionalization reactions of Phase I are reactions which generate functional group as in hydroxylation, or "unmask" functional group as in ester hydrolysis.

Oxidations carried out by P450's can be: aromatic oxidations (propranolol, phenobarbital, phenytoin, phenylbutazone, amphetamine, warfarin); aliphatic oxidations (amobarbital, secobarbital, chlorpropamide, ibuprofen, meprobamate, glutethimide, phenylbutazone, digitoxin); epoxidations (carbamazepine); N-dealkylations (morphine, caffeine, theophylline); O-dealkylations (codeine); S-dealkylations (6-methylthiopurine); N-oxidations, primary amines (chlorphentermine), secondary amines (acetaminophen), tertiary amines (nicotine, methaqualone); S-oxidations (thioridazine, cimetidine, chlorpromazine); deaminations (amphetamine, diazepam).

There are also non-P450 oxidations: monoamine oxidase reactions, different mechanism with the same result as P450 deamination (formation of imine followed by hydrolysis); flavin monooxygenase reactions (FMO) (but P450 reductases also use flavin as FAD, flavin adenine dinucleotide, and FMN, flavin mononucleotide) [8, 9, 10].

Other phase I reactions: reductions, e.g. nitro reduction (chloramphenicol, clonazepam), and azo group reduction (prontosil, tartrazine); hydrolysis: derivatives of carboxylic acid hydrolysis: esters (cocaine, procaine, tetracaine, benzocaine; succinylcholine), amides (lidocaine, mepivacaine, bupivacaine, etidocaine, prilocaine). Glucuronide hydrolysis gives rise to enterohepatic recirculation, significantly prolonging the life of some drugs, because their sufficiently lipophilic metabolites are reabsorbed into the portal circulation from which they can reenter the liver [8, 9, 10].

Compounds that remain in the circulation after undergoing Phase I metabolism often undergo Phase II metabolism. Phase II reactions are characterized by conjugation with small, endogenous substance, often taking advantage of functional group added in Phase I. The transferases that mediate Phase II reactions are important not only for eliminating drugs but also for detoxifying reactive drug metabolites, which are mostly produced by prior metabolism by cytochrome P450 enzymes. In some cases in which the parent drug has an appropriate site, Phase II metabolism may occur first [8, 9, 10].

Glucuronide formation is an important step in the elimination of many important endogenous substances from the body, including bilirubin, bile acids, steroid hormones,

and biogenic amines as serotonin. Many of these compounds are also substrates for sulfonyltransferases. The most common reaction occurs by transfer of a glucuronic acid moiety from uridine-diphosphate glucuronic acid (UDPGA) to an acceptor molecule. This process is termed either glucuronosylation or glucuronidation [11]. When enzymes catalyze this reaction, they are also referred to as UDP-glucuronosyltransferases (UGTs) (acetaminophen, ibuprofen, morphine, diazepam, meprobamate, digitoxin, digoxin).

Other Phase II reactions: sulfation (acetaminophen, methyldopa, 3-hydroxycoumarin, estrone); glutathione conjugation (ethacrinic acid); acetylation (sulfonamides, isoniazid, clonazepam, dapsone); methylation (dopamine, epinephrine, histamine, thiouracil) [8, 9, 10].

2.2. Cytochrome P450 System

The cytochrome P450 (CYP) family of heme monooxygenases comprises the most important group of phase 1 enzymes [12, 13]. These enzymes are characterized by a maximum absorption wavelength of 450 nm in their reduced state in the presence of carbon monoxide.

The term cytochrome P-450 refers to a group of enzymes which are located on the endoplasmic reticulum. The metabolic enzymes are also present in high concentrations in the enterocytes of the small intestines with small quantities in extrahepatic tissues (kidneys, lungs, brain etc). The nomenclature employs a three – tier classification consisting of the family (> 36% homology in amino acid sequence), subfamily (70% homology), and individual gene (ex. CYP3A4). Naming a cytochrome P450 gene includes root symbol "CYP" for humans ("Cyp" for mouse and Drosophila), an Arabic numeral denoting the CYP family (e.g. CYP2), letters A,B,C indicating subfamily (e.g. CYP3A) and another Arabic numeral representing the individual gene/isoenzyme/isozyme/isoform (e.g. CYP3A4) [12]. Each isoenzyme of CYP is a specific gene product with characteristic substrate specificity. These enzymes oxidate a wide range of both endogenous and exogenous compounds using atmospheric oxygen (O_2) [13].

The cytochrome P450 gene family contains 60 to 100 different genes, of which only a small group is involved in drug and chemical transformations. In the human liver there are at least 12 distinct CYP enzymes. At present it appears that from about 30 isozymes, only six isoenzymes from the families CYP1, 2 and 3 are involved in the hepatic metabolism of the most drugs. The most important P450 isoenzyme is CYP3A4 (50% of the P450 metabolism) followed by CYP2D6 (20%), CYP2C9 and CYP2C19 (together 15%). The remaining is carried out by CYP2E1, CYP2A6 and CYP1A2. The genes for CYP2D6, CYP2C9, CYP2C19 and CYP2A6 are functionally polymorphic. Therefore approximately 40% of human P450 dependent drug metabolism is carried out by polymorphic enzymes (for a list of all currently known cytochrome P450 gene alleles refer to http: //www.imm.ki.se/ CYPalleles/).

2.3. Genetic Polymorphisms in Drug Metabolism and Disposition

Genetic polymorphism with clinical implications has been described for 2D6, 2C19, 2C9, 1A2, 3A4 [e.g. 14, 15, 16].

The human genome contains three billions base pairs of nucleotides in the haploid genome of which about only 3% are genes [17]. A gene is the basic unit of heredity that contains the information for making one RNA and in most cases, one polypeptide. The number of genes in humans is estimated at 40.000 to 100.000. Polymorphism is defined as the existence of two or more genetically determined forms (alleles) in a population in substantial frequency. A polymorphic gene is one at which the frequency of the most common allele is less than 0.99. It has been estimated that in each human individual 20% of the proteins and hence the genes exist in a form that is different from the majority of the population. In a sample of 71 human genes it was observed that 28% were polymorphic and that the average heterozygosity was 0.067. Heterozygosity is defined as the proportion in a population of diploid genotypes in which the two alleles for a given gene are different [18].

Polymorphism in drug metabolizing enzymes is caused by mutations in genes that code for specific biotransformation enzyme [17]. Generally they follow the autosomal recessive trait that means that the mutations are not sex linked (autosomal) and that one mutated allele does not express the phenotype when combined with a normal, not mutated (dominant) allele [19].

Genes can be mutated in several ways: a nucleotide can be changed by substitution, insertion or deletion of a base. If changes refer to one or few bases, these mutations are called point mutations. Larger changes can exist also, deletion of the entire gene or duplication of the entire gene. Some point mutations are silent mutations: they have no consequences at the point level. Other point mutations will affect amino acid sequence and will affect the biological function of the protein [20].

For drug metabolizing enzymes, the molecular mechanisms of inactivation include splice site mutations resulting in exon skipping (CYP2C19), micro satellite nucleotide repeats (CYP2D6), gene duplication (CYP2D6), point mutations resulting in early stop codons (CYP2D6), enhanced proteolysis (TPMT), altered pro-moter functions (CYP2A5), critical amino acid substitutions (CYP2C19), or large gene deletions (CYP2D6). Conversely, gene duplication can be associated with enhanced activity of some drug metabolizing enzymes (CYP2D6). For many genes encoding drug metabolizing enzymes the frequency of single nucleotide polymorphisms (SNPs) and other genetic defects appears to be more than the 1 per 1000 nucleotide. It may be that genetic polymorphisms of drug metabolizing enzymes are quite common because these enzymes are not essential from evolutionary perspective. However some essential receptors have more mutations than would be predicted from the 1 in 1000 rate.

