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The Role of Biotransformation in Drug Discovery and Development

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Abstract

Biotransformation is a process by which organic compounds are transformed from one form to another, aided by organisms such as bacteria, fungi and enzymes. It plays a major role and determines the fate of the prospective drugs. Biotransformation must take place only after the drugs reach their specific target site and produce the desired effects. In addition, the nature of the metabolites produced from the drug, must be thoroughly studied; otherwise, the drugs would be rejected during the screening process. Hence, drug metabolism is a major criterion in the high-throughput screening of prospective drugs.

Biotransformation has an important role in the determination of the pharmacokinetic parameters like oral bioavailability, drug-drug interaction, clearance and the half-life of the entity within the cell. It is very essential in the toxicity studies too. Biotransformation is used as a valuable strategy to build molecules, similar to parent drug in the drug discovery programme. It can play an important role in identifying factors underlying the problems, facilitate the optimal selection of compounds for further development, provide information on metabolites for possible improvement in drug design, and contribute to the identification of the appropriate animal species for subsequent toxicity testing. Hence, some of the metabolites of biotransformation were already developed as a drug and are currently in clinical use.

Keywords: Drug discovery and development; Biotransformation; Enzymes; Liver

Abbreviations: DMPK: Drug Metabolism and Pharmacokinetic; ADME: Absorption, Distribution, Metabolism and Excretion; Pka: Negative Logarithm of Acid Dissociation Constant; CYP: Cytochrome P450; FMO: Flavin-Containing Monooxygenase; cDNAs: Complementary DNA Strands; Idrs: Idiosyncratic Drug Reactions; LC/MS/MS: Liquid Chromatography Coupled with Tandem Mass Spectrometry; LC/MS/NMR: LC/MS Nuclear Magnetic Resonance; AUC: Area under the Plasma Concentration–Time Curve

Introduction

Biotransformation can be defined as the use of biological systems to produce chemical changes on compounds that are not their natural substrates [1]. To defense the body against xenobiotic substances, an array of biotransformation reactions (or metabolic reactions) is undergone. Due to the biotransformation, the molecular structure of a drug is commonly changed to be more hydrophilic and the substances can be readily eliminated from the body [2].

In general, biotransformation reactions are divided into two broad categories known as 'phase I' and 'phase II' reaction. Phase I reactions are functionalization reactions that introduce polar chemical moieties either by inserting new polar functional groups or by interchanging or unmasking existing functional groups via oxidation, reduction and hydrolytic reactions. Phase I reactions are mediated by enzymes such as CYP, FMO, esterases and amidases. CYP enzymes are by far the most important enzymes responsible for the pharmacological activation of many drugs. In phase II reactions, small endogenous polar molecules (e.g. glucuronic acid and sulfate) conjugate with the functional groups formed during phase I reactions. Direct conjugation of the endogenous molecules can also occur if the compound already contains appropriate functional groups. These conjugative reactions are mediated by enzymes such as glucuronosyltransferase, sulfotransferase and N-acetyltransferase. In a traditional prodrug-based approach to drug discovery, biotransformation reactions are used to convert pharmacologically inactive compounds to pharmacologically active metabolites. These reactions are usually mediated by a broad class of hydrolytic enzymes, such as esterases, amidases and phosphotases, although the conversion of a prodrug to the corresponding active drug can also occur non-enzymatically [3-6].

Methods for generation of metabolites

In vitro methods

Subcellular fractions: Subcellular fractions prepared from organs expressing drug metabolizing enzymes include the cytosolic fraction, the S9 fraction and microsomes. Organs such as intestine, liver, kidney, lung, and skin are known to mediate xenobiotic metabolism. Liver is the major site of drug metabolism. As a result, liver subcellular fractions are often employed for studying metabolic reactions and generation of metabolites. Subcellular fractions can be used to prepare metabolites formed by a number of enzymes such as CYP, FMO, myeloperoxidase, ketoreductase, alcohol dehydrogenase, sulfotransferase, etc. [7].

Primary cell-based systems: Intact cells such as primary hepatocytes, contain both soluble and membrane-bound enzymes including the relevant cofactors at or near the appropriate physiological concentrations. As a result, they have greater physiological relevance and can mediate both phase I and phase II metabolism. Cryopreserved

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hepatocytes are now available for convenient use, and the hepatocytes can be pooled together from different donors. Immortalized human liver cell lines in which enzymes such as CYPs are individually expressed can also be used for relatively large-scale generation of metabolites. Tissue slices such as liver slices contain both phase I and II drug metabolizing enzymes can also be used to generate sufficient quantities of metabolites [8].

Cell lines containing heterologously expressed drug metabolizing enzymes: Recently, several cellular systems that express high levels of CYP enzyme have been used as "CYP bioreactors" for rapid and large-scale biosynthesis of metabolites. These include baculovirus-infected insect cells, [9] immortalized human liver epithelial cells, and E.coli [10] that have been engineered to express large quantities of individual CYP enzymes. These CYP bioreactor systems are capable of generating appreciable quantities of CYP-derived metabolites using simple equipment. Although CYP bioreactors are still at an early development stage, it has been demonstrated that they are a promising technology for large-scale and low cost production of metabolites. These techniques have the following advantages over conventional metabolism systems and chemical synthetic approaches including rapid and stereospecific synthesis of human CYP-derived metabolites at very high yield and ease of metabolite isolation and identification, as bioreactor mediums are relatively clean compared to other biological fluids.

Metabolite production by the insect cell CYP bioreactor consists of three steps: growth and maintenance of insect cells in suspension culture until a desired cell density is reached; transfection of the insect cells with baculovirus containing cDNA for a CYP enzyme; and metabolite production by the addition of the parent drug to the suspension culture. High metabolite yield is achieved using this system [11].

Immortalized human liver cell lines transfected with individual human CYP genes were originally developed for identifying potential mutagens, cytotoxins, and chemotherapeutics agents. More recently, the system has been applied for the biosynthesis of milligram quantities of CYP-dependent metabolites. These systems are relatively clean, and the metabolites in the incubation media can be directly identified by LC/MS or LC/NMR with no further purification [12,13].

***In vivo* methods:** Metabolites can be generated in vivo following compound administration to preclinical animals or human. In vivo samples such as plasma, urine, and bile can be used to obtain metabolites. Metabolites can also be obtained from drug-metabolizing organs such as liver obtained from animals pretreated with the desired compound. In vivo methods of generating active metabolite can be especially useful when active metabolites are formed extrahepatically because extrahepatic systems are not as widely used as hepatic systems for in vitro generation of metabolites [6].

Microbial cell-based systems: CYP-dependent monooxygenases capable of carrying out the oxidative biotransformations of xenobiotics have been found in bacteria, yeast, and fungi. Although phase I metabolism is of more importance for identifying metabolites with pharmacological and toxic effects, some microbial phase II enzymes have been described, including glucuronosyltransferase, arylsulfotransferase, and glutathione S-transferase [14-16]. Thus microorganisms are one of the most efficient biocatalytic agents with ability to metabolize a wide range of substrates.

Bioactive compounds can be obtained from a natural source by extraction or microbial transformation or biotransformation of currently marketed drugs. Extraction from natural sources presents some disadvantages such as dependency on seasonal, climatic and possible ecological problems involved with the extraction, thus calling for innovative approaches to obtain such compounds.

