17

Drug Interactions

17.1 Introduction

When two or more drugs are used together, the pharmacological result is not necessarily the sum of the effects obtainable from the drugs used individually in the same doses. This is because one drug may affect the action of another. This is termed 'drug interaction'.

With thousands of drugs available, the statistical probability of interactions when drugs are used in combination is high. There are many mechanisms of drug interaction. Some are chemical, some are biochemical, and some are physiological/pharmacological. Many of the most easily understood drug interactions are mediated by drug-induced changes in drug absorption, distribution, metabolism and excretion. Certain drug interactions are of great clinical importance, usually involving a drug with a narrow therapeutic window, while others are no more than interesting pharmacological curiosities.

Sometimes a distinction is made between drug–drug interactions, drug–food (e.g. grapefruit juice) interactions, and drug–chemical (e.g. alcohol) interactions. A widespread example of a drug–chemical interaction is illustrated by the fact that pharmaceutical oral syrup preparations of most amine drugs produce an ugly precipitate with the tannin content of the beverage when, with good intent, these syrups are diluted with tea (Curry *et al*., 1991). This chapter considers pharmacological, pharmacokinetic and pharmaceutical interactions, including examples of drug–drug, drug–food, and drug–chemical interactions. However, the influence on food *per se* on drug absorption in particular was considered in Chapter 9.

17.2 Terminology

When two (or more) interacting drugs have the same effect, for example central nervous system (CNS) depressant drugs and ethanol, the interaction is described as *homergic*. More often, only one of the drugs produces the effect being studied, although each drug in the combination may have an effect that is modified. This can be described as a *heterergic* interaction. Much discussion occurs in regard to whether drug interactions should be described under the headings of *antagonism*, *addition* (*or summation*), *potentiation* or *synergism*. Additionally, the use of the terms *supra-additive* and *infra-additive* has been proposed.

Antagonism is when one drug reduces the effect of another. This is clear for a heterergic inhibition interaction when the resultant effect is less than that recorded before the interaction. However, in a homergic interaction, the two doses together will generally produce an effect greater than that produced from either of the individual doses on its own. But if the new effect is less than that predicted from sum of the two individual contributions, then this is classified as antagonism (Section 17.6.4).

According to its Greek origins, the word '*synergism*' merely means 'working together', so it could be used to describe any interaction, but it has evolved in meaning to be synonymous with potentiation. Summation

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and addition also appear to be synonymous. If the effects of two drugs in a homergic interaction add together and lead to the effect expected by simple arithmetical addition, then summation and addition are appropriate terms. However, some writers differentiate between these two terms, on the basis of summation being used exclusively for a combined effect at the centre of the sigmoid log dose–response curve. Summation sometimes becomes a special form of addition in this argument, and other additive effects can be described as supra-additive if they are below the inflection of the curve, and infra-additive if they are above. Some authors stress the *mutual* nature of drug interactions, in that an effect of one drug on another is often reciprocated by an effect of the other on the one.

Life is obviously simpler with a heterergic interaction involving enhanced effect. If the effect of the one drug causing the effect is increased, then potentiation is the appropriate description. Fortunately, most interactions are heterergic. The drug affected in an interaction is sometimes called the 'victim' drug.

17.3 Time action considerations

It is easy to consider an interaction in relatively simple terms when thinking of it as occurring *in vitro* in a pharmacologist's organ bath, with an effect occurring immediately the drug is added to the bath and continuing until the bath is washed out. However, *in vivo* effects have onset, peak intensity and duration characteristics (Chapters 4, 13 and 14). These are functions of the drug concentrations in blood and tissues, and these concentrations are in turn controlled by the balance of absorption, tissue distribution, metabolism and excretion. This can introduce added terminology issues. In the introduction to Chapter 4 it was noted that modifications of drug absorption, metabolism and excretion could all affect a drug response differently. For example, an increase in the rate of absorption with no change in the degree of absorption, will lead to an earlier onset of effect and a greater and earlier maximum effect, but a shorter duration of effect. Slightly different, but completely analogous considerations are relevant to fluctuating concentrations during long-term treatment. Thus by reference to onset of effect and intensity of effect, an increased rate of absorption might be termed a potentiation, but the shorter duration of effect would be an antagonism. Given this set of circumstances, it is best to discuss the results of an interaction in terms of measured phenomena and pharmacological mechanisms, and be careful not to be too dogmatic in use of terminology. It is now recognized that every interaction of importance should be evaluated, where possible, in terms of the effect of the interacting drug on the absorption rate, bioavailability, time and height of maximum concentration, half-life, area under the curve, apparent volume of distribution, clearance, maximum and minimum concentrations in the fluctuating pattern of drug concentrations seen during long-term dosing, and in changes in the effect of the victim drug.

For the most part, the results of interaction studies tend to be expressed in terms of changes in the descriptive pharmacokinetic parameters listed in the previous paragraph, such as half-life and bioavailability. However, the more advanced clearance concepts discussed in Chapter 7 make it possible to perform calculations that provide greater insight into the underlying mechanism than is possible with descriptive pharmacokinetics alone. For example, propranolol has been shown to lower the hepatic clearance of lidocaine. This occurs because lidocaine has a high extraction ratio, and its hepatic clearance is very much affected by liver blood flow. Propranolol reduces cardiac output, which in turn reduces hepatic blood flow, reducing the clearance of lidocaine. This translates into a longer half-life of the lidocaine. Similarly, enzyme induction interactions are most important with low extraction ratio drugs, and protein binding interactions commonly involve drugs that demonstrate the saturability of protein binding within the clinical range of concentrations.

Similar approaches to the above permitted a perceptive study of the interaction of theophylline and enoxacin. Enoxacin inhibits theophylline metabolism in the liver, leading to nausea as an adverse effect of the increased theophylline concentrations. In the absence of enoxacin, the half-life of theophylline is

approximately 9 hours, so steady-state conditions should be established in approximately 2 days in a long-term dosing regimen (Section 4.2.6). However, under the influence of enoxacin, 4 days were required in order to achieve the new steady-state, consistent with the half-life having doubled. The experimental design permitted calculation of the new half-life as 22 hours, consistent with a reduction in unbound clearance by 56%. Although the raised theophylline concentrations were shown in the experimental protocol to take place within the clinically accepted range, it was clear that the same interaction occurring in patients would cause the plasma concentrations of theophylline to rise above the upper limit of this range and hence result in nausea.

17.4 Interactions involving drug distribution and metabolism

As already stated, a great many interactions can be explained in terms of effects on drug absorption, plasma protein binding, metabolism and excretion. Others can be explained in terms of classical theories of drug–receptor interaction (Chapter 13). Yet others are at present unexplained. The three most important mechanisms involving changes in drug disposition and fate are associated with drug metabolism, both induction and inhibition, and with binding of drugs to plasma protein and sequestration in tissues.

17.4.1 Enzyme induction

Induction of drug metabolizing enzymes is probably the longest established of the three major pharmacokinetic mechanisms of drug interaction. This phenomenon was discovered when the influence of phenobarbital on the effects and plasma concentrations of anticoagulants, in particular, was first observed. Enhanced enzyme activity can be detected in animals after just two or three doses of phenobarbital. Affected drugs typically show shortened half-life values, reduced areas under the curve, lower steady-state concentrations on multiple dosing, increased metabolite formation, and reduced effects. In addition to phenobarbital and virtually all of the other barbiturates, enzyme inducers include 3-methylcholanthrene (a laboratory model), rifampicin (rifampin), carbamazepine, valproic acid and phenytoin, among many other compounds, and most of them induce microsomal P-450 activity (Table 3.1). With the passage of time the major clinical focus has changed from phenobarbital to rifampicin in this context.

17.4.2 Enzyme inhibition

Although mutual inhibition of metabolism by various substrates for the P-450 system had been known for many years previously, it was the observation in the early 1980s that cimetidine caused major changes in the responses to a wide variety of drugs that focussed attention on this mechanism of drug interactions. Other H_2-blocking drugs were found to have lesser abilities to inhibit the P-450 system, and this distinction was soon exploited in the marketing of ranitidine, in particular, as a relatively non-interacting drug. The list of drugs that inhibit CYP3A4 and/or CYP2D6 in particular, now includes ketoconazole, fluoxetine, fluvoxamine, quinidine, theophylline and erythromycin (Table 3.1), with several examples causing interactions that affect prescribing practices.

17.4.3 Plasma protein binding and tissue uptake

Competition between different compounds for the same binding sites on albumin and other proteins can lead to the incumbent drugs being displaced. In a balanced therapeutic situation, this in turn can lead to an increased amount of displaced drug being available in plasma water and in other body fluids, for

pharmacological action. However, tissue uptake is also affected by concentrations in plasma water, so the overall effect may be a transient increased effect, but this is likely to be rapidly compensated by increased localization in tissues, and, sometimes, by enhanced elimination.