In the case of CYP2D6 gene some polymorphic modifications are known [20, 21].

Individuals with normal metabolic enzyme activities are often called extensive metabolizers (EM). Ultra-rapid metabolism (CYP2D6*2xN) is caused by multiple functional CYP2D6 genes, causing an increased amount of CYP2D6 to be expressed. Gene duplication or sometimes multiplication

leads to the ultra-rapid (UR) phenotype. A homozygous combination of non-coding alleles leads to the poor metabolizer (PM) phenotype, whereas heterozygous wild type or combinations of alleles with diminished enzyme activity lead to reduced CYP2D6 activity. The prevalence of CYP2D6 PM phenotype differs per race and is reported to be 5 to 10 % in white populations and 1 to 2% in Orientals [22].

The 2D6 isoenzyme represents <5% of total CYP proteins, but is the most intensively studied because of its large number of substrates (30-50 drugs) and its genetic polymorphism [24, 25, 61, 62]. Some of the cardiovascular agents and psychoactive drugs are metabolized *via* CYP2D6. Therefore, the clinical impact of impaired metabolism is thought to be the greatest in these classes of drugs. Some CYP2D6 substrates are: encainide, flecainide, mexiletine, propafenone; metoprolol, propranolol, timolol, amitriptylline, clomipramine, desipramine, imipramine, nortriptylline, fluoxetine, fluvoxamine, maprotiline, mianserine, paroxetine, trazodone, etc [23, 24, 25].

The 2C subfamily consists of isoenzymes 2C9, 2C10, 2C19 and others. CYP2C9 has a polymorphic distribution in the population and is missing in 1% of Caucasians. The isoenzyme CYP2C19 also exhibits genetic polymorphism. Its genetic absence in such a high percentage of Asians (20-30%) is notable.

In the case of CYP2C19 gene, two null-alleles, *2 and *3, have been described to account for approximately 87% of all PM in Caucasians and 100% of all PMs in Orientals [25, 26]. Three non-coding alleles (19*4, 19*5 and 19*6) have been described but the frequencies of these alleles are expected to be below 1% in Caucasians. Deficiency of CYP2C19 occurs with a prevalence of PMs of 2-5% among Europeans, 4-5% Black Africans, 6% Black Americans and 12-23% among Orientals [20]. Well known substrates of CYP2C19 are drugs like the sedative drug diazepam, and the proton pump inhibitor omeprazole, or lansoprazole.

The variability of CYP3A4 activity is quite severe. The intrinsic clearance for CYP3A4 metabolized substances can vary among individuals, with interindividual differences of factors of 10 or higher [28].

CYP3A4 is an isoenzyme involved in Phase I oxidative metabolism of many substances. It is the most important hepatic CYP-enzyme accounting for approximately 25% of all liver CYP450s [27]. Since CYP3A4 is also present in the small intestine, it has a significant effect on the first-pass metabolism of CYP3A4 substrates. A number of drugs metabolized chiefly by CYP3A4: fentanyl; carbamazepine; azitromycin, clarithromycin, erythromycin; fluconazole, ketoconazole, miconazole; indinavir, ritonavir, saquinavir; tamoxifen; amiodarone, lidocaine, quinidine; amlodipine, diltiazem, felodipine, nifedipine, nimodipine, nitrendipine, verapamil; fluvastatin, pravastatin; loratadine, terfenadine; cisapride; cyclosporine, tacrolimus; sertraline; alprazolam, midazolam, triazolam, zolpidem; dexamethasone, prednisone, testosterone, etc [23,24,25]. Although CYP3A is not polymorphic in its distribution, its activity varies over 50-fold in the general population [72].While polymorphisms in CYP3A4 are not recognized, CYP3A5 has known ethnic differences in its expression and there is ongoing interest in whether these differences manifest themselves in altered pharmacokinetics and clinical consequences of therapy with substrates for CYP3A [79].

CYP1A2 is an important drug metabolizing enzyme in the liver that metabolizes many commonly used drugs and this is the only isoenzyme affected by tobacco. Cigarette smoking may lead to a three-fold increase in 1A2 activity. Their clearances are all increased by smoking. Thus the people who smoke may require higher doses of some of the medications that are substrates of CYP1A2 [24, 25, 61, 62].

The individual status of the activity of drug metabolizing enzymes which in its turn is a method of phenotyping, can be assessed using enzyme specific probe drugs [28,29]. The drug is administered to a patient and the excretion rate (metabolic rate) is measured after several hours. Simultaneous assessment of *in vivo* activities of more than one enzyme may be performed by a multi-enzyme probe approach or by the cocktail approach [30]. For CYP2D6 dextrometorphan, sparteine, debrisoquine and metoprolol have been described as probe drugs [29]. *In vivo* enzyme activity of CYPsC19 gene can be assessed by measurement of the metabolic ratio of an enzyme specific probe, as mephenitoin, omeprazole and proguanil [30].Examples of multi-drug cocktails to assess P450 activity are: dextromethorphan, mephenytoin, or sparteine, mephenytoin, or debrisoquine, mephenytoin, or dextromethorphan, proguanil, for CYP2D6 and CYP2C19; dextromethorphan, caffeine for CYP2D6, NAT2, XO,CYP1A2; "Pittsburgh cocktail" caffeine, chlorzoxazone, dapsone, debrisoquine, mephenytoin, for NAT2, CYP1A2, CYP2E1, CYP3A4, CYP2D6 and CYP2C19 [26,31].

Genotyping is another tool to describe populations. Detection of mutations in genomic DNA is difficult to realize because one single point mutation has to be determined in the midst of three billion base pairs. The classical method is restriction fragment length polymorphism (RFLP) followed by Southern blotting. The polymerase chain reaction (PCR) has revolutionized the analysis of genetic diseases and polymorphisms, being the basis for almost all methods for the detection of single nucleotide polymorphisms (SNPs).

There are three ways to get information on metabolizing enzyme activities: study the genes that code for the enzyme; study the level of enzyme expression in a certain tissue, and assess actual enzyme activity using an enzyme specific probe. Genotyping is a more simple procedure compared to phenotyping. But in population studies, phenotyping might be helpful in detecting interethnic differences, or in studies to detect enzyme induction or inhibition [32].

Such polymorphisms may or may not have clear clinical significance for affected medications, depending on the importance of the enzyme for the overall metabolism of a medication, the expression of the other drug metabolizing enzymes in the patient, the therapeutic index of the drug, the presence of concurrent medications or illnesses, and other polygenic factors that impact drug response.These common polymorphisms in drug receptors and drug metabolizing enzymes are often major determinants of interindividual differences in drug response. The adverse drug reactions

could be related to genetically determined variation of drug-metabolizing enzymes in the liver [18, 33, 34, 35].

2.4. Ontogeny of Metabolic Enzymes

Earlier studies considered the presence of CYP enzymes in the embryo and fetus to be a kind of adaptive response toward exposure to environmental challenges. Other studies have suggested a number of forms of CYP450 may be present constitutively in the conceptus [36]. The developmental pharmacology has an important impact on the drug disposition, action and therapy in infants and children [37].