The notable toxicity due to bacterial metabolism in the intestines is related to bone marrow aplasia of a metabolite of the antibiotic chloramphenicol. This metabolite is only generated by a small percentage of patients who take the drug orally and have a high percentage of coliform bacteria that are capable of metabolizing chloramphenicol to the toxic metabolite p-aminophenyl-2-amin-1,3-propanediol [17].

Role of pharmacologically active metabolites in drug discovery and development

With the recent advances in molecular biology, genomics and chemical synthetic techniques, a number of new drug candidates have tremendously been discovered. However their possibility to be developed to the market product is extremely low because of poor pharmacological activity, unfavorable pharmacokinetic and pharmacodynamic behaviors or high toxicity. As an alternative approach in new drug development, pharmacologically active metabolites might be used as potential resources for drug discovery and development. Pharmacologically active metabolites can be used as candidates during optimization phase of drug discovery. Through some structural modification, the enhancement of metabolic stability and receptor binding affinity can successfully be achieved. In particular, improvement of the half-life in plasma and the metabolic stability of drugs have been widely investigated with active metabolites [2].

Biotransformation reactions can be accompanied by various events, such as the formation of chemically stable metabolites, which are devoid of pharmacological or toxicological activities, or the generation of short-lived chemically-reactive metabolites, which can lead to toxicological activation [4,5,18]. Biotransformation reactions can also result in the formation of chemically stable metabolites with pharmacological activity (on or off target) [3-6].

Formation of pharmacologically active metabolites

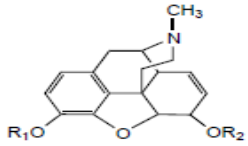
Pharmacologically active metabolites are generated through mainly primary and/or secondary, and tertiary metabolism by phase I and phase II reactions. Although metabolites are chemical structurally different from the parent drug, if they have structural similarities to the parent molecules, they might attain biological activities similar to the parent drug in some cases. Table 1 lists the active metabolites produced from currently marketed drugs. As shown in Table 1, simple reactions mediated via CYP generate active metabolites in many cases such as aromatic or aliphatic hydroxylation, O- or N-dealkylation, and dehydrogenation or in combinations. Many of the pharmacologically active metabolites are generated via hydroxylation or dealkylation reactions [19].

There are also numerous examples of active metabolites that are produced by phase II enzymes (Figure 1). Glucuronidation is normally considered to be detoxifying process, because glucuronides usually possess less intrinsic biological or chemical activity than their parent forms and exhibit higher polarity and excretability. However, some glucuronide conjugates are active and may contribute to pharmacological activities. The 6-O-glucuronide of morphine constitutes the most well-known example of a glucuronide possessing pharmacological activity greater than the parent drug. And, the sulfation of minoxidil, N-acetylation of acebutol or procainamide

Drug	Metabolite	Biotransformation	Therapeutic action
Atomoxetine	4-Hydroxyatomoxetine	Aromatic hydroxylation	Treatment of attention-deficit hyperactivity disorder
Atorvastatin	2- or 4-Hydroxyatorvastatin		Anti-hypercholesterolemia
Chlorpromazine	7-Hydroxychlorpromazine		Anti-depressive
Clomiphene	4-Hydroxyclomiphene		Anti-uterotrophic
Granisetron	7-Hydroxygranisetron		Anti-nausea
Indapamide	5-Hydroxyindapamide		Anti-hypertensive
Levamisole	4-Hydroxylevamisole		Anthelmintic
Propafenone	5-Hydroxypropafenone		Anti-arrhythmic
Propranolol	4-Hydroxypropranolol		Anti-arrhythmic
Alprazolam	4-Hydroxyalprazolam	Aliphatic hydroxylation	Anti-depressant
Bupropion	Hydroxybupropion		Anti-mental depression
Clarithromycin	14-Hydroxyclarithromycin		Anti-biotic
Cyclosporin A	Hydroxycyclosporin A		Immunosuppressive
Ibuprofen	Hydroxyibuprofen		Anti-inflammatory
Itraconazole	Hydroxyitraconazole		Anti-mycotic
Metronidazole	Hydroxymetronidazole		Anti-biotic
Midazolam	1′-Hydroxymidazolam		Anxiolytic eŠect
Nebivolol	4- or 4′-Hydroxynebivolol		Vasodilation
Nefazodone	Hydroxynefazodone		Anti-depressant
Praziquantel	4-Hydroxypraziquantel		Anti-neurocysticercosis
Quinidine	3-Hydroxyquinidine		Anti-arrhythmic
Risperidone	9-Hydroxyrisperidone		Anti-psychotic
Simavastatin	Simavastatin hydroxylic acid		Anti-hypercholesterolemia
Amiodarone	Desethylamiodarone	N-Dealkylation	Anti-arrhythmic
Azonafade	N-Desmethylazonaˆde		Anti-tumoral
Chlordiazepoxide	Desmethylchlordiazepoxide		Anxiolytic
Citalopram	Desmethylcitalopram		Anti-depressant
Clobazam	N-Desmethylclobazam		Anti-epileptic
Clomipramine	N-Desmethylclomipramine		Anti-depressant
Clozapine	Norclozapine		Anti-psychotic
Ferroquine	N-Demethylferroquine		Anti-malarial
	N,N-Didemethylferroquine		
Fluoxetine	Nor‰uoxetine		Anti-depressant
Ivabradine	N-Desmethylivabradine		Bradycardia
Lumefantrine	Desbutyllumefantrine		Anti-malarial
Mianserin	N-Desmethylmiaserin		Anti-depressant
Verapamil	Norverapamil		Anti-arrhythmic
Oxybutynin	N-Desethyloxybutynin		Smooth muscle relaxant
Rosuvastatin	N-Desmethylrosuvastatin		Anti-hypercholesterolemia
Sertraline	N-Desmethylsertraline		Anti-depressant
Sibutramine	N-Desmethylsibutramine		Treatment of obesity
	N,N-Didesmethylsibutramine		
Zolmitriptan	N-Demethylzolmitriptan		Treatment of migraine
Zopiclone	(S)-Desmethylzopiclone		Treatment of insomnia
Artemether	Dihydroartemisinin	O-Dealkylation	Anti-malarial
Ivabradine	O-Demethylivabradine		Bradycardia
Tramadol	O-Demethyltramadol		Centrally acting analgesic
Venlafaxine	O-Desmethylvenlafaxine		Anti-depressant
Acebutol	Diacetolol	N-Acetylation	Anti-hypertensive
Albendazole	Albendazole sulfoxide	Sulfoxidation	Anti-parasitic
Aripiprazole	Dehydroaripiprazole	Dehydrogenation	Anti-psychotic
Bupropion	Threo/erythro-hydrobupropion	Carbonyl reduction	Anti-depressant
Carbamazepine	Carbamazepine-10,11 epoxide	Epoxidation	Anti-epilepsy
Diacerein	Rhein	Deacetylation	Treatment of osteoarthritis

Isosorbide Dinitrate	5- or 2-Isosorbide mononitrate	Denitration	Treatment of angina pectoris
Losartan	Carboxylosartan	Carboxylation	Anti-hypertensive
Minoxidil	Minoxidil sulfate	Sulfation	Stimulating hair growth
Oxcarbazepine	10-Hydroxy-10,11-dihydrocarbamazepine	Hydroxylation & Dehydrogenation	Anti-epileptic
Procainamide	N-Acetyl procainamide	N-Acetylation	Anti-arrhythmic
Rifampicin	25-Desacetylrifampicin	Deacetylation	Treatment of tuberculosis
Thiocolchicoside	3-O-Glucuronidated-Thiocolchicoside	Glucuronidation	Muscle relaxant
Tramadol	O-Demethyl-N demethyltramadol	N,O-Dealkylation	Centrally acting analgesic
Triamterene	Hydroxytriamterene sulfate	Sulfation	Diuretic

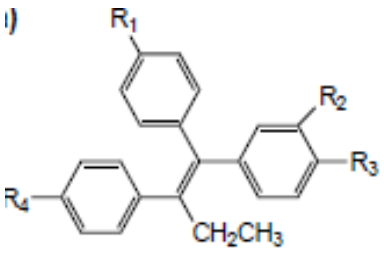
Table 1: Pharmacologically active metabolites of currently marketed drugs [2].



