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# Molecular toxicology and the medicinal chemist [1]

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**Abstract**

Drug metabolism has a number of pharmacodynamic and pharmacokinetic consequences which cannot be ignored even at the early stages of drug research. A number of aspects of drug metabolism are thus of interest to medicinal chemists, e.g. prodrug and soft drug design. This mini-review focuses mainly on toxication resulting from reactions of functionalization and conjugation. In the former case, oxidoreductases can reduce xenobiotics to nucleophilic radicals, or oxidize them to electrophilic and oxidizable metabolites. Conjugation reactions also play a role in toxication by generating lipophilic residues (e.g. hybrid triglycerides) or adduct-forming metabolites (e.g. some acylglucuronides), or by interfering with physiological pathways (e.g. Coenzyme A conjugates). Functional moieties undergoing such reactions are known as toxophoric groups. Because they are the biochemical endpoint of several toxication reactions, macromolecular adducts are now of special significance in molecular toxicology. But, as discussed in the conclusion, the substrate specificity of drug-metabolizing enzymes, the many biological factors that influence metabolism, and various repair and removal mechanisms all contribute to decrease toxicological risks and to protect organisms. 

*Keywords:* Reactive metabolites; Toxication; Detoxication; Functionalization; Conjugation; Adducts

## 1. Introduction: drug metabolism and the medicinal chemist

Drug design as practised today is mainly a ligand design aimed at discovering compounds with high affinity towards predefined biological targets. Modern high-throughput techniques have rendered this strategy immensely successful, but they have done nothing, to say the least, to shorten the path leading from a high-affinity ligand to a pharmacokinetically and toxicologically well-behaved drug candidate. To decrease the costly and time-consuming development of active compounds ultimately doomed by hidden pharmacokinetic and toxicological defects, medicinal chemists are beginning to integrate relevant considerations into drug design strategies.

Reactions of biotransformation play a major role in influencing the nature, intensity and duration of wanted and unwanted effects of drugs and drug candidates [1–3]. Such influences can schematically be classified into two categories, as shown in Table 1. A good understanding of drug metabolism is thus an essential component of the medicinal chemist's intellectual assets. In more practical terms, Table 2 summarizes various aspects of drug metabolism of interest to medicinal chemists who, starting from hits or lead compounds, have as their objective to design promising drug candidates with reduced risks of pharmacokinetic or toxicological failure.

The changes in physicochemical properties resulting from metabolic reactions represent a poorly explored field [4]. It is often assumed that metabolism markedly increases water solubility and facilitates urinary excretion, but quantitative data are very scarce indeed. Systematic studies are in progress to assess the influence of metabolic N- and S-oxygenation on the lipophilicity and electronic distribution of model and medicinal compounds [5,6]. Similarly, glucuronidation is generally assumed to generate highly hydrophilic metabolites, but experimental investigations indicate that the decrease in lipophilicity is largely structure dependent [7,8].

Prodrug design is also of relevance in the present context. All too often, inadequate solubility or pharmacokinetic properties lead to a valuable drug candidate being abandoned when a carefully designed prodrug could have prevented failure. It is therefore difficult to make sense of the observation that most papers on prodrug design appear to have been contributed by academic researchers. Certainly the number of potential pro-moieties is immense and perhaps intimidating, but a few simple concepts can orient medicinal chemists [2]. It is suggested that solubility and permeability problems

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Table 1
Major pharmacodynamic and pharmacokinetic consequences of drug metabolism

(A) *Pharmacodynamic influences*, when
- (a) the drug yields one or more metabolites which contribute to its therapeutic effects
- (b) the drug is inactive per se (prodrug) but is transformed into an active metabolite responsible for the therapeutic effects
- (c) the drug yields one or more metabolites which are responsible for unwanted or downright toxic effects (toxication)

(B) *Pharmacokinetic influences*, when
- (a) the rate of metabolism affects the duration and intensity of action of the drug, a general occurrence when inactive metabolites are formed (inactivation)
- (b) the drug induces one or several enzymes mediating its metabolism (auto-induction), resulting in a therapeutic response that changes over days or weeks
- (c) a metabolite acts as inhibitor of one of the metabolic pathways, resulting in complex kinetics
- (d) one or more metabolites have physicochemical properties vastly different from those of the parent compound, for example a very high lipophilicity resulting in tissue accumulation and residue retention