Pharmacokinetics and Pharmacodynamics are very different in children and adults [37].The pharmacokinetics of many drugs vary with age [37].Infants and children are very different from adults in terms of societal, psychosocial, behavioral and medical perspectives. Developmental changes affect profoundly the responses to medications and produce a need for age-dependent adjustments in doses. The levels of most phase I and phase II enzymes rise during the first weeks after the birth, regardless of gestational age at birth. The capacity of the human liver to eliminate xenobiotic compounds during the neonatal period is effective and the intensity of biotransformation depends primarily on the level of maturation of phase I enzymes. This makes it hazardous to extrapolate data for adults to children.

The use of pharmacokinetic data to examine the ontogeny of a drug metabolizing enzyme is well illustrated by theophylline, a substrate for the P450 cytochrome CYP1A2. It was reported that the elimination half-lives of theophylline ranged between 9 and 18 hours in term infants who are 6 to 12 weeks old. [38]. The dramatic alterations in theophylline plasma clearance occurring between 30 weeks (approximately 10 ml/h/kg) and 100 weeks (approximately 80 ml/h/kg) of postconceptional age, is primarily the result of age-dependent differences in metabolism of theophylline by CYP1A2 [39].

When administered intravenously, midazolam clearance reflects the CYP3A activity in the liver. The clearance and thus hepatic CYP3A activity is markedly lower in neonates less than 39 weeks of gestation (1.2 ml/kg/min) and greater than 39 weeks of gestation (1.8 ml/kg/min) relative to clearance of 9.1 ±3.3 ml/kg/min observed in infants greater than 3 months old. These data suggest that CYP3A activity increases approximately five fold over the first 3 months of life [40].

In addition to the P450 cytochromes, apparent age dependence exists for several phase II enzymes that are of quantitative importance for drug biotransformation. For example, the pharmacokinetics of selected substrates for UGT2B7 (e.g. lorazepam, morphine, naloxone) support a marked reduction in the level of activity for this isoform around the birth (approximately 10 to 20 % of the levels in adults), with attainment of competence equivalent to that in adults between 2 months and 3 years of age [41, 42].

Using published literature a children's pharmacokinetic database has been compiled which compares pharmacokinetic parameters between children and adults for 45 drugs. These comparisons indicate that premature and full-term neonates tend to have 3 to 9 times longer half-life than adults for the drugs included in the database. This difference disappears by 2-6 months of age. Beyond this age, half-life can be shorter than in adults for specific drugs and pathways [43]. The range of neonate/adult half – life ratios exceeds the 3.16-fold factor commonly ascribed to interindividual pharmacokinetic variability. Pharmacokinetics of xenobiotics can differ widely between children and adults due to physiological differences and the immaturity of the enzyme system and clearance mechanisms. This make extrapolation of adult dosimetry estimates to children uncertain, especially at early postnatal ages [43].

Such data suggest the importance of the study of targeted pediatric populations versus the entire pediatric population, to design of age (developmentally) – appropriate drug dosing regimens.

2.5. The Role of P-Glycoprotein and ABC-Transporters in Drug Metabolism and Drug-Drug Interactions

In addition to P450 enzymes, transporters play an important role in drug disposition. It is possible that drug-drug interactions at the site of transporters alter the plasma concentration-time profiles. Transporters mediate the membrane transport of a great number of drugs and endogenous compounds. The number of binding sites of transporters for drugs is limited, so the transport process is saturated at concentrations higher than the Km value. When drugs share the same binding sites of transporters, drug-drug interactions may occur depending on their pharmacokinetic properties. Interactions involving membrane transporters in organs of elimination (liver, kidney) and absorption (intestine) alter blood concentration time profiles of drugs.

Transporters can be classified into several families: secondary or tertiary active transporters (organic cation transporter, OCT; organic anion transporting polypeptide family, OATP; organic anion transporter, OAT; peptide transporter; sodium phosphate co transporter) and primary active transporters (P-glycoprotein, Pgp; multidrug resistance associated protein 1,MRP1; canalicular multispecific organic anion transporter, cMOAT/MRP2/cMRP; MRP3) [44].

Transport proteins mediate the translocation of specific molecules across various membranes. The translocation of their substrates can be either primary active using ATP hydrolysis as an energy source or secondary active using an existing cellular electrochemical gradient. Examples are the ATP-binding cassette transporters (ABC-transporters) or the solute carrier (SLC), respectively. The ABC-binding cassette transporters are a large and diverse superfamily of proteins comprising around fifty members with many and varied functions. Now a consistent nomenclature has been introduced, based on the sequence homology between these proteins. In this system the ABC genes are grouped into seven subfamilies, based on the similarity in the gene structure, order of the domains and sequence homology in the two nucleotide binding domains (NBDs) and two transmembrane domains (TMDs) (ABCA ABCB ABCC, ABCD, ABCE, ABCF, ABCG, each of them having different number of members, known also with different common names) [45,46,47].

One of the most important members is P-glycoprotein (ABCB1) known also as MDR1, a protein over expressed in

tumor cells with a multi-drug resistance (MDR) phenotype where it confers resistance to many unrelated cytotoxic drugs [48].

Now it is recognized that P-gp is a widely distributed constitutive protein that plays a pivotal role in the systemic disposition of a wide variety of hormones, drugs and other xenobiotics. Recent investigations have uncovered a large family of efflux proteins with diverse overlapping substrate specificities that play a critical role in the disposition of therapeutic agents the scope of these proteins is just beginning to be recognized. In the context of this article the discussion is on the P-gp as a prototype of the efflux pump family.

The over expression of this protein is often associated with conferring the multidrug-resistance (MDR) phenotype that involves the removal of a variety of structurally unrelated compounds from within cells. Human MDR1 gene contains 28 exons encoding for a 1280 amino acid transporter, consisting of two homologous halves. Presence of the highly conserved ATP binding site in each of the homologous half as well as the linker region clearly makes this protein a member of the so-called ATP-Binding Cassette (ABC) transporter superfamily [49, 50, 51, 52].

P-gp is constitutively expressed in nearly all barrier tissues: adrenal cortex, kidney, liver, intestine and pancreas, endothelial cells at blood-tissue barriers, namely the central nervous system, the testis, and the papillary dermis. P-gp is found at the apical canalicular surface of hepatocytes, in the apical membrane of the columnar epithelial cells of colon and intestine, and at the apical brush border of the renal proximal tubule epithelium [53].

The transporter action is quite different from classical enzyme-like mechanism used to describe several other transporters. P-gp interacts with its substrates, like other ABC transporters, but can bind to its substrates while they are associated with the plasma membrane; P-gp can efflux substrates directly from the membrane [51].

P-gp plays a role in the oxidative metabolism of its substrates that are also substrates of CYP3A4. These barrier proteins are co-localized to the apical region of the enterocytes that form the epithelial lining of the small intestine. Combined actions of P-gp and CYP3A4 could account in some part for the low oral bioavailability determined for many of these dual substrates [54]. Since P-gp blocks absorption in the gut, it should be considered part of the "first-pass effect".

In vitro experiments involving cyclosporine-A transport across Caco-e cell monolayers have shown how P-gp and CYP3A4 may act coordinately to enhance the attenuation of apical to basolateral transport of the drug. The reduction in the apical to basolateral flux of the drug caused by appically directed P-gp efflux, enhanced the exposure of the drug to CYP3A4, and thus a greater amount of metabolism was achieved [55].

In terms of P-gp drug substrates, a number of drugs used in cancer chemotherapy, immunosuppressant, hypertension, allergy, infection and inflammation are substrates of this transporter (amprenavir, cimetidine, colchicines, cyclosporine, dexamethasone, digoxin, diltiazem, domperidone, doxorubicin, erythromycin, etoposide, fexofenadine, indinavir, itraconazole, ivermectin, loperamide, morphine, nelfinavir, paclitaxel, etc).