$R_1 = CH_3, R_2 = H$ (Codeine)
$R_1 = CH_3, R_2 = C_6H_8O_6$ (Glucuronide)(Codeine-6-glucuronide)
$R_1 = CH_3, R_2 = SO_3$(Codeine-6-sulfate)
$R_1 = H, R_2 = H$(Morphine)
$R_1 = H, R_2 = C_6H_8O_6$ (Glucuronide)(Morphine-6-glucuronide)
$R_1 = H, R_2 = SO_3$(Morphine-6-sulfate)

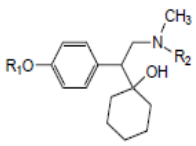
Figure 1: Examples of metabolites obtained through 'phase II' (conjugative) biotransformation reactions leading to pharmacological activation.

a)



$R1 = O(CH_2)_2N(CH_3)_2$, $R_2 = H$, $R_3 = H$, $R_4 = H$ (Tamoxifen)
$R1 = O(CH_2)_2NCH_3$, $R_2 = H$, $R_3 = H$, $R_4 = H$ (Desmethyltamoxifen)
$R1 = O(CH_2)_2NH$, $R_2 = H$, $R_3 = H$, $R_4 = H$ (Didesmethyltamoxifen)
$R1 = OH$, $R_2 = H$, $R_3 = H$, $R_4 = H$ (Desdimethylaminoethyltamoxifen)
$R1 = O(CH_2)_2NO(CH_3)_2$, $R_2 = H$, $R_3 = H$, $R_4 = H$ (Tamoxifen-*N*-oxide)
$R1 = O(CH_2)_2N(CH_3)_2$, $R_2 = OH$, $R_3 = H$, $R_4 = H$ (3-Hydroxytamoxifen)
$R1 = O(CH_2)_2N(CH_3)_2$, $R_2 = H$, $R_3 = OH$, $R_4 = H$ (4-Hydroxytamoxifen)
$R1 = O(CH_2)_2NCH_3$, $R_2 = H$, $R_3 = OH$, $R_4 = H$ (4-Hydroxydesmethyltamoxifen)
$R1 = O(CH_2)_2N(CH_3)_2$, $R_2 = H$, $R_3 = H$, $R_4 = OH$ (4'-Hydroxytamoxifen)

b)



$R_1 = CH_3, R_2 = CH_3$ (Venlafaxine)
$R_1 = CH_3, R_2 = H$ (*N*-Desmethylvenlafaxine)
$R_1 = H, R_2 = CH_3$ (*O*-Desmethylvenlafaxine)

Figure 2: Examples of biotransformation reactions that result in the conversion of a parent drug to metabolites with broad range of pharmacological activities.

has also contributed the production of the pharmacologically active metabolites [2,20].

Multiple active metabolites can also be formed from a single compound. For example, tamoxifen, a drug used for the treatment of breast cancer, forms multiple active metabolites by undergoing CYP-mediated aromatic hydroxylation, N-demethylation and FMO catalyzed N-oxidation, resulting in a 3-hydroxyltamoxifen (droloxifene), and 4-hydroxy tamoxifen, N-desmethyl tamoxifen and tamoxifen N-oxide, respectively (Figures 2a and 2b) [21,22]. The secondary metabolites, N-didesmethyltamoxifen and 4-hydroxy-desmethytamoxifen (endoxifen), are also pharmacologically active [21, 23] (Table 1).

Structure-activity relationships

Although metabolites are chemically distinct from the parent drug, they have structural similarities to the parent molecules that might help them, to some degree, attain activities similar to the parent drug. This is particularly true for those biotransformations that involve

simple functionalization reactions, such as hydroxylation, O- or N-demethylation and dehydrogenation or heteroatom oxidation, as is the case with many CYP-mediated reactions. Examples, in addition to those discussed above, are the simple hydroxylation or reduction or successive N-demethylation products of several antidepressant drugs that result in one or more active metabolites with significant therapeutic effect [24,25].

In general, if the functionalization reaction occurs at the auxophoric group (a non-pharmacophoric group) that is not crucial for the proper binding of the parent molecule to the receptor or enzyme, or if the reaction leads to optimization of the binding, it is very likely that the metabolite will maintain or enhance the potency of the parent compound. If, by contrast, the functionalization reactions lead to the formation of auxophoric groups that interfere with proper binding to the receptor or enzyme, or if the pharmacophoric group undergoes biotransformation, a decrease in activity is expected. In addition, a significant change in physicochemical properties or molecular size and shape can cause a significant loss in potency. An illustrative example is the biotransformation of tamoxifen (Figure 2a) to multiple metabolites that have different degrees of potency. For example, the N-desmethyltamoxifen was found to have a lower binding affinity and antiestrogenic activity compared with the parent drug in vitro. Removal of the second methyl group led to the didesmethyl metabolite that has even lower antiestrogenic activity than the N-desmethyl metabolite. This is because of the successive demethylation from the side chain (the alkylaminoethoxy) that is important for the antiestrogenic activity of the drug. In fact, further removal of the whole dimethylaminoethane side chain results in the conversion of the molecule to a full agonist [26].

By contrast, the aromatic hydroxylation of the drug results in the formation of the 4-hydroxytamoxifen, a metabolite that is 30–100 fold more potent than the parent compound in terms of binding affinity to the estrogen receptor and in suppressing estrogen-dependent cell growth [21]. Interestingly, the secondary metabolite, N-desmethyl-4-hydroxytamoxifen (endoxifen), has shown estrogen receptor-binding affinity comparable with the 4-hydroxytamoxifen. Endoxifen has similar potency in suppressing estrogen dependent cell proliferation to 4-hydroxytamoxifen [21,23]. In this case, the partial loss in activity of tamoxifen as a result of demethylation has been overcompensated by the higher potency built in through the hydroxylation of the aromatic ring.

As described above, when metabolites have significant structural similarities to the parent molecules, it is not surprising that they have biochemical actions similar to their parents. However, a minor structural modification can result in loss of potency or modification of the biochemical mode of action of the parent drug. For example O-demethylation of venlafaxine leads to an active metabolite, O-desmethylvenlafaxine, which has similar pharmacology as the parent drug, whereas N-demethylation of venlafaxine results in an inactive metabolite (Figure 2) [24].