17.4.4 Mechanisms of enzyme induction

The mechanism of enzyme induction has received considerable attention. The mechanism of microsomal oxidation of drugs was considered in Chapter 3. When enzyme induction occurs, the liver increases in weight and in protein content, the smooth membranes of the endoplasmic reticulum (microsomes) increase in quantity and in content of protein, RNA and phospholipid (especially when phenobarbital is the inducing agent) and the microsomal messenger RNA and the rate of microsomal incorporation of amino acids from transfer RNA is increased (especially when 3-methylcholanthrene is the inducing agent). This activation is reduced by ethionine, puromycin and actinomycin D, all of which inhibit protein synthesis. In laboratory animals, enzyme induction can be demonstrated as a decrease in hexobarbital sleeping time, and an increase in biphenyl 4-hydroxylation, in ethylmorphine *N*-desmethylation, and in *p*-nitrobenzoate reductase activity. Hexobarbital sleeping times have been widely used to assess undifferentiated P-450 activity. The liver concentrations of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome *c* reductase, cytochromes P-450, and cytochromes b_5 increase. However, there is no effect on glucose-6-phosphatase dehydrogenase, 6-phosphogluconate dehydrogenase or glucose-6 phosphatase. This was taken as evidence that the mechanism of enzyme induction involves changes in the enzymes specifically present as part of the drug metabolizing microsomal system, rather than changes in rate of NADPH generation. It appears that enzyme induction involves an increase in the rate of synthesis rather than a decrease in the rate of destruction of drug metabolizing enzymes. As the enzymes are relatively non-specific, induction by one substrate often leads to other substrates being more rapidly metabolized, thus providing a means of one drug affecting the response of another. Microsomal enzymes are constantly undergoing synthesis and degradation, with half-lives of turnover varying from hours to days, so induction results in increased amount of enzyme and, consequently, increased V_{max} values for the metabolic reactions. The full effect of the inducing agent may be quite rapid, or delayed, depending on the turnover rate of the enzyme isoform involved.

In the case of CYP3A4 induction, the process is believed to involve ligand binding to the nuclear pregnane X receptor (PXR). The activated PXR complex then forms a heterodimer with the retinoid X receptor (RXR), which binds to the XREM region of the CYP3A4 gene. XREM is a regulatory region of the CYP3A4 gene, and binding causes a co-operative interaction with proximal promoter regions of the gene, resulting in increased transcription and expression of CYP3A4 mRNA and protein. Induction of CYP2D6 involves the CAR/RXR nuclear receptor heterodimer. This process can be viewed as a defence mechanism of the body; a method of preserving homeostasis, that involves the body recognizing the ligand (in the current context, a drug) as an unwanted chemical that might be toxic, and automatically responding by increasing the amount of enzyme that can help rid the body of that drug.

17.5 Extent of drug interactions

The common feature of all of the compounds shown to be potent enzyme inducers is that they are highly lipophilic and are substrates for the enzymes concerned. It seems that important enzyme inducing agents must persist in the extracellular fluid in sufficient quantities for sufficient time, so they tend to be compounds with relatively long half-lives (generally resulting from relatively slow metabolism and limited renal excretion as unchanged drug). In addition, they must bind to P-450 as substrates without destroying the enzyme. This is illustrated well by the barbiturates. While barbital, phenobarbital, allobarbital, pentobarbital, quinalbarbital and thiopental are all inducing agents, phenobarbital is the most potent, as barbital is

relatively polar and can be excreted most easily without prior metabolism, allobarbital binds to P-450 but destroys the enzyme, pentobarbital and quinalbarbital are intermediate in potency and thiopental is weak because of its high degree of tissue localization reducing the amount available in extracellular fluid at any time.

The list of compounds affected by enzyme inducing agents is long, potentially including any compound that is a substrate for the enzymes. However, a change in the rate of metabolism does not necessarily bring about a change in drug response and any list of compounds for which an important change in response is likely, consequent on enzyme induction, is relatively short. Historically, the focus was on anticoagulants and anticonvulsants, and also chlorpromazine, for which enzyme induction has been shown to change autonomic nervous system responses without changing antipsychotic effects. Antipyrine has been used extensively in demonstrating enzyme induction. The following appear to be the conditions for maximal influence of enzyme induction, all of which are satisfied by the drugs mentioned here:

- The affected compound is slowly metabolized, often with dose-dependent kinetics.
- Termination of the effect is dependent on metabolism by microsomal enzymes, rather than on redistribution, excretion, or physiological effects.
- The effect is closely related to the concentration of the drug in the body.
- The compound affected has a low therapeutic index.

17.6 Key examples

In this section, four examples illustrating the most important pharmacokinetic mechanisms of drug interaction are described. The results of research into all four examples have, to a smaller or larger extent, affected the practice of medicine.

17.6.1 Warfarin

This coumarin anticoagulant, which can be considered as the quintessential victim drug, is mentioned in almost every drug interaction discussion because:

- It is a weak acid which is bound to plasma albumin, and is displaced from binding sites by other weak acids such as phenylbutazone; this interacting drug, obsolete as a therapeutic agent, has been a key compound in aiding our understanding of drug interaction mechanisms.
- It has a low volume of distribution and capacity-limited hepatic clearance.
- It is a substrate for the non-specific liver microsomal drug oxidase system, so its metabolism is accelerated by enzyme inducing agents such as phenobarbital, and its metabolism may be inhibited by competing substrates such as ketoconazole and imipramine.
- It has a low therapeutic index so small influences caused by a wide variety of drugs can assume great importance.
- It exerts its effect on a delicately balanced physiological control mechanism, thus maximizing the opportunity for clinical demonstration of any interaction.

However, it should be appreciated that while the effect of warfarin is closely related to the concentration of warfarin in plasma, or even more closely related to its concentration in plasma water, there is a 2–3 day lag between a change in concentration and a change in effect, except in situations of acute overdosage (Chapter 14).

The classical warfarin interactions are with phenobarbital and with various anti-inflammatory drugs, such as phenylbutazone. Table 17.1 shows a summary of the results of these interactions. It is of interest because of the fact that both the protein binding and the enzyme induction interactions lead to a reduction in total warfarin in plasma, but one of these interactions leads to an increased response and the other leads to a decreased response. A barbiturate added to a prevailing warfarin regimen will lead to accelerated warfarin metabolism. The result will be lower concentrations of warfarin in plasma, both free in plasma water and bound to protein, but with virtually the same proportion bound. This will lead to a slow change in warfarin concentrations and so to a reduced overall response. In contrast, addition of a displacing agent such as salicylate or phenylbutazone to a warfarin regimen will cause an immediate reduction in the amount and proportion of warfarin bound and a corresponding increase in unbound warfarin, with the total at first unchanged. If dosing with the displacing drug is continued then the total concentration of warfarin in plasma decreases as increased amounts of unbound drug are taken up into tissues (including, possibly liver, where the site of action of warfarin lies), until tissue/plasma concentrations equilibrate. The new equilibrium will then be: (i) increased tissue warfarin, (ii) decreased total and bound warfarin in plasma, (iii) increased unbound warfarin in plasma water, and (iv) consequent enhanced effects.

Table 17.1 Drug interactions and warfarin

Measure	Type of interaction	
	Protein binding	Enzyme induction
Total warfarin	Reduced	Reduced
Bound warfarin	Reduced	Reduced
Unbound warfarin	Increased	Reduced
Effect (short-term)	None	None
Effect (long-term)	Haemorrhage	Clotting[a]

[a] If barbiturate added to warfarin regimen.

The increased unbound fraction, *f*u, may result in increased metabolism of a drug such as warfarin, however the interaction with phenylbutazone is more complex than a simple displacement interaction. Warfarin is marketed as the racemate and the *S*-isomer, which is some three to four times more potent than the *R*-isomer, is metabolized to 7-hydroxywarfarin whilst the *R*-isomer is metabolized to warfarin alcohol. Normally the *S*-isomer is more rapidly metabolized than the *R*-, but in the presence of phenylbutazone the production of 7-hydroxywarfarin is reduced. In fact, phenylbutazone inhibits the clearance of *S*-warfarin but increases the clearance of the *R*-isomer so that the 'total' remains more or less the same (Figure 17.1).

In reality, the greatest clinical danger with the enzyme induction interaction has been when a barbiturate was withdrawn from an established regimen of barbiturate plus warfarin, such as occurred when patients admitted to hospital for anticoagulation therapy, were also given sleeping tablets, and became stabilized on the combination. On returning home, the patients no longer required the hypnotic drugs and their enzymes reverted to lower activity, leading to the warfarin then being metabolized more slowly. The increased concentrations of warfarin could lead to haemorrhage. Today's hypnotics do not affect anticoagulation therapy in the same way, but this example provides a lesson of lasting significance in the history of, and potential for, drug interactions.