Some drugs may act as inhibitors (atorvastatin, bromocriptine, carvedilol, cyclosporine, erythromycin, itraconazole, ketoconazole, meperidine, methadone, nelfinavir, pentazocine, progesterone, quinidine, ritonavir, saquinavir, tamoxifen, verapamil, etc), and others as inducers (amprenavir, clotrimazole, dexamethasone, indinavir, morphine, phenotiazine, retinoic acid, rifampicin, ritonavir, saquinavir, St. John's wort, etc.) [52, 56].

Because of the importance of P-gp in multi-drug resistance, considerable effort has been made to identify compounds that inhibit P-gp function in an attempt to improve the efficacy of chemotherapeutic agents (cyclosporine A, tamoxifen, quinidine, quinine; dexniguldipine, dexverapamil, S9788; GF120918, valspodar, CGP41251) [51].

Polymorphism of the MDR1 gene has been reported to be associated with alterations in disposition kinetics and interaction profiles of clinically useful drugs like digoxin, fexofenadine, cyclosporine etc. P-gp expression correlates inversely with phenotype indices (less protein on the apical surface of intestinal enterocytes to pump substrates back into intestinal lumen, resulting in increased bioavailability, and vice versa). A single polymorphism (G2677T/A0 or C3435T) was shown to be of importance, from single dose-pharmacokinetic studies, but the MDR1 gene has multiple polymorphisms [56, 57]. The information on the functional characteristics of drug transporters provides important information in allowing improvements in drug delivery or drug design by targeting specific transporter.

3. DRUG-DRUG INTERACTIONS

Metabolic drug interactions between drugs represent a major concern clinically for health care professionals and their patients. It has been estimated that some of the clinically significant drug-drug interactions may be the causes of adverse drug reactions (ADR) or causes of death [58].

There are many reasons for so many ADRs. First, more drugs, and many combinations of drugs, are being used to treat patients than ever before. Secondly, a great number of prescriptions are filled out for one person. Finally, the rate of ADRs increases exponentially after a patient is on 4 or more medications [59]. Efforts to reduce polypharmacy are important but for many patients, the number of medications cannot always be reduced without doing harm. That is why it is important to understand the basis for drug interactions.

During the past few years a revolution has taken place in our understanding of drug interactions, mostly as a result of advances in the molecular biology of the CYP enzyme system. This will allow us to make the most appropriate choices in prescribing and avoiding preventable ADRs. Many drug interactions are a result of inhibition or induction of CYP enzymes.

3.1. Enzyme Inhibition

Inhibition based drug interactions constitute the major proportion of clinically important drug interactions. Drug metabolism by CYP450 can be inhibited by any of the following three mechanisms [83].

The first is mutual competitive inhibition caused by co-administration of drugs metabolized by the same CYP450 is enzyme. Inhibition most often occurs as a result of competitive binding at the enzyme's binding site. In this case, blood concentrations of both drugs may be increased. Competitive inhibition depends on the affinity of the substrate for the enzyme being inhibited, the concentration of substrate required for inhibition, and the half-life of the inhibitor drug. The onset and offset of enzyme inhibition are dependent on the half-life and time to steady the state of the inhibitor drug. The time to maximum drug interaction (onset and termination) is also dependent on the time required for the inhibited drug to reach a new steady state.

$$E + S \leftrightarrow ES \rightarrow E + P$$
$$\text{Km (Michaelis constant for S)}$$

$$E + I \leftrightarrow EI$$
$$\text{Ki (Inhibition constant for I)}$$

where E is the enzyme, S is the substrate, ES is the enzyme-substrate complex, P is the product, I is the inhibitor, and EI is the enzyme-inhibitor complex. In the case of competitive inhibition, the metabolic rate (v) can be expressed by the equation:

$$V = \frac{Vmax.\ S}{Km\ (1+I/Ki) + S}$$

where Vmax in the maximum metabolic rate. The inhibition by a given concentration of I is marked when the substrate concentration is low and becomes less marked with an increase in the substrate concentration.

The second inhibition mechanism, and less common mechanism of inhibition, is the inactivation of CYP450 by the drug metabolite forming a complex with CYP450. Noncompetitive inhibition is a pattern of inhibition where the inhibitor binds to the same enzyme as the drug but the binding site is different, resulting in a conformation change of the protein, etc.

$$E + S \leftrightarrow ES \rightarrow E + P$$
$$\text{Km}$$

$$E + I \leftrightarrow EI$$
$$\text{Ki}$$

$$EI + S \leftrightarrow EIS$$
$$\text{Km}$$

$$ES + I \leftrightarrow EIS$$
$$\text{Ki}$$

where EIS is the enzyme-inhibitor-substrate complex.

The metabolic rate can be expressed by the equation:

$$V = \frac{\{Vmax / (1+I / Ki\}.\ S}{Km + S}$$

The degree of inhibition does not depend on the substrate concentration.

The third mechanism of inhibition is the uncompetitive inhibition, a pattern of inhibition where the inhibitor binds only to the enzyme forming a complex with the drug:

$$E + S \leftrightarrow ES \rightarrow E + P$$
$$\text{Km}$$

$$ES + I \leftrightarrow EIS$$
$$\text{Ki}$$

The metabolic rate can be expressed by the equation:

$$V = \frac{\{Vmax / (1+I / Ki\}.\ S}{Km / (1 + I / Ki) + S}$$

The inhibition becomes more marked with increasing substrate concentration. In clinical situations, the substrate concentration is usually much lower than Km [83].

3.2. Enzyme Induction

Drug interactions involving enzyme induction are not as common as inhibition based drug interactions, but equally profound and clinically important [24, 25, 61]. Enzyme induction occurs when hepatic blood flow is increased, or the synthesis of more CYP450 enzymes is stimulated. Like inhibitors, inducers tend to be lipophilic, and the time course of the interaction is dependent on the half-life of the inducer. A complicating factor is that the time course of induction is also dependent on the time required for enzyme degradation and new enzyme production. The half-life of CYP450 enzyme turnover ranges from 1-6 days. Enzyme induction is also influenced by age and liver disease. The ability to induce drug metabolism may decrease with age, and patients with cirrhosis or hepatitis may be less susceptible to enzyme induction [61].

The most common mechanism is transcriptional activation leading to increases synthesis of more CYP 450 enzyme proteins. If a drug induces its own metabolism, it is called autoinduction. If induction is by other compounds, it is called foreign induction. Metabolism of the affected drug is increased leading to decreased intensity and duration of drug effects. If the drug is a prodrug or is metabolized to an active or toxic metabolite, then the effect or toxicity is increased.

Some drugs–called "enzyme inducers"–are capable of increasing the activity of drug metabolizing enzymes, resulting in a decrease in the effect of certain other drugs. Examples of enzyme inducers include aminoglutethimide, barbiturates, carbamazepine, glutethimide, griseofulvin, phenytoin, primidone, rifabutin, rifampin, and troglitazone. Some drugs, such as ritonavir, may act as either an enzyme inhibitor or an enzyme inducer, depending on the situation. Drugs metabolized by CYP3A4 or CYP2C9 are particularly susceptible to enzyme induction. In some cases, especially for drugs that undergo extensive first-pass metabolism by CYP3A4 in the gut wall and liver, the reduction in serum concentrations of the object drug can be profound. Some drugs are converted to toxic metabolites by drug metabolizing enzymes. For example, the analgesic acetaminophen is

converted primarily to non-toxic metabolites, but a small amount is converted to a cytototoxic metabolite. Enzyme inducers can increase the formation of the toxic metabolite and increase the risk of hepatotoxicity as well as damage to other organs [24, 25, 62].