As is the case of desdimethylaminoethyl tamoxifen, biotransformation reactions that lead to significant structural modification of drugs will most likely result in loss of potency or a change in the biochemical action of drugs in an opposite or unrelated manner. For example, nefazodone (an antidepressant drug withdrawn recently from the market) undergoes hydroxylation at the ethyl side chain of the triazolone core to form hydroxynefazodone that has a similar in vitro pharmacological profile and is considered to have similar clinical efficacy to the parent drug. By contrast, N-dealkylation at the piperazinyl nitrogen leads to the loss of a big portion of the molecule and forms m-chlorophenylpiperazine, a metabolite that is an agonist at the serotonin 5-HT2C receptor, whereas nefazodone or hydroxynefazodone are antagonists at this receptor [25,27].

Most metabolites that result from the major phase II drug metabolizing reactions, such as glucuronidation, are not pharmacologically active. This is presumably because these reactions entail a significant change in the physicochemical properties and molecular size and shape of the metabolites relative to the parent compounds. There are few examples of conjugated metabolites, such as morphine-6-O-glucuronide or morphine-6-sulfate that are more potent than the parent drug [28,29].

The significance of active metabolites in drug discovery and development

There are a number of advantages for screening drug candidates for active metabolites during drug discovery. The primary reason is that the process may lead to the discovery of a drug candidate with superior drug developability attributes such as: improved pharmacodynamics,(e.g, Desloratadine------ loratadine; Morphine ---Morphine-6-O-glucuronide); improved pharmacokinetics,(e.g, Desloratadine------ loratadine, longer $t_{1/2}$); lower probability for drug-drug interactions; less variable pharmacokinetics and/or pharmacodynamics, (e.g. Desloratadine--loratadine); improved overall safety profile,(e.g Morphine ---Morphine-6-O-glucuronide, less nausea; Fexofenadine --terfenadine, no effect on QTc interval and ventricular rhythm) and improved physicochemical properties (e.g., solubility).

Furthermore, early discovery of active metabolites provides a more complete patent protection of the parent molecule [6]. There are several reasons why it is important to be aware of the significance of pharmacologically active metabolites. Described below are some of the roles that active metabolite might play at the various stages of drug discovery (e.g. lead compound selection and optimization) and development.

Active metabolites as lead candidates during lead optimization

Pharmacologically active metabolites can be conveniently used as leads where they are subjected to further structural modification to obtain improved lead candidates during the lead optimization phase of drug discovery. Such approach was used, for example, in the discovery of ezetimibe, a cholesterol absorption inhibitor. In this work, an active metabolite that was already ~30- fold more potent than the parent was further modified to give the final drug candidate (ezetimibe), which was 400-fold more potent than the original lead. This approach can also be effectively exploited when a given chemotype in a drug discovery program suffers from issues such as solubility or metabolic stability. As discussed previously, metabolites generally have improved aqueous solubility or metabolic stability compared with their corresponding parent molecules. Thus, if during the lead optimization stage a metabolite with sufficient biological activity is identified, the metabolite can be used as a lead with an added advantage of improved properties and might be optimized further [30-32].

Modern analytical techniques such as LC/MS/MS, LC/NMR and LC/NMR/MS are now available to structurally characterize the desired metabolites in a timely fashion. These techniques have the capability of online analysis combined with great sensitivity, specificity and speed. For example, it is now possible to obtain qualitative information on the metabolites while simultaneously acquiring quantitative information on the parent in in vivo and in vitro samples [33,34].

Active metabolites as drugs in their own right

If an active metabolite has improved pharmacological, pharmacokinetic and toxicological properties compared with the parent, it should be advanced to the clinic in its own right as a viable drug candidate. In this regard, there are several active metabolites on the market that have already been developed as drugs and some examples are listed in Table 2. For some of these drugs, only the metabolite forms (e.g. acetaminophen, fexofenadine and phenobarbital) are currently in clinical use [19].

In most cases, further metabolism of active metabolites leads to the formation of fewer total metabolites compared to the parent compound. As a result, the use of an active metabolite as drug may lessen the chance for off-target toxicity that may be caused by one or more extra metabolites formed from the parent compound, even when the parent compound itself has no intrinsic toxicity. Moreover, most active metabolites are products of phase I functionalization reactions and as such are more susceptible to phase II conjugation reactions. Phase II conjugation reactions result in the formation of secondary metabolites that, in general, are safely cleared from the body. For example phenacetin, which is no longer in use in humans, is metabolized to a number of metabolites. Of the man phenacetin metabolic pathways, the O-deethylation pathway leads to the formation of acetaminophen (a more analgesic agent than phenacetin), whereas N-hydroxylation of phenacetin leads to the formation of a toxic metabolite that induces methemoglobinemia and hemolytic anemia. On the other hand, the corresponding active metabolite, acetaminophen, is predominantly cleared via phase II conjugation reactions (sulfation and glucuronidation) and has a greater margin of safety relative to phenacetin [6]. Therefore, whenever possible, an effort should be made to characterize an active metabolite in terms of intrinsic potency, bioavailability, rate of clearance, tissue distribution and safety to determine whether the metabolite can be a viable clinical candidate.

Extrapolating the pharmacodynamics data observed in the preclinical animal model to human

It is known that differences can exist among species in the rate and extent of drug metabolism, with quantitative differences being more common than qualitative ones. As a result, the nature (qualitative) and amount (quantitative) of active metabolites formed in an experimental animal model and their relative contribution to the overall pharmacological activity can be very different from those in human. For example, dogs are unable to acetylate N-arylamines, therefore active metabolites such as acetyl procainamide are not formed in dogs; cats are unable to glucuronidate compounds that are well known to undergo glucuronidation in other species including human. In rats and mice only the inactive metabolite of morphine-3-O-glucuronide is produced, whereas in human both the morphine-3-O-glucuronide and the 6-O-glucuronide (the active metabolite) are formed. The plasma AUC ratios of hydroxybupropion (an active metabolite) to bupropion in mice, rats, dogs and humans were ~3, 0.3, 1, 16, respectively, after oral administration of bupropion, clearly indicating significant species differences in the extent of formation of the active metabolite. This was also consistent with the differences seen in the pharmacology of the compound in rats and mice. Oral administration of bupropion prevented tetrabenzine-induced sedation in mice but not in rats. Therefore, to improve the decision making process at the discovery stage and to design appropriate clinical studies early on, it is important to appreciate the considerable differences between species in terms of rate of formation and clearance of metabolites. Under these circumstances, it is recommended that a correlation of in vitro and in vivo metabolism and pharmacodynamic data are established in the experimental animal model and these data are used in combination with the human in vitro metabolism systems to project human pharmacokinetics and efficacious dose [20,35].

S.No.	Parent drugs	Metabolite drugs
1.	Phenacetin	Acetaminophen
2.	Hydroxyzine	Cetirizine
3.	Imipramine	Desimipramine
4.	Loratadine	Desloratadine
5.	β-methyldigoxin	Digoxin
6.	Terfenadine	Fexofenadine
7.	Thioridazone	Mesoridazone
8.	Codeine	Morphine
9.	Amitriptyline	Nortriptyline
10.	Diazepam	Oxazepam
11.	Primidone	Phenobarbital

Table 2: Active metabolites developed as drugs [2].