Table 2
Aspects of drug metabolism of major interest to medicinal chemists

- (a) The chemistry and biochemistry of reactions of toxication and detoxication [1–3]
- (b) Changes in physicochemical properties (acidity, basicity, lipophilicity, etc.) resulting from biotransformation [4–8]
- (c) Prodrug and soft drug design [2,9,10]
- (d) Predictions of drug metabolism based on expert systems, quantitative structure–metabolism relationships, and molecular modelling of enzymatic sites [11,12]

of new bioactive compounds should be identified in the very early stages of drug development, and that prodrug design should be initiated rapidly if other chemical attempts to solve these problems result in decreased activity [9].

Toxication resulting from biotransformation is a major issue in the molecular toxicology of xenobiotics [13]. It is also of great significance in drug design [14], and as such constitutes the main argument of this mini-review, as discussed below.

## 2. Toxication resulting from metabolic reactions

### 2.1. General aspects

The relation between metabolism and toxicity must first be seen in a broad context comprising both partners (the drug and its metabolites) and the two types of pharmacodynamic effects they can produce, namely, wanted (therapeutic) and unwanted (unfavourable and toxic) effects. As shown in Fig. 1, both the drug and its metabolites can contribute to wanted and unwanted effects.

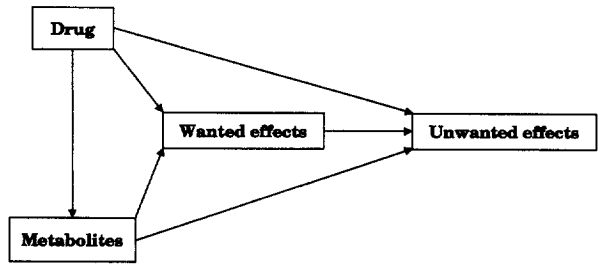


Fig. 1. Metabolism and pharmacodynamic activity. A priori, both the drug and its metabolites can produce wanted and unwanted effects. (Reproduced with permission.)

Biotransformation can conveniently be classified into reactions of functionalization (phase I reactions, which create or modify a functional group), and reactions of conjugation (phase II reactions) which covalently attach an endogenous moiety to a xenobiotic or a metabolite thereof [1–3]. The two classes of reactions can produce toxic metabolites which may elicit various types of cellular damage, but by essentially different mechanisms. Major molecular mechanisms of toxicity include:

- covalent binding to biological macromolecules (reactive intermediates produced by phase I and II reactions);
- oxidative stress produced by oxygen-activating phase I metabolites;
- interference with physiological pathways;
- accumulation of lipophilic residues formed by conjugation reactions.

### 2.2. Metabolic reactions of functionalization

A number of functional groups (the so-called toxophoric groups) are well known for their potential to undergo metabolic toxication. Important toxophoric groups activated by a reaction of oxidation or reduction are listed in Table 3 [1–3,13]. Thus, the cytochrome P450 catalysed activation of ethynyl groups (Fig. 2; R′ = H; reaction **a**) leads to reactive products such as oxirenes (reaction **b**), ketenes (reaction **c**), or intermediates that immediately bind covalently to the catalytic site of the enzyme (reaction **d**). The oxidation of aro-

Table 3
Some potential toxophoric groups activated by reactions of functionalization (A) or conjugation (B)

| | |
|---|---|
| A | • aromatic rings oxidized to epoxides and quinones |
| | • aromatic ethynyl groups oxidized to ketenes |
| | • polyhalogenated alkyl groups reduced to radicals |
| | • primary aromatic amines oxidized to reactive N-oxygenated species and nitrenium ions |
| | • aromatic nitro compounds reduced to reactive N-oxygenated species and nitrenium ions |
| | • thiocarbonyl compounds oxidized to sulfenes |
| | • thiols oxidized to mixed disulfides |
| B | • carboxylic acids forming reactive acylglucuronides |
| | • carboxylic acids yielding Coenzyme A conjugates which can interfere with lipid biochemistry and/or form lipophilic residues |