3.3. Isoenzymes and Drug Interactions

Understanding the mechanisms of enzyme inhibition and induction, is extremely important in order to give appropriate multiple therapy. The advances in our understanding of the CYP enzyme system has made it possible to associate specific enzyme activity with the formation of a particular metabolite and in some cases to identify the major isoenzyme responsible for the total clearance of a drug. However, it is impossible to remember all of the drug interactions that can occur. Some books, reviews and up-to date databases are available [24,25,62-71]. Table **1** shows important substrates, inhibitors and inducers of the six most common drug-metabolizing CYP isozymes [25, 72-78].

4. PHARMACOKINETICS AND METABOLISM

4.1. General Pharmacokinetic Principles and Parameters

Pharmacokinetics is the mathematics (kinetics) of the time course of Absorption, Distribution, Metabolism and Excretion (ADME) of drugs in the body. The biological, physiological, physico-chemical and pharmaceutical factors

Table 1. Cytochrome P450 Enzymes Involved in Drug Metabolism: Substrates, Inducers and Inhibitors

Isozyme	Substrates	Inhibitors	Inducers
CYP1A2	Clozapine Cyclobenzaprine Fluvoxamine Imipramine Mexiletine Propranolol Theophylline	Cimetidine Ciprofloxacin Clarithromycin Enoxacin Erythromycin Fluvoxamine Ofloxacine Ticlopidine	Polyccyclic Aromatic Hydrocarbons (Cigarette Smoke) TCDD(dioxin)
CYP2C9	Diclofenac, Flurbiprofen, Ibuprofen, Losartan (not telmisartan or candesatan) Naproxen, Phenytoin Piroxicam, Sulfamethoxazole, Tolbutamide, Warfarin	Amiodarone, Fluconazole, Fluoxetine, Isoniazid, Paroxetine, Ticlopidine, Zafirlukast	Phenobarbital, Rifampin
CYP2C19	Amitriptyline, Clomipramine, Ciclophosphamide, Diazepam, Imipramine, Lansoprazole, Nelfinavir, Omeprazole, Phenytoin	Cimetidine, Fluoxetine, Fluvoxamine, Ketoconazole, Lansoprazoel, Omeprazole, Paroxetine, Ticlopidine	Carbamazepine, Norethindrone
CYP2D6	Amitriptyline, Clomipramine, Codeine, Desipamine, Dextromethorphan, Imiporamine, Metoprolol, Nortriptyline, Oxycodone, Paroxetine, Propranolol, Risperidone, Thioridazine, Timolol	Amiodarone, Fluoxetine, Haloperidol, Indinavir, Paroxetine, Quinidine, Ritonavir, Sertraline	Rifampin
CYP2E1	Aceaminophen, Chlorzoxazone, Ethanol, Enflurane, Halothane, Isoflurane	Disulfiram	Chronic Ethanol Isoniazid
CYP3A	Alprazolam, Astemizole, Buspirone, Calcium Chanel Blockers Carbamazepine, Cisapride, Cyclosporine, Protease Inhibitors Lovastatin, Midazolam, Simvastatin, Tacrolimus, Triazolam	Amiodarone, Cimetidine, Clarithromycine, Erythromycine, Grapefruit Juice, Iraconazole, Ketoconazole	Carbamazepine, Glucocorticoids, Phenytoin, Rifampin Ritonavir

which influence the transfer process of drugs in the body also influence the rate and extent of ADME of those drugs in the body. In many cases the pharmacological action as well as the toxicological action are related to plasma concentration of drugs.

After the administration of a drug, the concentration of the drug in the plasma appears to be described by exponential equations. The kinetics of ADME processes are frequently described by first order kinetics. The resulting concentration is proportional to the dose. When this is true, the kinetics are linear. When these kinetics are applied to the safe and effective therapeutic management of an individual patient, it is called clinical pharmacokinetics. In clinical pharmacokinetics we monitor plasma concentrations of drugs and suggest dosage regimens which will keep the concentration of drug within the desired therapeutic range. Pharmacodynamics refers to the relationship between the drug concentration at the receptor and the intensity of pharmacological or toxicological response. This can be controlled indirectly by controlling plasma concentrations, assuming that there is a predictable relationship between drug concentration in the plasma and drug concentration at the receptor site. This assumption is the basis for all clinical therapeutics.

After a single intravenous dose of a drug solution, the rate at which the drug is eliminated is proportional to how much is present (first order). Thus the assumptions are: body homogeneous (one compartment); distribution instantaneous; concentration proportional to dose (linear); rate of elimination proportional to how much is there (first order) [80,81,82].

Intravenously administered drugs distribute very rapidly throughout the body without an absorption step. For other extravascularly routes of administration, absorption needs to be considered. When the drug reaches the systemic circulation, it can undergo both distribution and elimination. Elimination can be by metabolism or by excretion. Each of these processes is associated with a rate constant and they govern the plasma drug concentrations at any time.

Clearance (Cl) is one of the most useful pharmacokinetic parameters. It depends on the intrinsic ability of the organs such as the liver and kidneys to metabolize or excrete, and also clearance is a function of the blood flow rate to these organs. Total or systemic clearance is the sum of all individual organ clearances that contribute to the overall elimination of the drug. For certain drugs that become metabolized completely, renal clearance is negligible. In this case systemic clearance can be approximated to hepatic clearance by the liver. Hepatic clearance can be estimated approximately from *in vitro* metabolism kinetics data. The clearance can be estimated *in vivo* too indirectly and directly (although it is more difficult). Rate of metabolism is generally defined by the Michaelis-Menten enzyme kinetics relationship [80, 81, 82].

4.1.1. Compartmental Analysis

Pharmacokinetic data analysis using mathematical models is known as compartmental pharmacokinetics. The body is represented as a system of compartments, but they have no anatomical meaning. The rate transfer between these compartments and the rate of elimination are assumed to be following first-order kinetics.

The one-compartment model is the simplest and it represents the body as a single compartment. It is applicable to drugs that distribute through the body rapidly. After an intravenous administration, all the pharmacokinetic parameters can be obtained using the appropriate equations. The elimination rate decreases as the drug concentration (C) decreases:

$$C=C_0.e^{-K.t}$$

where Co is the concentration at time zero, and K is the rate constant of elimination.

Administration of a drug by an extravascular route, will involve an absorption step. The plasma concentration at any time is given by the equation:

$$C = \frac{Ka.F.D}{(Ka-K).Vd}\left(e^{-K.t} - e^{-Ka.t}\right)$$

where F is the fraction of the administered dose D that is absorbed after extravascular administration, Ka is the absorption rate constant and K is the elimination rate constant.

The rate constants can be estimated by fitting concentration-time data to the above equation using a non-linear least squares regression program. Commercially software packages are available. (Kinetica, WinNonlin, SAS, etc).

The pharmacokinetic constants and parameters commonly used are: Ka, Kel,$T_{1/2}$, Vd (apparent volume of distribution), Cl (clearance), AUC (area under the curve), etc [80, 81, 82].