Understanding the mechanism of actions of drugs

In most cases, active metabolites, particularly those formed as primary metabolites, have biochemical actions similar to their parents. However, an active metabolite can also have biochemical actions that are synergistic or inhibitory to the action of the parent drug or act on off-target pharmacology that is completely unrelated to that of the parent drug. Recognition and appreciation of the role of active metabolites is thus important to fully understand and tease out the mechanism of action of drugs early on [19].

Challenges of metabolism in drug development

The numbers related to success in producing new medicines are very challenging. Two of the major problems related with drug development are bioavailability and toxicity, both of which can be related at least in part to metabolism. The goal is to do a better job of selecting candidate drugs early in the development process (even as part of discovery) to avoid problems later in clinical trials, when more resources (including time) have been invested, by restricting the development process to the compounds with the highest likelihood for success. There are about five contexts of drug toxicity are on-target toxicity; Hypersensitivity and immunological reactions; Off-target pharmacology; Bioactivation of drugs to reactive intermediates; and Idiosyncratic drug reactions [36].

On-target toxicity is based on the drug's mechanism of interaction with its intended target. An example is the statins. As a class, these drugs have been very safe, and almost all of the toxicities have been documented to result from inhibition of the enzyme target 3-hydroxymethyl glutaryl CoA reductase in muscle instead of liver [37].

Hypersensitivity and immunological reactions are associated with penicillins and other β-lactum antibiotics. The reaction of these compounds with proteins can lead to autoimmune responses, in which the body's immune system begins to respond to the compounds as foreign entities. This phenomenon is the basis of the "hapten" and "danger" hypotheses, in which enough of a load triggers adverse responses [38].

Off-target pharmacology is a drug's interaction with a system other than that for which it was intended. One example is terfenadine, which can accumulate and block human ether-a-go-go (hERG) channels

in addition to the target H_1 (histamine) receptor (this example also involves metabolism, in that the accumulation of the parent drug terfenadine is associated with impaired metabolism to fexofenadine, which is the normal circulating product) [39,40].

The fourth context of toxicity involves bioactivation of drugs to reactive intermediates that bind covalently to macromolecules including DNA and proteins. The basic reactions are governed by two chemical reactions: the reaction of an electrophile (formed from the drug) and a nucleophile (in the tissue), and free radical propagation (oxidative stress) [36]. Bioactivation of acetaminophen, a classic example, is shown in Figure 3.

The drug-protein adducts cause toxicity either by impairing physiological functions of the modified proteins or through immune-mediated mechanisms, and that reducing this level of covalent damage is an effective approach to avoiding toxicity. The means to this end are changing the chemistry of the molecule either to increase the biological efficacy (thus reducing the dose needed) or to block the enzyme activation. Table 3 gives examples of drugs whose toxicities are attributed to covalent binding of metabolites to proteins. Advances in analytical instrumentations, notably LC/MS/MS and LC/NMR, have markedly expanded our capability to detect and identify adduct formation via reactive intermediates [41].

The extent of covalent binding of a drug or other chemical to tissue macromolecules can be used as a measure of potential toxicity, but many exceptions exist. For instance, 3-hydroxyacetanilide is relatively nontoxic but yields as much covalent binding as 4-hydroxyacetanilide (acetaminophen). The modified proteins differ for the two compounds, but a satisfying mechanistic explanation for the differential toxicity is still not available. Thus it remains difficult to accurately predict potential adverse drug reactions due to formation of these protein adducts, since not all of them result in drug toxicity [42].

Other issues in toxicity are receptor-mediated and signaling events, cell proliferation, repair, and immune responses. A view of the complexity of events involved in cell toxicity is presented in Figure 4 below.

Majority of drugs withdrawn from market or with "black box" warnings were characterized to have reactive metabolites. For instance, of a set of six drugs withdrawn from the market (in the time frame examined), five (benoxaprofen, iproniazid, nefazodone, tienilic acid, and troglitazone) are known to have reactive products. Of another 15 drugs with "black box" warnings, eight (dacarbazine, dantrolene, felbamate, flutamide, isoniazid, ketoconazole, tolcapone, and valproic acid) have reactive metabolites. Thus, 62% of the problem drugs in this group involve metabolism and reactive products [36].

The fifth and final classification is idiosyncratic drug reactions. These are more sporadic (~ $1/10^4$ cases) and often unrepeatable,

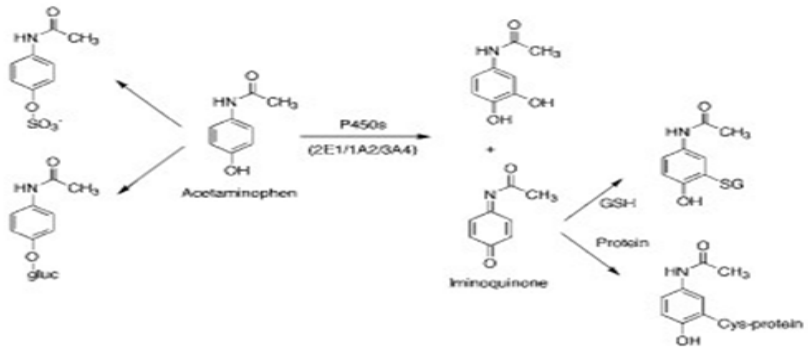


Figure 3: Metabolism of acetaminophen.

Drugs	Reactive intermediate	Toxicity
Acetaminophen	Quinine-imine	Hepatotoxicity
Carbamazepine	2-hydroxyl,quinine-imine	Agranulocytosis, aplastic anemia
Clarithromycin	Nitroalkane	Hypersensitivity
Clozapine	Desmethylclozapine	Agranulocytosis
Dapsone	Hydroxylamine, nitroso	Hemolysis, Hypersensitivity
Diclofenac	Acyl glucuronide, benzoquinone imine	Hepatotoxicity
Halothane	Trifluoroacetyl	Hepatesis
Indomethacin	Iminoquinone	Hypersensitivity, Agranulocytosis, Hepatotoxicity
Isonizaid	Isonicotinic acid, acetylating species	Hypersensitivity
Phenacetin	p-nitrosophenetole	Hepatotoxicity
Procainamide	Hydroxylamine, nitroso	Agranulocytosis
Tacrine	7-OH-tacrine	Hepatotoxicity
Tamoxifene	N-oxide, N-oxide-epoxide	Carcinogenicity
Ticlodipine	Keto, S-oxide	Agranulocytosis, aplastic anemia
Tienilic acid	Thiopene S-oxide	Hypersensitivity, hepatitis
Troglitazone	Conjugates, benzoquinone, quinine epoxide	Hepatotoxicity
Valproic acid	Acyl glucuronide, 2-N-propyl-4-pentenoic acid	Hypersensitivity, Hepatotoxicity

Table 3: Example of drugs that form reactive intermediates and exhibit toxicity [41].