Non-barbiturate drugs which can affect anticoagulants by virtue of their enzyme inducing properties include rifampicin, haloperidol and griseofluvin, as well as several obsolete compounds mentioned in older literature. Non-anticoagulant drugs affected by barbiturates are many in number, e.g. phenytoin. Other drugs which affect anticoagulants by protein binding effects include a variety of older drugs, plus aspirin.

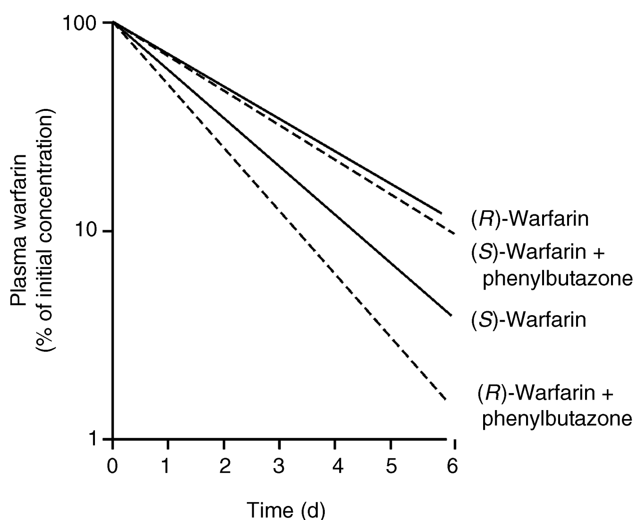

Figure 17.1 Schematic diagram of changes in plasma concentrations of warfarin enantiomers after single doses before and after phenylbutazone, 100 mg three times a day for 10 days. (Redrawn after Lewis *et al.*, 1974.)

17.6.2 Cimetidine and ketoconazole

These drugs are ideal examples of enzyme inhibitors. Although it has long been known that there is considerable potential for drug interactions caused by inhibition of isoforms of the P-450 system, and, now, grapefruit juice is known to have similar properties, it was only in the late 1970s and early 1980s, when cimetidine was introduced as the first of a series of highly innovative drugs that specifically inhibits gastrointestinal acid secretion (the H_2-blocking drugs), that clinically-important interactions related to this mechanism started to emerge. Cimetidine was found to inhibit the metabolism of a wide range of P-450 substrates, including theophylline, diazepam, tricyclic antidepressants, and metoprolol. Other inhibitors have since been found. These have included the unrelated antifungal drug, ketoconazole. One consequence of the observations with cimetidine was a vigorous search among its analogues and successors, especially ranitidine, for similar properties. These, basically, were not found, leading to vigorous competitive marketing, on the basis of these differences, among the makers of H_2-blocking drugs. As knowledge of the isoforms of P-450 emerged, it was seen that cimetidine, and later ketoconazole, particularly inhibited CYP3A4, the isoform especially responsible for first-pass metabolism, so that parent drugs were found to have enhanced bioavailability under the influence of cimetidine and ketoconazole, while the metabolic products of first-pass metabolism had reduced plasma concentrations.

One of the cimetidine interaction studies of particular interest involved amitriptyline, the tricyclic antidepressant at one time commonly co-prescribed with cimetidine in the hope of effectively treating both ends of the 'gut–brain' axis responsible, in part, for gastrointestinal ulcers. The first-pass desmethylation of amitriptyline to nortriptyline was inhibited in the interaction. Plasma amitriptyline concentrations were higher, and plasma nortriptyline concentrations were lower, as Figure 17.2 shows. Because this work was conducted with single doses of amitriptyline in healthy volunteers, it was possible to measure changes in blood pressure and heart rate, as well as performance in digit symbol substitution tests of brain function, in relation to plasma drug concentrations. The effects were greater when the amitriptyline concentrations were higher. However, this work also revealed that higher plasma amitriptyline concentrations resulted in more intense reductions in both heart rate and blood pressure, which was not, at first, explicable in terms of the known central and autonomic nervous system effects of the drug, which were expected to reduce blood

pressure, but raise pulse rate by both an anticholinergic affect and a reflex response to the reduction in blood pressure. It was only when dose-dependent pharmacological effects of amitriptyline were studied in a separate experiment that it was found, in healthy volunteers, and therefore in newly diagnosed patients, that the drug caused heart block, with reduction in both blood pressure and heart rate at the same time, at least partially explaining the known but poorly understood risk of acute cardiac complications of amitriptyline in naïve subjects. This risk was made worse by cimetidine but not by ranitidine. This study illustrates the extreme need for integrated pharmacokinetic/pharmacological studies.

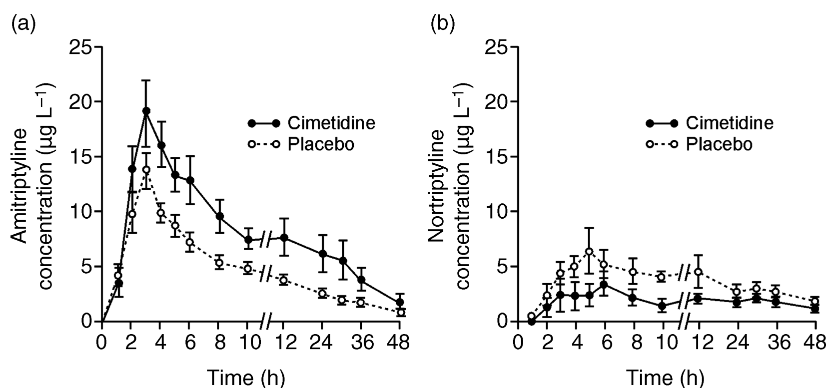

Figure 17.2 Interaction of cimetidine and amitryptiline. (a) Co-administration of cimetidine increased amitriptyline concentrations compared with placebo, whereas (b) the corresponding concentrations of the metabolite, nortriptyline were reduced. Note that the area under the curve for amitriptyline was increased, and that for nortriptyline was reduced, consistent with inhibition of CYP3A4 during presystemic metabolism of the oral doses. (Redrawn from Curry *et al.*, 1985.)

Ketoconazole, like the effect of the cimetidine on amitriptyline, inhibits the first-pass conversion of terfenadine to its metabolite fexofenadine by CYP3A4 (Figure 17.3). The raised terfenadine concentrations in plasma are sufficient to cause a severe toxic effect of terfenadine-induced prolongation of the QT interval

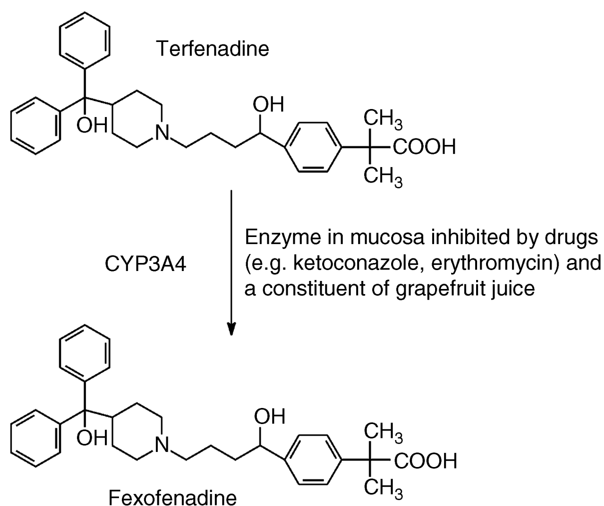

Figure 17.3 Inhibition of terfenadine prevents conversion to the antihistamine fexofenadine.

in the electrocardiogram. Both the C_{max} and the half-life of terfenadine are increased, greatly increasing the patient exposure to the drug (Table 17.2).

Table 17.2 Effect of ketoconazole on terfenadine and fexofenadine kinetics

Treatment	Pharmacokinetic parameters		
Terfenadine alone	$C_{max} < 10\,ng\,mL^{-1}$	Metabolite half-life	13.2 h
		Metabolite C_{max}	471 ng mL^{-1}
		Metabolite t_{max}	2.8 h
Terfenadine + ketoconazole	C_{max} 27 ng mL^{-1}	Metabolite half-life	35.7 h
		Metabolite C_{max}	134 ng mL^{-1}
		Metabolite t_{max}	7.0 h

The pharmacokinetics of the metabolite, fexofenadine, are also affected, partly because of its slower formation from terfenadine, but also because fexofenadine itself is a substrate for the affected P-450 isoforms. Fexofenadine formed from terfenadine experienced a delay in t_{max}, an increase in C_{max}, and a longer $t_{1/2}$. The interaction with terfenadine was deemed to be so significant that it had two dramatic consequences: (i) terfenadine was withdrawn from the market, and replaced with fexofenadine, which, although sensitive to the effect of ketoconazole, has much lesser potential, if any, to cause prolongation of the QT interval; and (ii) testing of the QT interval has become routine in the safety testing of new drugs, both in preclinical studies and in the Phase I and Phase II human studies, and in Phase III pivotal clinical trials, creating a whole new testing culture and industry at the same time.

Ketoconazole also inhibits first-pass metabolism of ciclosporin (cyclosporine), enhancing its effect.