The two-compartment model is applicable when a drug, following an intravenous injection, will distribute rapidly to the central compartment into the peripheral tissues (the peripheral compartment). A bi-exponential decline can be associated with a two-compartmental model. Plasma concentrations are a sum of two exponential terms:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

The equations for rate constants α and β are described in the pharmacokinetic texts cited. Also from these constants pharmacokinetic parameters as AUC, Cl and Vd can be estimated [80, 81, 82].

In order to differentiate between the most suitable of the models (one versus two compartment), the Schwartz criteria and the Akaike information criteria (AIC) are standard useful methods [98].

4.1.2. Non-Compartmental Analysis

In non-compartmental analysis, the pharmacokinetic parameters are obtained without fitting the data to any specific compartmental model, but assuming that the data follow linear pharmacokinetics. The analysis is based on the theory of statistical moments.

The mean residence time (MRT) is obtained by the equation:

MRT = AUMC / AUC.
$K = 1 / MRT_{iv}$
$T_{1/2} = 0.693\ MRT_{iv}$
Vdss = Cl.MRT

where Vdss is the volume of distribution in the steady state, AUMC is the area under the first moment of the curve. Other parameters, as clearance and bioavailability, etc, can be obtained from AUC and AUMC the same way as for the compartmental treatment data. In the non-compartmental analysis, some data are derived directly from the plasma concentration-time profiles, as for example the rate of absorption can be estimated from Cmax (maximum plasma concentration) and Tmax (time for Cmax) [80, 82].

4.1.3. Enzyme Kinetics

Systemic clearance of a drug that is eliminated by metabolism in the liver is a function of the hepatic blood flow and the intrinsic clearance of the liver. Administration of drugs that are cytochrome P450 enzyme inducers or inhibitors can therefore influence the systemic clearance [83].

The basis for the extrapolation of *in vitro* data to the *in vivo* is the parametric intrinsic clearance (Clint). Intrinsic clearance is a measure of enzyme activity and is independent from other physiological factors. It is a proportionality factor between the rate of metabolism (V_0) and the drug substrate concentration at the enzyme site (Cs):

$V_0 = Clint.Cs$

Rate of metabolism is defined by the Michaelis –Menten enzyme kinetics relationship:

$$V_0 = \frac{Vmax.\ Cs}{Km + Cs}$$

where Km is the Michaelis-Menten constant, Vmax is the maximum rate of metabolism; Km is the substrate concentration at the half the maximum velocity (Vmax).

When the drug concentration is much smaller than Km (Km>>Cs) Clint can be expressed as:

$Clint = V_0 / Cs = Vmax / Km$

and in this case Clint is constant and independent of the drug concentration. To calculate Clint, enzyme activity should be measured at time points over the time period that V0 is linearly changing with concentration. Enzyme activity is measured by measuring the increase in metabolite and/or the decrease in the concentration of the parent drug.

Michaelis-Menten equation can be arranged to give Lineweaver-Burk equation as shown in equation:

$1/V = [Kmax/ Vmax].\ 1/Cs + 1 / Vmax$

The parameters Km and Vmax are obtained from a plot of 1/V versus 1/Cs.

Some other equations are used for studying the enzyme kinetics [83].

Michaelis –Menten (hyperbolic) kinetics can be verified using an Eadie-Hofstee transformed plot (v/S ratio on the x-axis and v on the y-axis) which becomes a straight line with a negative slope equal to –Km as shown in Fig. (**2**) [84].

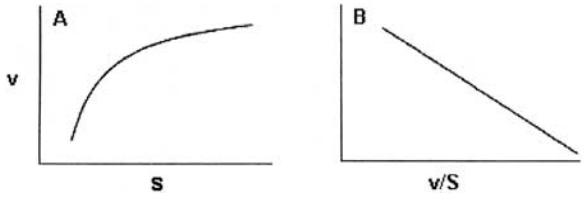

Fig. (2). Concentration-velocity plots (A) and corresponding Eadie-Hofstee transformation (B) of enzyme kinetics for a single Michaelis-Menten enzyme (A, B); v is the reaction velocity; S is the substrate concentration.

Cl_{int} can be scaled to *in vivo* clearance $Cl_{int\ invivo}$, using scaling factors, as: microsomal protein content, number of hepatocytes per gram of liver, liver weight and liver blood flow. A liver model can be used to estimate the intrinsic *in vivo* clearance, and Cl *in vivo*, the hepatic clearance of a drug can be estimated. More detailed data are available [85, 86].

Using *in vitro* models to study human drug metabolism is expanding. The *in vitro* systems are depending on the proper conditions. Cell culture systems with single human drug metabolizing enzymes expressed are commercially available and in use.

4.1.4. Metabolite Pharmacokinetics

Drugs are eliminated from the body by excretion and by transformation to metabolites. These processes occur simultaneously, but the extent of each process changes from one drug to another. Metabolites are eliminated by excretion and further metabolism. The amount of drug in the body depends on the rate of presentation of the drug to the body and its rate of elimination. Analogously, the amount of a metabolite in the body depends on its rates of formation and elimination. The quantitative assessment of metabolite kinetics is similar to approaches used to describe the parent drug kinetics: compartmental or non-compartmental analysis [80, 81, 82, 87, 88, 89].

The majority of the drug biotransformation reactions are carried out in the liver by enzymes which are located mainly in the smooth endoplasmic reticulum of the hepatic cells. The result of the metabolic transformation of drugs is metabolites that are more polar and less lipid soluble than the parent drug, therefore more easily excreted.

But drugs that are administered to humans may be biotransformed to yield metabolites that are pharmacologically active. Some drugs are inert and depend on metabolism for activation. Some metabolites have pharmacologic properties in common with the parent drug and augment its effect. Some have a different pharmacologic profile and may even be the cause of toxicity. Some are inactive but may, by acting as inhibitors, prolong or augment the response to a drug. Still others may affect the disposition of a drug by competing for plasma and tissue binding sites. In all these cases, unless a sufficient concentration exists at the appropriate site, the presence of metabolites is of little

therapeutic concern. The utility of metabolite pharmacokinetics is evident for an explication of the biological activity, with a clinical efficacy or even toxicity.

The principle of bioactivation has long been recognized in prodrug therapy. The undesirable properties of an active compound, such as poor lipid or water solubility, lability, or poor patient acceptance, are overcome by slight structural modification of the active compound (esterification). By definition, prodrugs are inert substances, and through bioactivation, the active moiety is released to trigger the pharmacological response. One particularly important case is that of prodrugs where the pharmacologically active metabolite is formed *in vivo* and knowledge of its kinetics following oral administration of the precursor is crucial.

In the simplest case a linear one-compartment system describing the pharmacokinetics of a metabolite, the parent compound (D) is administered intravenously and eliminated solely by metabolism to a metabolite (M) formed *via* a linear first-order process with rate constant k_f. The elimination rate of parent compound, k, is equal to the formation rate of metabolite, i.e. $k = k_f$. The elimination of the metabolite in the urine is a linear process with rate constant k_{mu}:

$$k = k_f \qquad k_{mu}$$
$$D \rightarrow M_{body} \rightarrow M_{urine}$$

$$dD / dt = -k.D = - k_f. D$$

$$dM/dt = kf.D-k_{mu}.M$$

The amount of metabolite in the body with time (t) can be described by the equation:

$$M = (dose.\ k_f / k_f - k_{mu}).\ (e^{-kmu.t} - e^{-\ kf.t})$$

The influence of the ratio of rate of formation of metabolite to its rate of elimination, on the terminal slope of the metabolite-time profile is of particular interest [90].

In some instances metabolites formed from the parent compound can inhibit their own metabolism, and non-linear elimination occurs. Nonlinearity could also be a result of capacity limited protein binding, or the result of time-dependent kinetics such as enzyme induction or nonlinear protein binding of the parent compound or metabolite [88].