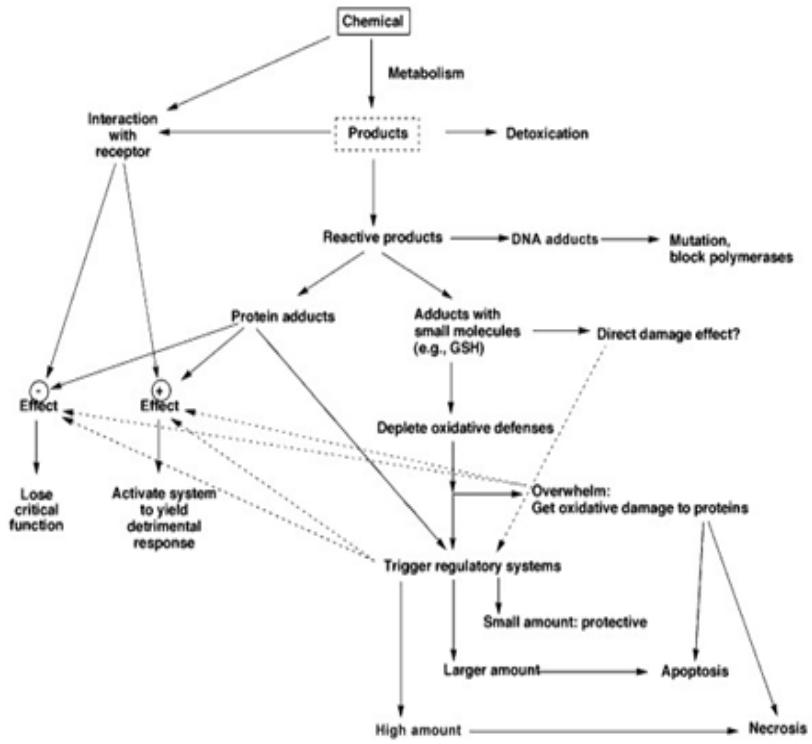


Figure 4: Biological events possibly relevant to chemical toxicity.

apparently dose-independent, and very problematic in that prediction has been difficult and these problems do not appear until late clinical trials or after a drug enters the market. As their name implies, the nature of idiosyncratic toxicities is not understood. As more knowledge develops, many of these toxicities should fit into the other four contexts of drug toxicity. Although idiosyncratic toxicities are often considered dose-independent, the data to support this view are very limited and the point has been correctly made that very few of these problems occur with drugs administered at daily doses of 20 mg or less [38].

A large fraction of failures due to toxicity problems may be attributable to (uncharacterized) off-target pharmacology or even to previously unrecognized detrimental effects of down-regulation of the main target itself (on-target). The problem in analysis is that in most cases mechanisms are not determined; leads are simply scrapped, and there is no incentive to establish a mechanism, at least in the short term [36].

Identification of metabolism related drug toxicity

Rather than provide an alternative to traditional toxicity studies, toxicogenomics is currently being applied successfully to elucidate molecular-based mechanisms responsible for toxicological observations and also to identify biomarkers for the early detection of toxicity during clinical trials. Adverse drug reactions (ADR) can either be pharmacologic or idiosyncratic. Pharmacologic reactions result from augmentation of the normal/expected pharmacologic response of the drug and are sometimes referred to as an exaggerated response. Idiosyncratic reactions on the other hand, are of unknown etiology and are not manifestations of the expected pharmacologic response. It is currently difficult to accurately predict which drugs will be associated with a significant incidence of idiosyncratic drug reactions [43].

Formation of reactive metabolites and covalent adduct formation of these intermediates with cellular components such as proteins and DNA has been associated with IDR and is used as a screening tool to predict which molecules can potentially cause IDR. However, since not all reactive metabolites covalently bind to cellular components and even when they bind, not all cause IDR, predicting which metabolites will affect endogenous signaling pathways potentially leading to IDR, remains a challenge. In addition, some reactions are species-specific, making it difficult for predictions based on preclinical data to be made [44].

Recently, it has been suggested that genetic factors play a crucial causative role in IDR and studies have shown that drugs associated with IDR also up-regulate protective genes in vivo. Thus polymorphisms in enzymes that catalyze bioactivation reactions, the proteins to which reactive metabolites covalently bind, receptor proteins and protective genes could potentially affect the formation of IDR and account for the individual differences in susceptibility to IDR. Use of genomics technologies to determine gene expression profiles and genotypes therefore has the potential to develop into a more effective screening tool for IDR, as genetically susceptible subsets of the population could be identified [41].

Loss-of-function polymorphisms have the potential to affect pathways not directly related to the predicted mechanism of action of a drug. Recent advances in nuclear receptors and signal transduction fields have increased our knowledge and understanding of the complex biological processes of gene regulation and their impact on normal physiology as well as the pathological outcome of variations in this network. We now know for instance that nuclear receptors, initially named orphan_ receptors because of lack of endogenous ligands, actually control a cascade of biochemical processes and pathways

essential for the normal functioning of organisms. Thus the nuclear receptor, LXR, acts as a cholesterol sensor and helps to maintain lipid homeostasis. CAR, AhR and PXR have the ability to modulate thyroid hormone levels by modulating the expression of UGT enzymes, which are involved in the conjugation of thyroid hormones. A non-steroidal progestin was shown to cause transcriptional induction of microsomal UGTs, which are predominantly responsible for the conjugation and elimination of thyroid hormones in the rat [45,46].

Metabolism and drug design

From toxicological and pharmacological points of view, it is desirable to design a "safer" drug that undergoes predictable metabolic inactivation or even undergoes no metabolism. Several approaches have been used for the design of safer drugs.

Hard drugs

The concept of non-metabolizable drugs or so-called hard drugs design is quite attractive. Not only does it solve the problem of toxicity due to reactive intermediates or active metabolites, but the pharmacokinetics also is simplified because the drugs are excreted primarily through either the bile or kidney. If a drug is excreted mainly by the kidney, the differences in the elimination between animal species and humans will be dependent primarily on the renal function of the corresponding species giving highly predictable pharmacokinetic profiles. A few successful examples of such hard drugs include bisphosphonates and certain ACE inhibitors.

Bisphosphonates are a unique class of drugs. As a class, they are characterized pharmacologically by their ability to inhibit bone resorption, whereas pharmacokinetically, they are classified by their similarity in absorption, distribution and elimination. In the clinic, these drugs are used in patients as antiosteolytic agents for the treatment of a broad range of bone disorders characterized by excessive bone resorption. The discovery of bisphosphonates was based on earlier studies of inorganic pyrophosphate by Fleisch and his coworkers. They found that pyrophosphate bound very strongly to calcium phosphate and inhibited not only the formation of calcium phosphate crystals, but also the crystal dissolution in vitro. However, pyrophosphate exhibited no effect on bone resorption in vivo. This was later explained by the observation that pyrophosphate is hydrolyzed before it reaches the site of bone resorption. These findings led to a search for analogs that would display the activities similar to pyrophosphate, but would also resist enzymatic hydrolysis. It was found that the bisphosphonates, characterized by a P-C-P bond rather than the P-O-P bond of pyrophosphate, fulfilled these criteria. As hard drugs, bisphosphonates are not metabolized in animals or humans, and the only route of elimination is renal excretion. In general, these compounds are very safe with no significant systemic toxicity [47,48].

Similarly, enalaprilat and lisinopril are considered as hard drugs. These two ACE inhibitors undergo very limited metabolism and are exclusively excreted by the kidney. Unlike sulfhydryl-containing ACE inhibitors, such as captopril and its analogs, neither enalaprilat nor lisinopril exhibits significant side effects. The most common side effects accompanying the clinical use of captopril are rashes and taste dysfunction. Similar side effects are observed with penicillamine, which is a sulfhydryl-containing heavy metal antagonist. It is therefore speculated that captopril interacts with endogenous sulfhydryl-containing proteins to form disulfides that may act as haptens, resulting in immunological reactivity, which may be responsible for these side effects. Enalaprilat and lisinopril were designed to avoid these undesirable side effects by removal of the sulfhydryl group [49,50].