17.6.3 Digoxin and quinidine

The interaction of quinidine with digoxin illustrates two important issues: (i) inhibition of tissue uptake, and (ii) that drugs that are routinely co-prescribed may indeed interact. The clinical problem was that quinidine elevates plasma concentrations of digoxin two- to threefold, inducing arrhythmias as an adverse effect of the digoxin, in patients being treated for arrhythmias by the two drugs in combination. Quinidine is a potent inhibitor of P-glycoprotein (P-gp; Section 2.3.1.2) and in cultured cell lines containing P-pg was shown to inhibit transport of digoxin by 57%. Mice in which the gene expressing P-gp was disrupted ('knock-out', KO) were compared with wild-type mice. The quinidine doses were reduced in the study in KO mice to allow for impaired quinidine transport, so that exposure in both groups was the same. Quinidine increased digoxin plasma concentrations by 73% in wild-type mice, but only 19.5% in KO mice. Quinidine also increased digoxin concentrations in the brains of wild-type mice, by 73.2%, but decreased brain concentrations in KO mice by 30.7%. It was concluded that quinidine inhibits the transport of digoxin, both *in vitro* and *in vivo*, including at the blood–brain barrier, decreasing the apparent volume of distribution of digoxin, and raising concentrations in plasma in the wild-type mice. The effect on brain concentrations in the KO mice was not fully explained.

Rifampicin also has effects on digoxin, not through enzyme induction, but by a mechanism involving induction of P-gp. Plasma concentrations of digoxin are reduced, assessed by *AUC* measurements, with oral doses being affected more than i.v. doses. This has been investigated using i.v. and oral doses, with and without rifampicin, in human volunteers who underwent duodenal biopsies for P-pg assessment. It was found

that rifampicin increased intestinal P-gp threefold, with no effects on renal clearance or the half-life of digoxin. Thus the interaction is induction of digoxin efflux in intestinal tissue, reducing the bioavailability. A similar mechanism may apply to St John's wort, which reduced digoxin concentrations by approximately 20% over 16 days when 900 mg day^{-1} of hypericum (St John's wort) was co-administered. Hypericum is also a CYP3A4 inducer, with potentially catastrophic effects on ciclosporin in particular, because of its popularity as an over-the-counter treatment for the depression which is common in post-transplant patients.

17.6.4 Alcohol and other depressants (notably barbiturates)

This is the homergic interaction par excellence with almost infinite possibilities for discussion of addition, potentiation, etc. (see earlier). A number of facts are pertinent:

- Ethanol can accelerate or retard the absorption of other drugs.
- Other drugs can accelerate or retard the absorption of alcohol.
- Alcohol can accelerate or retard the metabolism of other drugs.
- Other drugs can accelerate or retard the metabolism of alcohol.

Interactions with alcohol often lead to concentration changes in plasma and appear to occur to some extent with alcohol and barbiturates, other depressant drugs, such as phenothiazines and antihistamines, phenytoin, and some antidepressants. There is a significant interaction with benzodiazepine drugs, but any changes resulting from mutual influences on the plasma concentrations of benzodiazepines and ethanol appear to be minimal, although the pharmacological consequences of this interaction are considerable. In regard to changes in plasma concentrations the arguments of earlier sections must be borne in mind, in that different conclusions may be drawn depending on whether the rate of rise or fall of the concentration is measured or whether the area under the curve is measured.

In addition to the above:

- Certain doses of drugs are sub-threshold (Chapter 13) and a dose may be sub-threshold for sedation but above threshold for, say, anticonvulsant therapy.
- Sub-threshold doses of two depressant drugs can combine to form an above threshold response – which will be an effect of unexpected magnitude.

The above considerations combined lead to the conclusion that an all-embracing description of the combination of alcohol and other depressant drugs is inappropriate, and precise terms such as addition, potentiation or antagonism should only be applied to precisely defined circumstances. Some other statements of fact reinforce this opinion:

- As already stated, diazepam and alcohol exert relatively small mutual effects on their various plasma concentrations (very little or no pharmacokinetic interaction).
- Sub-threshold doses of diazepam and alcohol cause a recordable effect when combined (potentiation or synergy, or addition within the limits of a common dose–response relation).
- Chlorpromazine increased arterial alcohol concentrations (potentiation of absorption).
- Chlorpromazine enhanced the effects of alcohol (potentiation, or addition within the limits of a common dose–response relationship).
- Phenobarbital and alcohol given in combination at 50% of doses which individually had shown responses gave a much enhanced response (potentiation).

- An analysis of deaths involving alcohol and barbiturates showed that death from barbiturates alone occurred at a mean concentration of 3.67 μg mL^{-1}, death from alcohol alone occurred at 65 g L^{-1}, and death from the combination occurred at 70% of the blood barbiturate concentration (2.55 μg mL^{-1}) plus 27% of the ethanol concentration (17.5 g L^{-1}) which is very close to an additive response.

Thus, it seems that the interaction is basically additive, but this will show as enhancement of effect or addition of effect depending on the interplay of dose–response and time–action factors.

One particular technique especially applicable to homergic interactions is isobolography. An isobol is a line linking equipotent doses of two drugs, in a graph in which the two axes are doses of the two drugs involved in the interaction. Thus, it is necessary to define an 'end-point' or 'criterion-effect' such as ED_{50}, or minimum inhibitory concentration, in the case of antimicrobial agents. Figure 17.4(a) is an isobologram for the interaction of diazepam and alcohol, studied by means of a digit symbol substitution test. Based on preliminary information, the protocol included high and low doses of the two drugs individually, a combination dose that gave an effect between those of these high and low doses, and placebo. Two-point dose–response graphs were thus established for the two drugs alone. The combination dose established the criterion-effect for the isobologram. The doses of the individual drugs that were expected to induce the criterion effect were then calculated, and the three-point isobologram was constructed using these calculated doses and the combination dose. This line was straight, indicating addition of the contributions from the two drugs. A concave line would have indicated potentiation, while a convex line would have indicated antagonism [Figure 17.4(b).] More complex isobolograms have been constructed in situations where the pharmacology was more complicated, and more data were available.

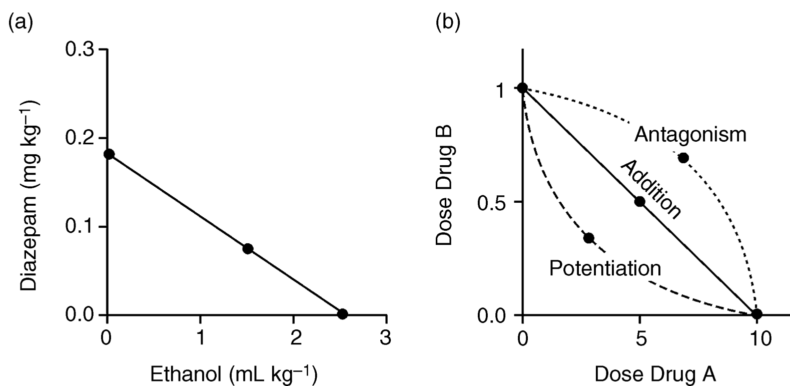

Figure 17.4 (a) Isobologram depicting effects of diazepam, ethanol and a combination of the two on digit substitution test (from Curry *et al.*, 1985). (b) Isobologram showing differentiation of potentiation, addition and antagonism for homergic drug interactions.

17.7 Further examples and mechanisms of a wide range of drug interactions

These are listed in detail in Table 17.3. The classification is by site of interaction and each interaction is considered in regard to whether it modifies the onset, intensity and/or duration of the effect being recorded. In some cases, no entry is made under these headings because of a lack of relevant information and the impossibility of making a useful prediction. The reader is recommended to refer to the time–action principles

Table 17.3 A table of drug interactions

Group	Site of interaction	Drug affecting the status quo (A)	Drug affected (B)	Overall effect of A on B	Modification of effect of B		
					Onset	Intensity	Duration
I	In pharmaceutical preparation of intravenous infusion fluids[a]	Tetracycline drugs	Penicillin drugs	Inactivated			
II	At the site of administration						
	(a) Parenteral[b]	Adrenaline/other vasoconstrictors	Local anaesthetics	Potentiation			Prolonged
	(b) In the gastrointestinal tract[b]	Liquid paraffin (mineral oil)	Fat soluble vitamins	Reduced absorption	Delayed	Decreased	Shortened
		Liquid paraffin (mineral oil)	Fat soluble drugs	Reduced absorption	Delayed	Decreased	Shortened
		Arachis oil and dietary lipid	Griseofulvin	Accelerated absorption	Earlier	Increased	Shortened(?)
		Monoamine oxidase inhibitors (e.g. tranylcypromine)	Dietary amines (e.g. tyramine in cheese)	Increased absorption	Earlier	Increased	Prolonged
		Smooth muscle depressants (e.g. barbiturates, atropine-like drugs and opium alkaloids)	Many drugs	Decreased absorption	Delayed	Decreased	Shortened
		Calcium and/or iron	Tetracycline	Reduced absorption		Reduced	Shortened
III	Distribution/protein binding[c]	Salicylates	(a) Anticoagulants (warfarin and other coumarins)	Increase in clotting time	Earlier	Increased	Prolonged
			(b) Tolbutamide	Hypoglycaemia	Earlier	Increased	Prolonged