Most drugs are metabolized to one or more metabolites. These metabolites in turn could be further biotransformed by a phase II enzyme and eliminated. Generally the parent compound is converted to inactive and more polar metabolites. In some instance the primary metabolite formed might be active and might substantially exceed the parent compound in terms of concentration and/or activity. In these cases it is imperative to elucidate the pharmacokinetics of the metabolite, especially when the parent drug is administered on a long term basis (accumulation of metabolite).

Many drugs that undergo extensive first pass metabolism in the gut are generally metabolized by phase I enzymes. It is difficult to characterize the true magnitude of the contribution of presystemic gut metabolism and hepatic metabolism to total metabolism. This is because of the inability to directly assess their respective contributions. Extraction ratio across the gut (Eg) is estimated indirectly by estimating the bioavailability (F), the fraction absorbed (f_{abs}) and the extraction ratio across the liver (Eh) [90]:

$$F = f_{abs}.\ (1-Eg).\ (1-Eh)$$

The amount of the amount of metabolite formed would depend on the release of the parent drug from the dosage form. Immediate release dosage forms could potentially saturate the metabolism of the gut resulting in increased bioavailability of the parent drug. Slow release dosage forms could result in more complete metabolism and thus lower the bioavailability of the parent drug.

In bioequivalence studies, the guidelines are in agreement that the parent drug must be measured. EMEA guidlines state that the metabolites are required if the concentration of parent drug is too low, and if the parent compound is unstable or half-life is too short [91]. The most recent guidline from the US FDA requests that the parent drug is measured. Only when a metabolite is formed as a result of gut wall or other presystemic metabolism and the metabolite contributes to safety and efficacy is the metabolite measured to provide supportive evidence [92].

4.2. Pharmacokinetic Analysis of Drug-Drug Interactions and their Clinical Significance

4.2.1. Introduction

There are several ways for analyzing data from a drug-drug interaction study. The noncompartmental pharmacokinetic analysis is the most used method, but compartmental analysis can also be useful and sometimes more informative than noncompartmental analysis [93].

The methodology in analyzing drug-drug pharmacokinetic data will be presented as follows. The description of methods and calculations will be exemplified using real data obtained from a study of pharmacokinetic interaction between fluoxetine and metoclopramide [94]. Briefly, the experiment consisted of two period administration of metoclopramide (20 mg) alone or in combination with fluoxetine after 8 days treatment with fluoxetine (60 mg/day) in twenty-four healthy volunteers. Metoclopramide plasma concentrations were analyzed by a validated high performance liquid chromatographic method with fluorescence detection.

The first step in analyzing a drug-drug interaction is the visual inspection of raw data. It can be done by plotting either the mean or the individual plasma levels of drug *vs.* time before and after pharmacokinetic interaction. In our example, the mean plasma concentrations of metoclopramide administered alone or in combination with fluoxetine after 8 days treatment with fluoxetine are shown in Fig. (**3**).

To be noted that the statistical analysis of metoclopramide levels when given alone or in combination with fluoxetine (t-test for paired values) results in significant differences for all the observation times (p value ranged between 0.0007 and 0.00002 (data not shown) except at 0.5 h (p=0.112). This is the first indication for a possible pharmacokinetic drug-drug interaction. Because sometimes a statistical significant difference in plasma levels at a certain

time does not necessary imply a statistical significant difference in pharmacokinetic parameters, the conclusion of evidence of interaction cannot be based only on that analysis.

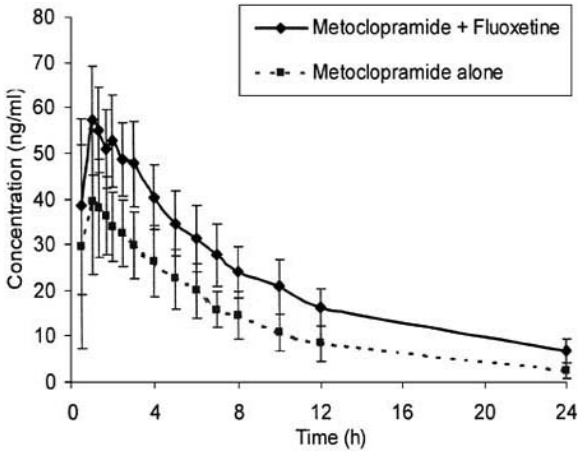

Fig. (3). Mean (±SD) plasma levels of metoclopramide (20 mg p.o.) given alone or in combination with fluoxetine (60 mg, p.o.) after pretreatment with fluoxetine for 8 days (60 mg p.o.), n=24 (with the permission of the publisher).

4.2.2. The Study of Drug-Drug Interaction by Noncompartmental Analysis

The most used method for analyzing a pharmacokinetic drug-drug interaction is noncompartmental analysis followed by the statistical comparison of some parameters. The method is relatively simple and rapid. The parameters used are AUC (observed and/or total), Cmax, Tmax and half-life. Statistical evaluation of differences between the calculated parameters, before and after drug-drug interaction, can be done using ANOVA or t-test for paired values.

The mean pharmacokinetic parameters of metoclopramide administered alone or in combination with fluoxetine, as well as the statistical significance following their comparison are given in Table **2**.

Peak plasma concentrations (Cmax) of metoclopramide before and after the fluoxetine multiple doses treatment (44.02 ng/ml *vs*. 62.72) is significantly different between the two treatments. The same statistical significance can be found when comparing $AUC_{0-\infty}$, Kel and $T_{1/2}$ parameters. However, the time to reach the peak plasma concentration (Tmax) does not significantly differ between treatments (p=0.5187).

The significant statistical difference between the most pharmacokinetic parameters analyzed, proved the presence of drug-drug interaction.

If we apply the bioequivalence test to the pharmacokinetic parameters of the drug administered alone (reference) versus drug in combination with another (test), we should be able to find a significant difference between these parameters, if it exists.

For our study, the pharmacokinetic parameters Cmax, Tmax and AUC0-inf were used for the bioequivalence evaluation of metoclopramide administered alone (Reference) or in combination with fluoxetine (Test). The parametric 90% confidence interval for the ratio Test/Reference period of the mean pharmacokinetic parameters Cmax and AUC (log transformed) of metoclopramide and the significance of the difference of Tmax (Test-Reference, mean values), are shown in Table **3**.

The 90% confidence intervals for geometric mean of metoclopramide in Test/Reference individual ratios for Cmax, and AUC0-∞ were outside the acceptable limits of bioequivalence. That means that the pharmacokinetic interaction between fluoxetine and metoclopramide may have a clinical significance. However, that affirmation has to be demonstrated by further clinical observations.

In literature many pharmacokinetic interactions are reported but some are without clinical significance. The clinical significance of a pharmacokinetic drug-drug interaction can be evaluated only by measuring and comparing a clinical parameter or effect of a drug administered alone or with another. Clinical parameters may be: the therapeutical efficacy, the frequency of side or adverse effects and so on. Without clinical data, we cannot know that a certain pharmacokinetic interaction is clinically significant. However, having only pharmacokinetic data such as in our example, we can still evaluate the possibility that the demonstrated drug-drug interactions have clinical significance.