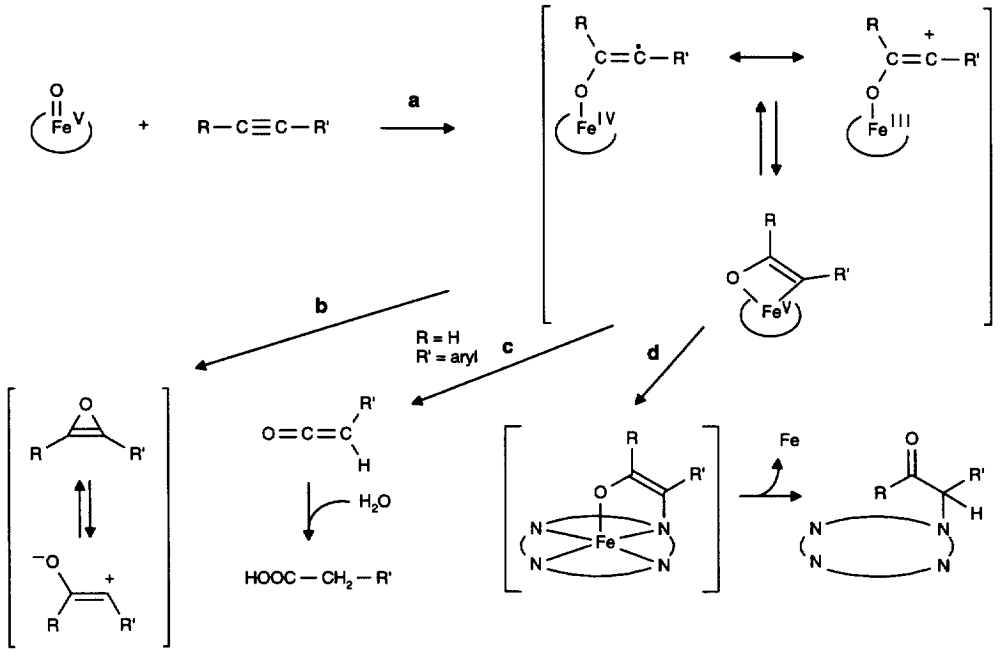


Fig. 2. P450-catalyzed oxidation of acetylenic derivatives (modified from Ref. [1]).

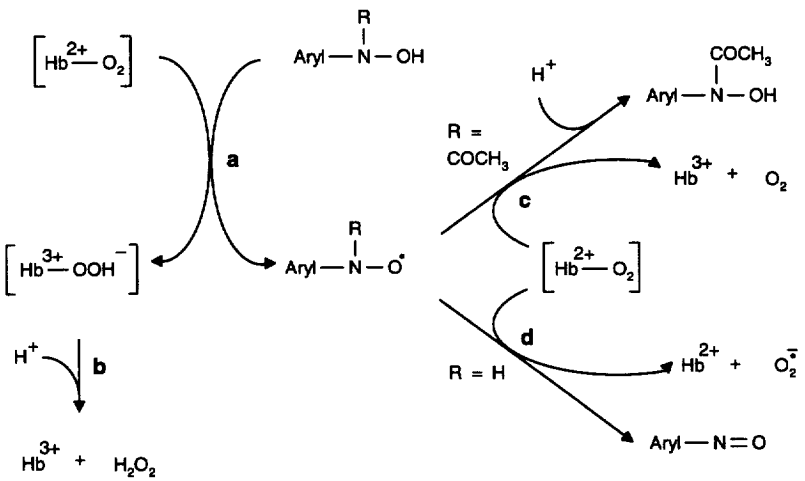


Fig. 3. Mechanisms of interaction of arylhydroxylamines (R=H) and *N*-hydroxy-*N*-arylacetamides (R=acetyl) with oxyhemoglobin (modified from Ref. [1]).

matic amines and the reduction of aromatic nitro compounds yields a variety of reactive metabolites, for example arylhydroxylamines that may interact in various ways with oxyhemoglobin (Fig. 3).