		Phenylbutazone	Warfarin	Increase in clotting time	Earlier	Increased	Prolonged
		Sulfonamides	(a) Tolbutamide	Hypoglycaemia			
			(b) Bilirubin in neonates	Kernicturus	Earlier	Increased	Prolonged
		Quinidine	Digoxin	Arrhythmias			
IV	Drug metabolism[d]						
	(a) Induction	Phenobarbital/rifampin	(a) Phenytoin	Reduced effect	Delayed	Decreased	Shortened
			(b) Warfarin	Reduced effect	Delayed	Decreased	Shortened
	(b) Inhibition	Phenylbutazone	Tolbutamide	Hypoglycaemia	Earlier	Increased	Prolonged
		Anticholinesterases	Suxamethonium	Paralysis			
		Tranylcypromine	Pethidine and morphine	Convulsions	Earlier	Increased	Prolonged
		Cimetidine and ketoconazole	Many drugs (e.g. amitriptyline and terfenadine)	Enhanced effects	Earlier	Increased	Prolonged
V	Drug excretion[e]	Probenecid	Penicillin	Prolonged effect			Prolonged
		Ammonium chloride	(a) Amphetamine	Reduced effect		Reduced	Shortened
			(b) Phenobarbital and salicylates	Increased effect		Increased	Prolonged
		Sodium bicarbonate	(a) Amphetamine	Increased effect		Increased	Prolonged
			(b) Phenobarbital and salicylates	Reduced effect		Reduced	Shortened
		Verapamil	Digoxin	Inhibits biliary excretion		Increased	
VI	Pharmacological; classical drug receptor studies[f]	(a) Atropine	Cholinergic drugs	Inhibited		Abolished	
		(b) Anticholinesterases	Muscle relaxants (curare-type)	Reversal of effect		Abolished	
		(c) Amphetamine	Guanethidine	Reversal of hypotensive effect			
		(d) Tranylcypromine	Imipramine	Hypertension			
		(e) Desipramine	Guanethidine	Reversal of hypotensive effect			

(*continued*)

Table 17.3 (*Continued*)

Group	Site of interaction	Drug affecting the status quo (A)	Drug affected (B)	Overall effect of A on B	Modification of effect of B		
					Onset	Intensity	Duration
VII	Pharmacological: unclassified[g]	(a) Salicylate	Insulin	Hypoglycaemia		Increased	Prolonged
		(b) Thiazide diuretics	Digitalis glycosides	Cardiac effects increased		Increased	
		(c) Tranylcypromine	Amphetamine	Hypertension	Earlier	Increased	
		(d) Corticosteroids	Anaesthetics	Hypotension			
VIII	Pharmacological: CNS[h]	(a) Amphetamine	Barbiturates	Euphoria			
		(b) Ethanol	Many drugs with central depressant actions				

[a] Chemical mechanism of pharmaceutical incompatibility; interactions of this type can also occur in body fluids through inadvertent co-prescribing.

[b] (a)Adrenaline causes vasoconstriction, so the rate of removal from the site of administration (which is also the site of action) is reduced. (b)Vitamins do not have acute effects; prolonged interaction leads to vitamin deficiency. Griseofulvin is unusual in that it is absorbed intimately mixed with dietary lipid. Normally, tyramine is extensively metabolized by monoamine oxidase (MAO) in the intestinal lumen and mucosa and during first passage through the liver. Tranylcypromine inhibits MAO and, hence first-pass metabolism of tyramine which enters the general circulation and displaces noradrenaline from stores in adrenergic neurons. Divalent metal ions (Ca^{2+}, Mg^{2+}, Fe^{2+}) react with tetracyclines to form less easily absorbed complexes.

[c] Drugs A displace drugs B from binding sites on plasma protein. This increases the amount of unbound B available for action at receptors. This effect may be short-lived, as the increased amount of unbound B can diffuse to tissue binding sites. Thiazides increase protein binding of pempidine but the significance of this is uncertain.

[d] Barbiturates (phenobarbital is the one most studied) and many other drugs stimulate the liver microsomal enzymes to greater activity in metabolizing warfarin, phenytoin and a wide variety of other drugs. Interestingly, phenobarbital and phenytoin are still used in combination. Problems with warfarin arise when patients are taken off barbiturates and their enzymes return to normal. For cimetidine and ketoconazole details see Sections 17.4.2 and 17.6.2.

[e] Probenecid blocks penicillin excretion by active transport into the proximal tubule – this has been used to prolong the action of penicillin. Ammonium chloride reduces reabsorption of amphetamine and other amines in the renal tubule by reducing pH; it increases reabsorption of phenobarbital, salicylic acid, and other weak acids. Sodium bicarbonate causes effects opposite to those of ammonium chloride, by increasing urine pH.

[f] (a) Inhibition of acetylcholine at cholinergic receptors; (b) Accumulation of acetylcholine displaces competitive neuromuscular blocking drugs; (c) Guanethidine is displaced from tissue binding sites; (d) Inhibition of metabolism of noradrenaline by tranylcypromine plus inhibition of reuptake of noradrenaline into adrenergic nerve endings by imipramine leads to excessive effects of endogenous noradrenaline.

[g] Salicylates exert a hypoglycaemic effect by enhancing beta-cell sensitivity to glucose and potentiating insulin secretion.

[h] (a) Appears as a qualitatively different CNS effect, but it is probably the result of partial pharmacological inhibition of the effects of each drug. (b) Special problems arise when two drugs have similar actions; opinions differ concerning whether to describe this interaction as addition, potentiation, or synergy.

discussed at the beginning of Chapter 4 in his or her assessment of an interaction. Readers may also find it useful to refer to electronic databases of information on particular interactions.

17.8 When are drug interactions important?

There is an immense literature on drug interactions. Almost any combination of drugs can be employed to demonstrate an interaction provided enough ingenuity is used in designing the experiments concerned. Thus the literature is packed with reports that one drug affects the plasma levels of another in man, the metabolism of another in mouse liver homogenates, the concentrations of another in plasma, the action of another in isolated enzyme preparations, etc. Many of these interactions are benign. For example, it was shown many years ago that of a group of 237 warfarin-treated patients who received chloral hydrate only about 10% showed clear potentiation of the effects of warfarin. As already partly discussed in relation to enzyme induction in particular, in practice drug interactions seem to be important when:

- One or more of the drugs concerned has a low therapeutic index.
- The effects of two of the compounds involved are similar (in contrast with the situation of an interacting drug with its own action of a type totally different from that of the drug affected).
- The compounds are interacting to influence delicately balanced physiological control mechanisms, e.g. those concerned with the heart, maintenance of blood pressure, blood coagulation, etc.

Thus, the interactions most obviously affecting the prescription of drugs are those involving anticoagulants, cardioactive drugs, antihypertensive drugs, antidepressants, cold cures, local vasoconstrictors, older hypnotics, anticonvulsants and ethanol.

In addition, it should be realized that a number of clinical procedures apparently involve auto-adaptation to the presence of interacting drugs (e.g. the combined use of phenobarbital and phenytoin, and digoxin, diuretics and potassium supplements).

Borrowing terminology from classical pharmacology Rowland and Tozer (1995) have stressed the 'graded,' rather than 'quantal,' nature of enzyme induction, (and also the opposite, metabolic inhibition), changes in enzyme concentration that occur during induction, occur in proportion to the concentration of the inducing ligand, and over a period of time, thus rarely, if ever, creating an *acute* medical emergency. Rather, they cause a gradual change in drug response that can be recognized and controlled in the patient by appropriate dosage adjustment. However, it should be noted that there have been cases of acute haemorrhage after addition of phenylbutazone to a warfarin regimen.

17.9 Desirable drug–drug interactions

The term 'drug interaction' is often associated with alarm, as it engenders feelings related to potential lack of control over the effects of drugs in the body. There is some validity in this, as some drug interactions are, indeed, the cause of great difficulty in therapeutics, as we have observed in the previous sections. However, it should not be forgotten that some interactions can be turned to advantage, and far beyond the use of combination therapy. For example:

- The use of vasoconstrictors, both adrenaline and octapressin, in prolonging the effect of local anaesthetics thus allowing larger doses to be given safely.
- The use of carbidopa to enhance the effect of L-dopa, allowing lower doses and reduced unwanted effects
- The use of probenecid to prolong the effect of penicillin.
- The effect of cilastin to reduce the renal metabolism of imipenem, enhancing its duration of action and preventing formation of nephrotoxic metabolites.