Although metabolically inert compounds are highly desirable candidates for drug design, the versatility of the drug-metabolizing enzymes presents quite a challenge to achieve this goal. For example, CYPs are known to catalyze numerous oxidative reactions involving carbon, oxygen, nitrogen, and sulfur atoms in thousands of substrates with diverse structures. In addition, CYPs are unique in that metabolic switchings can occur when the primary metabolic site of a compound is blocked. Thus, considering the broad substrate specificities and the versatilities of CYPs and other drug-metabolizing enzymes, designing drug candidates that are metabolically inert may not always be feasible.

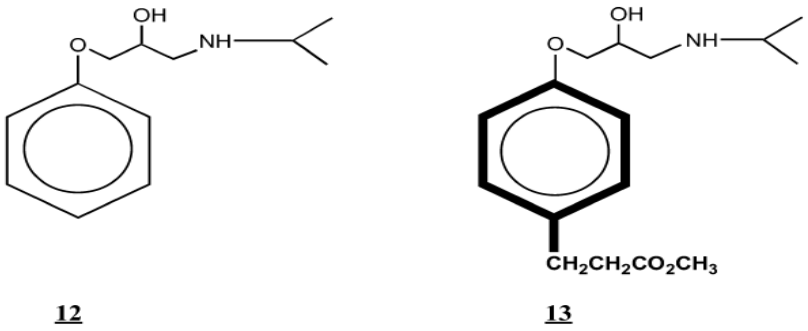
Soft drugs

In contrast to the concept of hard drugs, a soft drug undergoes a predictable and controllable metabolism to nontoxic and inactive metabolites. The main concept of soft drug design is to avoid oxidative metabolism as much as possible and to use hydrolytic enzymes to achieve predictable and controllable drug metabolism. Most oxidative reactions of drugs are mediated by hepatic cytochrome P-450 enzyme systems that are often affected by age, sex, disease, and environmental factors, resulting in complex biotransformation and pharmacokinetic variability. In addition, P-450 oxidative reactions have the potential to form reactive intermediates and active metabolites that can mediate toxicity. These undesirable effects attributed to oxidative metabolism may be circumvented to some extent by incorporating metabolic structural "softness" so that the drug is hydrolyzed to nontoxic components that are rapidly eliminated from the body [51,52].

Although nature has provided numerous examples of soft drugs, esmolol has come to be regarded as the prototypical soft drug that was obtained via rational design. In this case, a methyl propionate was appended to the classical aryloxypropanolamine template associated with ß-adrenergic receptor blockade (Figure 5) in order to program the latter's metabolism along the ubiquitous esterase pathways such that the resulting ß-blocker would possess an ultra-short duration of action. Thus, a methyl 3-arylpropionate system (bolded atoms within Figure 3) represents a useful metabophore already having clinical proof of principle within the molecular context of an aryloxypropanolamine template. This metabophore can be used to program human drug metabolism by esterases [53].

Compound 12 represents the classical aryloxypropanolamine pharmacophore associated with blockade of β-adrenergic receptors. Compound 13 is esmolol, a soft drug version of 12 that has been programmed to have an ultra-short duration of action due to hydrolysis of the methyl ester by the ubiquitous esteases. The methyl 3-arylpropionate (bolded within 13) thus represents a useful metabophore for the associated human esterases [53].

Atracurium, a nondepolarizing muscle relaxant, can be also considered a soft drug. This drug contains quaternary N-functions and ester groups. Atracurium is metabolized in vivo by two nonoxidative processes: a nonenzymatic metabolism by Hofmann-degradation to form a tertiary amine and an alkene, and hydrolysis of the ester groups by esterases. Remifentanil, a novel short-acting m-opioid receptor agonist, may also be considered a soft drug. This drug is a methyl ester and is metabolized extensively by esterases to an inactive acid metabolite, GI-90291, of which over 90% is subsequently recovered in urine. To a much lesser extent, the drug also is metabolized by N-dealkylation to a second metabolite, GI-94219. The major metabolite GI-90291 is approximately 2000- to 4000-fold less potent compared with remifentanil [54,55]. Although both hard and soft drug designs



12 13

Figure 5: Esmolol as the prototypical soft drug.

are of academic interest, there are only a few successful examples in the drug market.

Active metabolites with better pharmacological and toxicological profile

For many years, the process of biotransformation was considered synonymous with the inactivation of pharmacologically active compounds. There is increasing evidence, however, that the metabolites of some drugs are pharmacologically active. Numerous examples of pharmacologically active metabolites being used as a source of new drug candidates exist because these metabolites often are subject to phase II reactions and have better safety profiles.

Perhaps the best known example is acetaminophen, which is an O-deethylated metabolite of phenacetin. Acetaminophen shows superior analgesic activity when compared with phenacetin. The main advantage of acetaminophen over phenacetin is that it does not produce methemoglobinemia and hemolytic anemia. Phenacetin is converted to at least one dozen metabolites by O-deethylation, N-deacetylation, and hydroxylation processes. N-hydroxyphenatidine, a metabolite of phenacetin, has been shown to be responsible for the formation of methemoglobin and hemolysis of red blood cells. Conversely, acetaminophen primarily undergoes glucuronidation and sulfation exclusively and is quite safe clinically at the recommended dose [56].

Although pharmacologically active metabolites are generally formed by phase I oxidative reactions, phase II conjugation reactions also can produce biologically active metabolites. Morphine 6-glucuronide is more potent as a m-opioid receptor agonist than morphine itself. Recent clinical studies in cancer patients given morphine 6-glucuronide indicated that useful analgesic effects are achieved without the side effects of nausea and vomiting that are often associated with morphine. These findings have led to the commercial marketing of morphine 6-glucuronide. Sulfation also produces biologically active metabolites. Minoxidil, a potent vasodilator, is a good example. Studies concerning the action of minoxidil revealed that the therapeutic activities were mediated by its sulfate conjugate [57,58].

In addition to the advantages that active metabolites may have in terms of efficacy with fewer unwanted side effects, active metabolites can also be preferred over the parent drugs for kinetic reasons. Many benzodiazepines form active metabolites with similar pharmacological properties. Oxazepam is the common active metabolite of chlordiazepoxide, halazepam, chlorazepate, and diazepam. Unlike other benzodiazepines, oxazepam undergoes only glucuronidation and has a shorter half-life than any of its precursors. This kinetic advantage has led to the marketing of oxazepam as a short-acting benzodiazepine in the treatment of sleeping disorders [59].

Experimental indicators of the presence and involvement of active metabolites

There are several experimental observations that can be used as indicators of the presence and involvement of active metabolites. Some of them are discussed below:

Apparent disconnect between *in vitro* and *in vivo* pharmacological data: Because of the presence of active metabolites, a greater pharmacological response can be observed that is inconsistent with in vitro biological data. For example, because of the contribution of active metabolites, significant differences might be observed in vivo in the animal model of choice among several compounds of the same chemical class, despite the fact that the compounds exhibit similar in vitro activity and a similar pharmacokinetic profile [6].