Such examples could be multiplied, and indeed they fill an entire book [1]. Of significance in the present context are the common characteristics of such toxication reactions. As represented in Fig. 4, nucleophilic radicals formed by reduction and electrophilic metabolites formed by oxidation can bind covalently to biomacromolecules. They can also reduce molecular oxygen, the first step in oxidative stress.

### 2.3. Metabolic reactions of conjugation

As a rule, reactions of conjugation involve the coupling of an endogenous moiety (the so-called endocon) to a substrate,

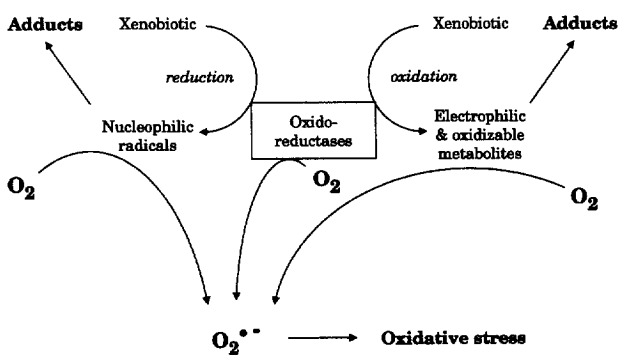


Fig. 4. A general scheme showing the interconnected roles of oxidoreductases in the initiation of oxidative stress and the toxication of xenobiotics (modified from Ref. [1]).

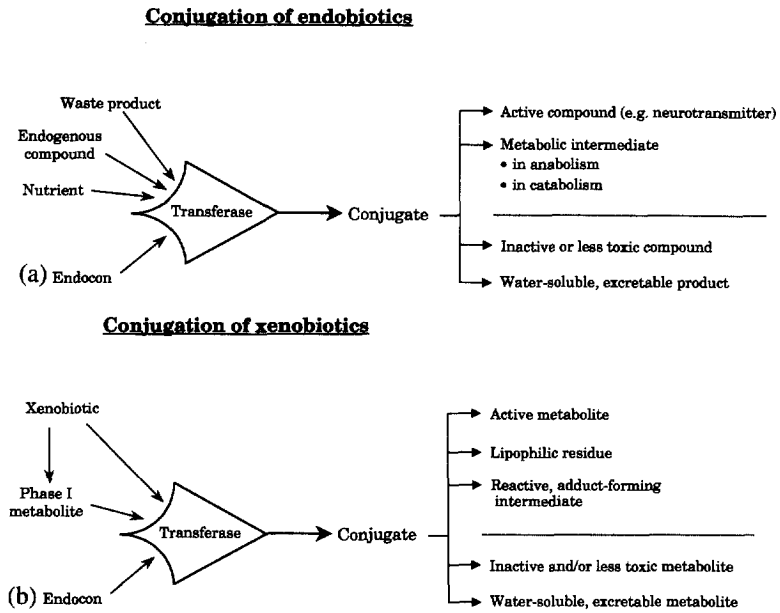


Fig. 5. Schematic representation of conjugation reactions and their consequences: (a) conjugation of endobiotics; (b) conjugation of xenobiotics. (Reproduced with permission.)

as mediated by an enzyme known as transferase. A few non-enzymatic conjugations are known (e.g. formation of Schiff's bases and some glutathione conjugations) [2]. Reactions of conjugation are all important in the metabolism of endogenous compounds, waste products and nutrients, with a variety of possible consequences, as shown in Fig. 5(a). They are also of major significance in the metabolism of xenobiotics and their phase I metabolites, with comparable but not identical consequences (Fig. 5(b)).

Xenobiotic carboxylic acids have received much interest since some of these compounds may yield reactive acylglucuronides that bind covalently to proteins by an Amadori reaction (Table 3). Also of interest is the fact that a few reactions of conjugation produce metabolites of high lipophilicity which tend to accumulate as tissular residues. The substrates of such reactions are xenobiotics that undergo esterification by fatty acids, or xenobiotic carboxylic acids which form hybrid triglycerides or cholesteryl esters. Some anti-inflammatory arylpropionic acids (e.g. ibuprofen) are a case in point [13,15].