Table 2. Pharmacokinetic Parameters of Metoclopramide Administered Alone or After Pretreatment with Fluoxetine and the Result of Statistical t Test Used for Comparison

Pharmacokinetic Parameter (±SD)	Metoclopramide Alone	Metoclopramide + Fluoxetine	p* Value, t-Test
Cmax (ng/ml)	44.02 (14.96)	62.72 (9.20)	0.0001*
Tmax (hr)	1.15 (0.46)	1.06 (0.41)	0.5187
$AUC_{0-\infty}$ (ng.hr/ml)	312.61 (112.72)	590.62 (140.01)	0.0001*
Kel (1/hr)	0.131 (0.029)	0.087 (0.021)	0.0008*
$T_{1/2}$ (hr)	5.52 (1.12)	8.469 (2.18)	0.0009*

* Significance for $p<0.05$

Table 3. Bioequivalence Evaluation of Metoclopramide Administered Alone or After Pretreatment with Fluoxetine

Pharmacokinetic Parameter	90% Confidence Intervals
$AUC_{0-\infty}$ (ng.h/ml)	1.66-2.29 (ANOVA, S)
Cmax (ng/ml)	1.30-1.70 (ANOVA, S)
Tmax (hr)	χ^2=3.841 (Friedman, NS)

S= significant, NS= non significant

4.2.3. The Study of Drug-Drug Interaction by Compartmental Analysis

The compartmental pharmacokinetic analysis can provide additional information besides the results offered by noncompartmental analysis. However, in many instances it is not easy to find an appropriate model for fitting the data, so that kind of analysis of drug-drug interaction is not usually performed in published papers.

Because metoclopramide metabolism in man is mediated through CYP2D6 [95] and fluoxetine has an inhibitory effect on this enzyme [96, 97], the observed pharmacokinetic interaction might be due to alteration of metoclopramide metabolism. Because of that, a change in both presystemic and systemic elimination of metoclopramide may explain the observed interaction. First, the presystemic metabolism is likely to decrease and that will result in a higher absorption extent of metoclopramide (higher Cmax and AUC0-∞). At the same time, the decrease in systemic metabolism will also contribute in increasing Cmax and AUC0-∞ as well as in increasing the half-life of the drug. All these suppositions are based on drug characteristics and general drug-drug interaction mechanism found in literature data. However, we can verify these suppositions using compartmental analysis.

From data in literature we know that the analyzed interaction is metabolic (chapter 3), so the pharmacokinetic parameters more likely to change are the bioavailability (F), due to change of presystemic metabolism and clearance (Cl), due to change in systemic metabolism. A change of volume of distribution is less probable and will not be considered.

The aim of compartmental modeling of data in the case of interaction between metoclopramide and fluoxetine is to find out if both F and Cl have a contribution to the interaction, and to what extent. For that, three pharmacokinetic models were built. All the models assumed a monocompartmental distribution of the drug and contained two sets of differential equations: one for the drug administered alone (parameters with indicative "R") and the second for drug administered in combination with another (indicative "T").

The first model (Model 1) assumes that the interaction is due both for changes in F and Cl. The second model (Model 2) and the last one (Model 3), is assumes that the interaction is due only to a change in one parameter, F or Cl respectively.

The equations written for Model 1 are presented in Fig. (**4**).

$$\text{Model1}\begin{cases}\dfrac{\partial Q_{absR}}{\partial t} = -k01 * Q_{absR}\\ \dfrac{\partial Q_{cR}}{\partial t} = k01 * Q_{absR} - \dfrac{Cl_R * Q_{cR}}{Vd}\\ \dfrac{\partial Q_{absT}}{\partial t} = -k01 * Q_{absT}\\ \dfrac{\partial Q_{cT}}{\partial t} = k01 * Q_{absT} - \dfrac{Cl_T * Q_{cT}}{Vd}\\ Q_{totT} = F_{rel} * Q_{totR}\end{cases}$$

Fig. (4). The equations written for Model 1.

where QabsR and QabsT means the quantity of metoclopramide at absorption place when administered alone or in combination with fluoxetine, k01 is the absorption rate (the same in both treatments), QcR and QcT means the amount of metoclopramide in central compartment, Vd is the apparent volume of distribution of central compartment (the same in both treatments), ClR and ClT the apparent clearance of drug in absence or administered with fluoxetine, QtotR and QtotT are the total quantities of drug absorbed, Frel is the relative bioavailability of drug administered in combination related to the drug administered alone, R and T are reference and test, respectively

The parameters evaluated by Model 1 are k01, Frel, ClT, ClR and Vd.

All three models were fitted to individual data of the subjects (simultaneous fitting of plasma concentrations of metoclopramide from the two treatment periods).

The first step is to choose the model that fits the data best, which is correlated with the most probable mechanism of interaction. For this purpose, the Akaike index [98] was calculated for each model as a means of individual values for every subject. A lower value of Akaike index means a better fitting of the model to the data, indicating the best model. The mean values for Akaike index for the three models studied were 98.4 for Model 1, 102.3 for Model 2 and 114.9 for Model 3. Thus, we can conclude that a change in both bioavailability and clearance (Model 1) is the most probable mechanism of observed pharmacokinetic interaction between fluoxetine and metoclopramide. The mean values of calculated pharmacokinetic parameters for Model 1 are presented in Table **4**.

Using compartmental pharmacokinetic analysis, we were able to estimate that the interaction between fluoxetine and metoclopramide may be due to changes in two factors: an increase of bioavailability of metoclopramide by a factor of 1.52 and a decrease of its apparent clearance from 71.4 mL/min to 55.8 mL/min (decrease by 28%). All these changes are expressed in relative terms, e.g. the interaction was related to normal physiological conditions.

Table 4. Mean Values of Pharmacokinetic Parameters of Metoclopramide Administered Alone or in Combination with Fluoxetine Using a Model that Calculates the Contribution of Two Factors (Bioavailability and Clearance) to Drug-Drug Interaction

Mean Pharmacokinetic Parameter (n=24)	Mean Value (±SD)
k01 (h^{-1})	3.52 (2.91)
Frel	1.52 (0.36)
Cl_R (mL/min)	71.4 (21.6)
Cl_T (mL/min)	55.8 (19.8)
Vd (L)	463.7 (116.9)

5. CONCLUSIONS

It is well known that the rate of adverse drug reactions increases exponentially after a patient has been on multiple medications, therefore it is very important to make efforts to reduce polypharmacy. However the number of medications cannot always be reduced without doing harm. This is why the understanding of the basis for drug interactions is so important.

Pharmacokinetics and drug metabolism play an important role as determinants of *in vivo* drug action. The CYP450 enzyme family plays a determinant role in the biotransformation of a vast number of structurally diverse drugs. Many drug interactions are a result of inhibition or induction of CYP enzymes. In addition to inhibition and induction, microsomal drug metabolism is affected by genetic polymorphisms, ontogeny of metabolic enzymes, age, nutrition, hepatic disease and endogenous chemicals.

The non-compartmental pharmacokinetic analysis is the most used method for analyzing data from a drug interaction study. Compartmental analysis can be also useful and sometimes more informative than non-compartmental analysis. A metabolic drug interaction may have different mechanisms and using compartmental pharmacokinetic analysis it may be possible to discriminate between different physiologic phases of drug metabolism, like presystemic or systemic, with consequences on bioavailability and clearance. Pharmacokinetics is a useful method to study the mechanism of drug interactions and to elucidate important aspects of human pharmacology.

Clinicians should be aware of the potential interactions and become familiar with the substrates, inhibitors, and inducers of the common enzymatic pathways responsible for drug metabolism. By understanding the unique functions and characteristics of CYP enzymes, physicians will be able to anticipate and manage drug interactions. This will enhance the use of rational drug therapy and better drug combinations.

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