Apparent disconnect between pharmacodynamic and pharmacokinetic data: When a compound with a short pharmacokinetic half-life shows unexpectedly prolonged pharmacological action, it can be indicative of the presence of active metabolite(s). For example, the pharmacodynamic half-life of atorvastatin is twofold longer than the pharmacokinetic half-life of the compound because of the contribution of its active metabolites [60].

Higher pharmacological response for compounds given extravascularly versus parenterally: If a parent compound undergoes extensive first-pass metabolism upon oral administration, its bioavailability and consequently its plasma concentration will be lower than the plasma concentration obtained, for example, via an intravenous route. However, the concentration of the metabolites following oral administration will be higher (with respect to intravenous administration). Therefore, under these conditions, there is a strong indication that active metabolites are involved, if a higher pharmacological response is observed after oral administration than after intravenous administration. Of course, this assumes that the first-pass organs are not the site of the pharmacological action. In addition, it might be necessary to adjust the doses to make sure that parent drug concentrations are independent of dose route [19].

Higher or lower pharmacological response in the presence of metabolism modulators: The presence of active metabolites might be inferred if an interaction with concomitantly administered drugs (inhibitors or inducers) or, for example, a genetic polymorphism results in: a decrease of pharmacological response in spite of an increase in the systemic concentration of the parent; an increase in pharmacological

response despite a decrease in the systemic concentration of the parent or either an increase or decrease in pharmacological activity, even when there is no change in the corresponding pharmacokinetic profile of the parent.

In this instance, particularly at the discovery stage, biotransformation of parent can be blocked by using a suitable inhibitory agent, such as 1-aminobenzotriazole, or an antifungal drug, such as clotrimazole. Inhibitory agents will increase parent drug levels, which contribute to increased pharmacological activity, therefore, such studies have to be conducted carefully [19].

Unintended pharmacological responses: If a compound shows an additional pharmacological response in vivo unrelated to or opposite to that expected from its interaction with a proven or established pharmacological target, it might be caused by active metabolites. For example, losartan was developed as an antihypertensive drug through the antagonism of the angiotensin II receptor, mainly by the action of its active carboxylic acid metabolite. However, the observation that the compound also had antiinflammatory and anti-aggregatory properties independent of the angiotensin II receptor antagonism led to the identification of another (aldehyde) active metabolite that was largely responsible for this additional pharmacological action [61].

Approaches used to assess pharmacological activity of metabolites: The presence and impact of active metabolites can be evaluated and confirmed using the following approaches:

In silico screening

Virtual screening can be used to assess the biological activity and binding affinity of active metabolites with commonly used computational techniques, such as ligand-based pharmacophore modeling and structure-based docking, which might have already been developed for the purpose of discovering or optimizing the parent compound. Ligand-based modeling can be built on existing knowledge of the biological activity of the parent molecule and/or other compounds with known activity against the pharmacological target of interest, whereas structure-based docking can utilize the 3D structure of the binding sites of the target (receptor or enzyme) of interest. For example, a search for molecular homology of metabolites of losartan to known anti-inflammatory drugs was used in the identification of an active metabolite with anti-inflammatory and anti-aggregatory properties [61].

In vitro screening

Metabolites can be tested for their biological activity by using targeted screening approach against a particular pharmacological target in high or medium throughput mode. This is particularly useful if classes of compounds within a drug discovery program are known to undergo extensive in vitro metabolism. In this instance, it is possible to generate sufficient quantities of metabolites more conveniently and in a timely fashion by using various biological methods. Their pharmacological activity can be tested either before or after isolation of the metabolites [6].

In vivo activity studies

In vivo pharmacological methods are used to assess and establish the actual contribution of the metabolite to the overall therapeutic outcome. This can be done, for example, by comparing the overall pharmacodynamic and pharmacokinetic profile of the metabolite and the parent compound. The actual contribution of any active metabolite to the overall pharmacological action of a drug can be unequivocally ascertained by dosing the metabolite itself in vivo via an appropriate route of administration. Exceptions to this are metabolites that either need to reach the site of action in the parent form, where they are released via pertinent biotransformation reactions, or metabolites with in vivo dispositions influenced by the presence of the parent. The direct dosing of the metabolite enables one to obtain pharmacokinetic and tissue distribution data for the metabolite. The resulting data can be used to determine whether the metabolite can be developed as a drug in its own right. Using appropriate study design, the data might also aid the assessment of any off-target pharmacological activity [19].

Evolving aspects of drug metabolism and future perspectives

Pharmacogenetics and genomics have profoundly impacted drug metabolism research by providing plausible mechanisms for interindividual variability in drug response and metabolism-related toxicity. They provide tools with which to understand enzyme regulation, identify factors that affect drug exposure, the potential for drug-drug interactions and species differences in drug disposition. Knowledge from these areas can form the scientific basis for designing appropriate clinical studies and data interpretation, which can lead to development of safer and more efficacious drugs [41].

Over time, drug metabolism has become more and more important in pharmaceutical research on drug discovery and development [41,62]. Where drug metabolism traditionally investigated the well-defined aspects of ADME, its focus has shifted towards areas on the genomics and genetics levels, aiding the early discovery or prediction of adverse effects of new drugs [63]. Advances from the past decade in fields such as pharmacogenetics, pharmacogenomics and toxicogenomics have increased our knowledge of the genetics and genomics of drug-metabolizing enzymes (DMEs), resulting – for example – in new insights in induction and inhibition, substrate specificities and polymorphisms of DMEs [64,65].

This information is useful in the development of novel in vitro cell models for the purpose of screening drug candidates for their efficacy and safety because capacity to metabolize chemicals is a prerequisite for such models. For instance, at present, there is an increasing interest in the development of stem-cell-derived models, such as hepatocyte-like cells, and the metabolic competence of such novel models is considered of utmost relevance [66,67].

Conclusion

The numerous examples discussed above demonstrate that biological transformation can be a useful approach for discovering drugs. It is therefore useful to screen drug candidates for possible conversion to active metabolites during the discovery stage. Active metabolites may show superior pharmacology, pharmacokinetics, and safety profiles in comparison to their parent molecules, and the inherent benefits that metabolites often display make their study a worthwhile endeavor. As discussed above, a number of active metabolites of marketed drugs have already been developed as drugs in their own right. Also discussed in this paper are several examples where active metabolites were identified during the discovery phase and either became lead compounds or led to the structural alterations of the chemotype. Consequently, the discovery of drugs through biological transformation can be an integral part of the drug discovery process and it may therefore be beneficial to have a process in place to screen drug candidates in search of active metabolites. There are a number of experimental observations during the preclinical pharmacokinetic and pharmacodynamic evaluations of drug candidates that can trigger the search for conversion of drug

candidates to active metabolites. Approaches such as the rapid bioassay guided method described herein can be used to generate and detect active metabolites. Different biological methods are now available for production of active metabolites. Large-scale synthesis of metabolites by biological methods is now feasible because of the recent advances in biotechnology, particularly in the area of CYP bioreactors and microbial methods. Recent advances in LC/ MS and LC/NMR technologies have also expedited the structural characterization of metabolites with high sensitivity, specificity, and speed. These advances have made the routine identification and evaluation of metabolites a reality.

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