## 3. Conclusion: beyond the chemical level

The formation of macromolecular adducts from reactive metabolites or metabolic intermediates has been mentioned repeatedly above. Some characteristics of macromolecular adducts, and how and why they may elicit toxicity, are summarized in Table 4.

At this stage of the argument, it might be concluded that potential toxophoric groups should be avoided at all costs by medicinal chemists. This is an unsustainable position for the reasons discussed below, and one just needs to count the

Table 4
Macromolecular adducts as a class of toxic metabolites

- Their *formation* may be:
  - non-enzymatic
  - enzymatic
  - post-enzymatic
- The *covalent bond* may be:
  - strong (C–N, C–O, C–S)
  - of medium energy (S–S)
- The *target macromolecule* may be:
  - soluble
  - membrane-bound
- The *fate of macromolecular adducts* is largely unknown
- Macromolecular adducts may be *toxic due to*, e.g.:
  - loss of the macromolecule's original functions
  - antigenic activity
  - toxic breakdown products

number of marketed drugs bearing a carboxylic group to suspect that there is much more to molecular toxicology than the mere presence or absence of a toxophoric group.

There are at least three main reasons for this state of affairs. First, the presence of a metabolically labile group is a necessary but not sufficient condition for a xenobiotic to undergo biotransformation. The molecule in its entirety must be a substrate of the metabolic reaction and must thus fulfil a number of structural conditions such as size, shape, lipophilicity and electronic properties, as revealed by structure–metabolism relationships.

The second reason is found in the many biological factors that can markedly influence the substrate specificity, rate and capacity of a metabolic reaction. Such factors include the animal species, phenotypes, sex, tissue, age, and external influences (diet, inducers or inhibitors).

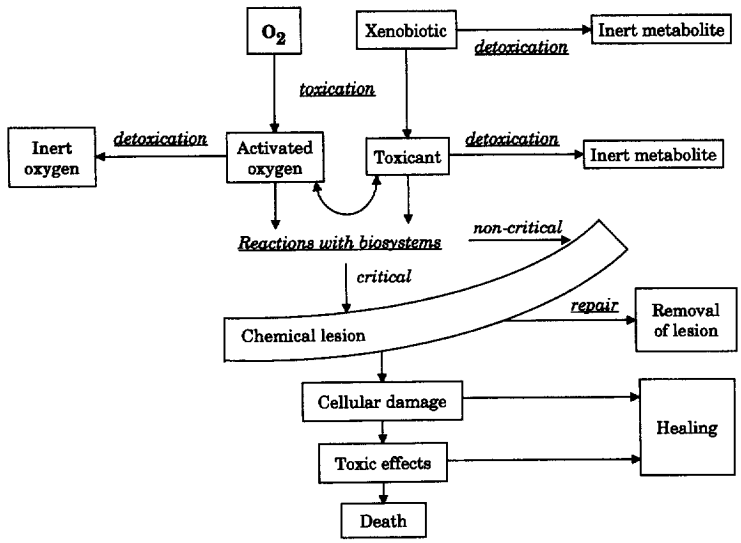


Fig. 6. General scheme placing reactions of toxication and detoxication in a broader biological context. Damaging and protective events are represented by vertical and horizontal arrows, respectively. (Reproduced with permission.)

The third reason emerges when one considers metabolic reactions in their biological context (Fig. 6). Indeed, the extent of a reaction of toxication for a given substrate will depend on the occurrence of competitive reactions of detoxication, while its toxicological impact at the level of the organism will be influenced qualitatively and quantitatively by many known and unknown events that may be detrimental (e.g. damage amplification) or protective (e.g. repair mechanisms, immunological removal).

Mastering a comprehensive knowledge of xenobiotic metabolism and its broad biological context is beyond any medicinal chemist, biologist or pharmacologist. Only teams of experts backed by extensive databases and expert systems can hope to offer fair previsions, but the last word will always remain with experiment.

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