17.10 Predicting the risk of future drug interactions with new chemical entities

With so many drugs in use, and with others in development, there is an obvious need for accurate and efficient evaluation of whether or not a new chemical entity (NCE) is likely to have significant effects on the actions of existing drugs when it is co-prescribed, and/or whether the NCE itself will cause unexpected effects in patients because of drug interactions. In recent years, several new drugs, as with terfenadine, have been withdrawn from the market because of drug interactions not anticipated before their introduction, discovered only through post-marketing surveillance, highlighting the risk of loss of the investment of time, money and hope represented by the NCE. It is not possible to conduct a comprehensive laboratory or clinical evaluation of this risk before approval for marketing, for reasons of both practicality and protection of the human subjects used in clinical pharmacological testing, yet the need is recognized by both research personnel and regulatory authorities. Three approaches to this problem exist: (i) *in vitro* experiments, (ii) use of drug mixtures and phenotyped subjects in Phase I studies conducted by clinical pharmacologists, and (iii) limited Phase III trials of relevant drug combinations.

17.10.1 In vitro prediction of drug interactions

It has been known since the 1980s that enzyme inhibitors, such as cimetidine, can be introduced into *in vitro* CYP 450 incubations, to determine K_i values, using conventional enzymology approaches. Also, using phenobarbital as an example, it is well-established that pretreatment of rats *in vivo* for as little as 2 days, with an enzyme-inducing agent, leads to an increase in microsomal intrinsic clearance of other compounds when measured *in vitro* using the livers from the pretreated animals. Some inducing agents take longer to exert an effect. Both inhibition and inducing techniques are widely used. The need is to utilize such information predicatively, as there is just as much risk of rejection of a useful NCE with a benign level of interaction risk, as there is of acceptance for further development of a NCE carrying with it serious future risk. Most of the current work in this regard is with the potential for enzyme inhibition, evaluated using K_i values.

Inhibition can be reversible or irreversible, competitive (competition for the active sites on the enzyme), non-competitive (such as induction of an allosteric change in the enzyme molecule reducing its activity), or 'un-competitive.' These three categories are differentiated by their different effects on the Km and V_{max} values, as determined using Lineweaver–Burk plots relating the rate of reaction (v) and substrate concentration (C). For competitive inhibition, the rate equation becomes:

$$v = \frac{V_{max} C}{K\mathrm{m}(1 + I/K_i)} \qquad (17.1)$$

in which I is the inhibitor concentration and K_i is the inhibition constant.

At one time the doctrine was simple. An inhibitor of microsomal P-450 was considered 'weak' if it showed K_i values against various substrates of more than $100\ \mathrm{mol\,L^{-1}}$, 'intermediate' if the K_i values were <100 but $>10\ \mathrm{mol\,L^{-1}}$, and 'potent' if the K_i values were $<10\ \mathrm{mol\,L^{-1}}$. It was considered in the 1990s that a K_i of $>10\ \mathrm{mol\,L^{-1}}$ was unlikely to lead to a clinically-important drug interaction. More recently, it has been recognized that several considerations other than *in vitro* potency must be taken into account, including:

- The concentration of inhibitors likely to occur *in vivo*.
- The concentration range of potential substrates (victim drugs) occurring *in vivo*.
- The therapeutic index of the victim drug.
- Whether the inhibitor and victim were likely to be co-prescribed.

As a result, *in vitro* experiments of this type have become more complex, with, for example:

- Recognition that inhibitor concentrations occurring at the active enzyme site should be incorporated into the calculations.
- Recognition that non-specific binding to the liver material can affect the active concentrations in the biophase.
- Particular focus on CYP2C9, CYP2D6 and CYP3A4 as these are especially important drug-metabolizing isoforms of P-450.
- Recognition that the fraction of the dose metabolized by the particular CYP isoform will affect the risk of the interaction studied being significant *in vivo*.
- Incorporation of the absorption rate constant of the inhibitor into risk assessment calculations because rate of absorption can affect whether or not the inhibitor and victim drugs are likely to interact with CYP3A4 at the same time.
- The average systemic plasma concentration of the inhibitor should be considered for interactions involving CYP2D6 in particular, incorporating evaluation of risk resulting from parallel drug elimination pathways for inhibitor and victim drug.

The result of this attention to detail in the prediction process has been a marked improvement in the *in vitro* predictability of CYP P-450 interactions in laboratory animals *in vivo* assessed using *AUC* values. In contrast, attempts to allow for potential protein binding and metabolism by intestinal mucosa in P-450 inhibition interactions have been less successful. Obviously, there can be no data on whether a NCE rejected in development would have caused interactions in patients, and there are no data at present on whether these more modern approaches are leading to drugs in clinical trials with lesser incidences of inhibition-based drug interactions. In reality, it is probably true to say that an inhibitor of CYP2D6 or CYP3A4 is unlikely to go forward into clinical trials, unless it is of such exceptional value that any risk of interactions would be seen as acceptable in relation to the potential clinical benefit.

An interesting case study in this context was presented by Chien and colleagues (2006) on the interaction between ketoconazole (the inhibitor) and midazolam (the victim drug), with focus on non-linear and non-steady state conditions such as first-pass effects and accumulation of inhibitor during multiple dosing regimens. This work used statistical probability, sensitivity analysis, and uncertainty concepts, and integrated kinetic models of the two drugs, and tested the *in vitro* prediction against five published *in vivo* studies. These investigators were able to:

- Reveal the optimal dose(s) and regimen for achieving inhibition (a regimen to avoid).
- Show that the most influential variable was the fractional clearance of midazolam by CYP3A4 – basically the proportion subjected to a first-pass effect.
- Demonstrate a saturable ketoconazole efflux process from the site of enzyme inhibition in the liver, as a key mechanistic component of the interaction.

17.10.2 **In vivo** *predictions in human subjects*

At one time, the half-life of antipyrine (phenazone) was commonly used to demonstrate enzyme induction in human subjects. Antipyrine is an obsolete analgesic drug that has rapid oral absorption, an oral systemic availability of close to unity, an apparent volume of distribution of total body water, and it is a substrate for human P-450. Thus changes in its half-life will be a result of changes in enzyme activity (clearance) rather than changes in volume of distribution. Test doses are safe, and it was popular to show that the antipyrine half-life became shorter in subjects pretreated with enzyme-inducing drugs such as phenobarbital.

There are numerous examples of drugs that have been tested with antipyrine, however dichloralphenazone, a drug product that is now virtually obsolete, is a curious, and probably unique, example to consider. This product combined the volatile compound chloral, which is still in use, with the (mostly) benign antipyrine, in a once popular sleeping tablet. Chloral is an enzyme inducer, and is metabolized to trichloroacetic acid, which displaces warfarin from its binding sites. Also, antipyrine, although pharmacologically of no significance in this product, induces P-450 activity after long-term treatment, shortening its own half-life. Thus dichloralphenazone, in one product, illustrated two major mechanisms of interaction, and provided a test method for demonstrating the consequences of its own properties!

Antipyrine was thus used as a non-specific test of P-450 activity. It has been superseded by compounds specific to particular isoforms. For example, a team of investigators at the Indiana University School of Medicine is credited with 'invention' of the 'Indiana Cocktail' comprising five test drug dosages that are notable test substrates for particular isozymes of cytochrome P-450 (Table 10.2). This has been used in the study of St. John's wort (Wang *et al.*, 2001) and clarithromycin (Bruce *et al.*, 2001) in particular. In the case of the clarithromycin study, the cocktail included tolbutamide, caffeine and dextromethorphan administered simultaneously. In the case of St. John's wort, it was shown that short-term administration had no effect on enzyme activities. Long-term administration caused a significant increase in the clearance of oral midazolam, and a lesser increase in the clearance of intravenous midazolam. The activities CYP1A2, CYP2C9 or CYP2D6 were unaffected. The clinical implications for management of patients using oral substrates of CYP3A4 are considerable.

Another clinical pharmacology approach to the study of specificity in drug interactions is the use of human subjects previously phenotyped for their levels of the various CYP isozymes. Thus, for an evaluation of a drug for its ability to inhibit CYP2D6 in particular is sought, then subjects with known CYP2D6 hepatic activity can be used, ensuring optimal experimental design. The human subjects with such a level of hepatic calibration would seem to possess a priceless commodity!

17.10.3 Innovative phase III clinical trial designs

Protocols that allow a drug to be tested as both inhibitor and inducer, and as both interacting drug and victim drug, in complementary investigations have been designed to produce comprehensive data for the purpose of risk assessment during clinical development of NCEs (Fox and van Troostenburg de Bruyn, 2007). One such design is shown in Figure 17.5. Human microdosing may also have a role in this work, eventually displacing

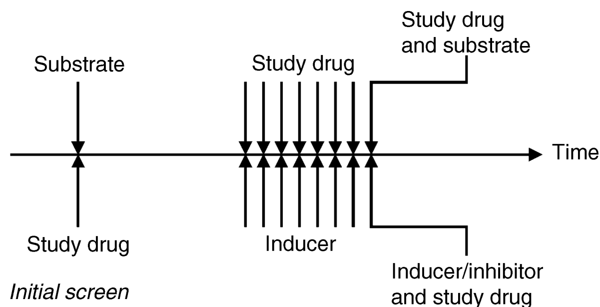

Figure 17.5 Two typical designs for a Phase I drug interaction trial. The horizontal line represents time, over several days. Above the line the study drug is being tested for any interaction, e.g. enzyme inhibition or induction, with a known isoenzyme substrate. Below the line the test drug is being examined for susceptibility to inhibition or induction by some other drug; note that in this protocol the roles of the drug inducing the effect, and the victim drug, are reversed. (Redrawn from Fox & van Troostenburg de Bruyn , 2007.)

the need for pharmaceutical innovators to conduct as many as 5–10 full-scale drug interaction clinical trials in the target population during Phase III development. These trials, historically, have focused on the drugs most likely to be co-prescribed in the patients with the indication to be treated with the NCE, and so have little value in predicting the risk of interaction with the thousands of other interacting drugs that might emerge after the drug has been approved for marketing.

References and further reading

Bruce MA, Hall SD, Haehner-Daniels BD, Gorski JC. In vivo effect of clarithromycin on multiple cytochrome P450s. *Drug Metab Dispos* 2001; 29: 1023–8.

Burk O, Koch I, Raucy J, Hustert E, Eichelbaum M, Brockmoller J, Zanger UM, Wojnowski L. The induction of cytochrome P450 3A5 (CYP3A5) in the human liver and intestine is mediated by the xenobiotic sensors pregnane X receptor (PXR) and constitutively activated receptor (CAR). *J Biol Chem* 2004; 279: 38379–85.

Chien JY, Lucksiri A, Ernest CS, 2nd, Gorski JC, Wrighton SA, Hall SD. Stochastic prediction of CYP3A-mediated inhibition of midazolam clearance by ketoconazole. *Drug Metab Dispos* 2006; 34: 1208–19.

Curry SH, DeVane CL, Wolfe MM. Cimetidine interaction with amitriptyline. *Eur J Clin Pharmacol* 1985; 29: 429–33.

Curry SH, Smith CM. Diazepam-ethanol interaction in humans: addition or potentiation? *Commun Psychopharmacol* 1979; 3: 101–13.

Curry ML, Curry SH, Marroum P. Interaction of phenothiazine and related drugs and caffeinated beverages. *DICP The Annals of Pharmacotherapy* 1991; 25: 437–8.

Fox AW, van Troostenburg de Bruyn A-R. Drug interactions. In: Edwards LD, Fletcher AJ, Fox AW, and Stonier PD, editors. *Principles and Practice of Pharmaceutical Medicine*, 2nd edn. Chichester: John Wiley & Sons, 2007.

Gibson GG, Plant NJ, Swales KE, Ayrton A, El-Sankary W. Receptor-dependent transcriptional activation of cytochrome P4503A genes: induction mechanisms, species differences and interindividual variation in man. *Xenobiotica* 2002; 32: 165–206.

Hussar DA. Drug interactions. In: Remington G, editor. *The Science and Practice of Pharmacy*. Philadelphia: Lippincott, Williams & Wilkins, 2006: 1889–1902.

Lewis RJ, Trager WF, Chan KK, Breckenridge A, Orme M, Roland M, Schary W, Warfarin. Stereochemical aspects of its metabolism and the interaction with phenylbutazone. *J Clin Invest* 1974; 53: 1607–17.

Rowland M, and Tozer TN. *Clinical Pharmacokinetics: Concepts and Applications*. 3rd. edn. (1995. Media, Pennsylvania, Lippincott, Williams & Wilkins.

Walsky RL, Boldt SE. In vitro cytochrome P450 inhibition and induction. *Curr Drug Metab* 2008; 9: 928–39.

Wang Z, Gorski JC, Hamman MA, Huang SM, Lesko LJ, Hall SD. The effects of St John's wort (*Hypericum perforatum*) on human cytochrome P450 activity. *Clin Pharmacol Ther* 2001; 70: 317–26.

18

Drug Metabolism and Pharmacokinetics in Toxicology

18.1 Introduction

The study of the drug metabolism and pharmacokinetic (DMPK) properties of xenobiotics provides critical support to the science of toxicology, from the point of view of (i) evaluation of exposure, (ii) determination of mechanisms, and (iii) guidance on treatment. At one time, in industrial drug discovery, DMPK studies were conducted at the same time as safety evaluation. Nowadays, they are a component of the new drug discovery process itself, conducted in parallel with preclinical chemical and biological studies. 'Toxicokinetics' is the study of pharmacokinetics in the context of exposure to doses associated with unwanted effects, in both animals and human beings. This includes the design, conduct and interpretation of safety evaluation protocols, and also validation of dosing in such studies. Toxicokinetics also plays a part in the process of extrapolation of data from animals to humans. 'Clinical toxicology' is the study of xenobiotic toxicity in human beings, especially patients, but also non-patient populations, exposed to therapeutic agents and environmental toxicants, at trace, therapeutic and excessive exposures. 'Analytical toxicology' focuses on methods of measurement of toxicants in biological material.

18.2 Terminology

An 'unwanted' drug effect is any effect occurring in the wrong circumstances. It can be an extension of the normal response, or something completely different. An unwanted effect can occur at the same or a higher dose than that inducing the wanted effect, or even, in special circumstances, at a lower dose. Almost any pharmacological mechanism can be involved in an unwanted effect, including that of the wanted effect. Thus, for example, unwanted effects can be ascribed to unusual drug metabolic routes and/or pharmacokinetics, unusual responses mediated through the nervous or endocrine systems, and many other events. However, there are some mechanisms that are exclusively associated with unwanted effects, and these are the subject of this chapter, illustrated by a number of notable examples. The objective of this chapter is to introduce the reader to some of the more important concepts of toxicology, as they relate to the DMPK properties of toxic molecules. It should be appreciated that all unwanted effects are related in some way to exposure, controlled by dosage and pharmacokinetic influences.

Various terms are used in relation to toxicology. 'Unwanted effect' is deliberately non-specific, but does allude to the fact that drugs have 'effects', which may be 'wanted' or 'unwanted'. Furthermore, an unwanted effect in one situation may be wanted in another. So, for example, constipation arising from the use of

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morphine postoperatively to treat pain is an unwanted effect, particularly after bowel surgery but the same effect is a wanted effect of morphine in antidiarrhoeal preparations. The term 'side effect' is similarly all inclusive, and frequently used, but it has the disadvantage that it suggests that effects and side effects are different and can be separated – this is often not the case. The term 'adverse reaction' has a connotation of greater severity than does unwanted effect, but that is not always intended when this term is used. 'Toxicity', similarly and often, but not always, implies a greater problem than does side effect or unwanted effect. An idiosyncratic unwanted effect is usually thought of as occurring in a definable subgroup of the population. The non-specific term 'secondary effect' is also sometimes found in scientific literature.

There have been many attempts to classify unwanted drug effects, based variously on mechanisms, clinical manifestations and other approaches. These have at times led to arguments about terminology and meanings of words that have not been helpful to scientific understanding. One relatively simple and useful classification is as follows:

1. *Pharmacokinetic unwanted effects*. Effects arising from the presence of excessive concentrations of molecules that exert only desirable effects in normal quantities. This group makes allowance for overdose, be it deliberate or accidental, acute or cumulative, absolute or relative, and resulting from anything to do with DMPK that can be described as idiosyncrasy or intolerance.
2. *Pharmacodynamic unwanted effects*. Effects occurring by pharmacodynamic mechanisms at normal clinical dosage, acutely or after prolonged exposure. This group allows for anything describable as a side effect, a secondary effect, or an idiosyncrasy or intolerance where physiological or biochemical sensitivity is a factor.
3. *Drug allergy.* Hypersensitivity with an immunological basis.
4. *Toxic drug reactions*. Effects caused basically by means of covalent chemical reaction between pharmacologically active foreign molecules and biologically important materials. This includes blood dyscrasias and tissue necrosis.
5. *Drug interactions*.

In this classification, groups 1 and 2 are considered to be reversible, in the sense that conventional dose–response relationships apply to both wanted and unwanted effects, so that reduction in concentrations consequent on removal of the drug and/or its metabolites from the body leads to the dissipation of the effects. Numbers 3 and 4 are thought of as irreversible, because, although they can clearly be reversed by a variety of medical interventions, and by the normal processes of body healing, covalent chemical reactions are key to their origins, and removal from the body of the offending molecules and the molecules with which they have reacted is presumed to be needed. Group 5 was the subject of Chapter 17. It should be appreciated that the clinical outcome with any particular drug can invoke a combination of these processes.

18.3 Dose–response and time–action with special reference to toxicology

Interpretation of toxicity studies is conducted, in part, in ways already discussed in regard to studies of the desired effects of drugs. Thus appropriate recognition is always given to dose–response relationships, and the growth and decay curves underlying the time–action relationships at the core of pharmacological science. So, the time course and dose relationships discussed in the opening paragraphs of Chapter 4, and in later chapters, are crucial. Similarly, pharmaceutical factors, special population factors, and disease factors potentially apply equally in toxicology as in pharmacology. There are, however, some variations on this theme in toxicology. For example, it is not uncommon for unwanted drug effects to arise only in subgroups of the treated population, and the characteristics of those subgroups become very important. Also, toxicity is often discovered only through epidemiological studies. For example, the most dramatic and arguably most tragic drug toxicology disaster involved exposure of foetuses to thalidomide given to their mothers. Its occurrence

involved disposition mechanisms such as placental transfer hitherto ignored in new drug discovery. Its exemplification was a rare congenital malformation already known to the medical professions, then linked to thalidomide when its incidence increased dramatically in the new-born children of the treated mothers. Similarly, the cardiac toxicity of the COX-2 inhibitors (anti-inflammatory analgesics) only emerged after patients in vastly larger numbers than those involved in the clinical trials had been prescribed the drugs.

18.4 Safety studies in new drug discovery

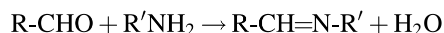
Safety studies in laboratory animals are a critical component of new drug discovery and preparation of new chemical entities for initial human exposure. These studies use various designs, including single dose with recovery, and two-week, one-month, three-month and six-month exposure regimens followed by autopsy. They commonly utilize one rodent (e.g. rat) and one non-rodent (e.g. dog) species in each project. In order to meet the requirements of good laboratory practice (GLP), exposure must be confirmed using a validated method of analysis specific for the compound under investigation. Generally, samples for toxicokinetic analysis are taken from a subgroup of animals from within the treatment group, but a parallel group is permitted if it is considered that sampling from the test group would unduly influence the outcome of the safety evaluation. Commonly, plasma samples for toxicokinetics are taken at the beginning of the dosing time, such as after the first dose, and after the last dose. The regulatory objective is solely to prove that there was exposure to the drug, but these studies commonly provide the first opportunity to investigate doses other than the single doses typically used in pre-clinical studies, and useful multiple-dose pharmacokinetic data can be obtained. For example, toxicokinetics can detect whether the long-term concentrations predicted from the single doses are reached, and whether there are any differences in metabolic handling as the result of long term dosing. Toxicokinetics can also test whether long term effects are more closely related to $C_{\max}$ concentrations or to overall exposure, and whether there is evidence of tissue accumulation.

Safety pharmacology is often restricted to single doses, and involves the study of general organ systems, such as heart, kidney and lung function, regardless of whether the drug is designed to have an effect on one of these organs. Because safety pharmacology is generally conducted to GLP, toxicokinetic studies are required. Doses are increased beyond those required to demonstrate the desired effect, so toxicokinetics can provide the opportunity to check for deviations from linearity in the pharmacokinetic properties of the compound in question. However, LD_{50} (the dose that kills 50% of a population of laboratory animals) values are no longer required or scientifically justifiable – modern emphasis is on such parameters as the maximum repeatable dose (MRD). Determination of the MRD is an iterative process. Laboratory animals are first given increasing doses up to a point where unwanted effects are seen. Dosing then continues at a level just below this toxic dose, for a period of time sufficient to show that it can be repeated without induction of toxicity.

18.5 Examples

18.5.1 Isoniazid

Primary amines and hydrazines undergo condensation reactions with aldehydes to form imines (Schiff's bases):



$$\text{R-CHO} + \text{R}'\text{NH}_2 \rightarrow \text{R-CH=N-R}' + \text{H}_2\text{O}$$

An analogous reaction occurs between isoniazid and pyridoxal and/or pyridoxal phosphate, the active form of vitamin B6 (Figure 18.1). Pyridoxal phosphate is an important cofactor for the synthesis of monoamine neurotransmitters and a reduction in its availability can produces a peripheral neuropathy and central nervous

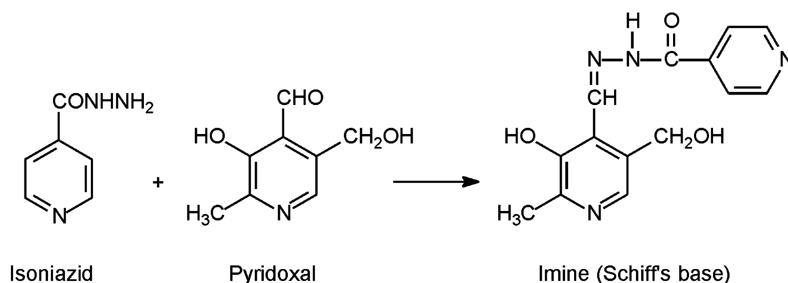


Figure 18.1 Condensation of pyridoxal with isoniazid.

system (CNS) effects. The neuropathy can be reversed by removal of the isoniazid, administration of pyridoxine, or both. It occurs most commonly in slow-acetylators (Section 10.3.1). These patients have unusually high plasma concentrations of isoniazid when treated with conventional doses. It has been suggested that the response of tuberculosis patients to isoniazid is associated more with the magnitude of peak concentrations in plasma than with overall exposure during daily dosing. The drug is activated by bacterial catalase which results in inhibition of the synthesis of mycolic acid required for mycobacterial cell walls.

Isoniazid is also hepatotoxic in certain individuals, shown by raised serum liver enzyme and bilirubin concentrations. In this case the toxicity is more prevalent in fast acetylators. The chemically-related monoamine oxidase inhibitor, iproniazid, causes similar toxicity, although this drug has a substituent on its terminal nitrogen atom, preventing metabolic acetylation (Figure 18.2). Phenobarbital pretreatment leads to increased incidence of this liver toxicity in rats treated with either isoniazid or iproniazid, and this toxicity can be prevented by treatment with inhibitors of drug metabolism. When [^{14}C-acetyl]-labelled acetylisoniazid was administered to rats, a large amount of covalently bound radioactivity was

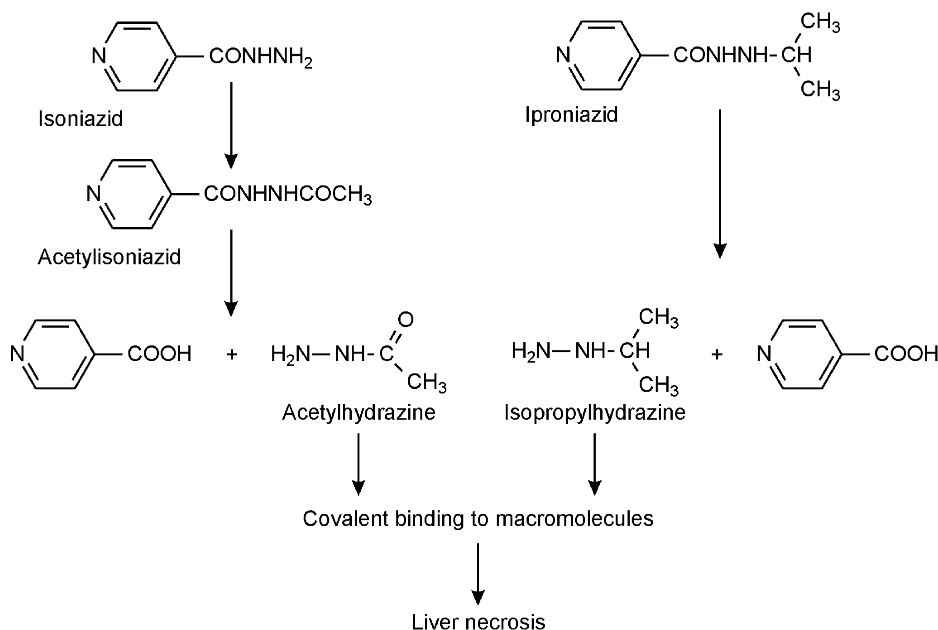


Figure 18.2 Proposed mechanism of hepatotoxicity of isoniazid and iproniazid.

found in the liver. No covalent binding of radioactivity resulted when the acetylisoniazid was ring-labelled. The conclusion was drawn that hepatic necrosis results in this case from cleavage of the substituted compounds, acetylisoniazid and iproniazid, producing acetylhydrazine and isopropylhydrazine respectively. These two products are highly toxic. Metabolic activation, induced by phenobarbital, and leading to reactive species, is needed to facilitate their toxicity by means of covalent binding (Figure 18.2).

Interestingly, isoniazid demonstrates most of the general ideas involved in drug toxicity, in that there is a basis for the use of the various terms including unwanted effect and adverse reaction: the toxicity occurs in a subgroup of the population thereby qualifying to be described as idiosyncratic; there is a pharmacokinetic influence under genetic control; there are drug metabolism sequences involved in its toxicity; there is a plausible physiological/biochemical explanation for the toxicity providing a basis for methods of successful treatment of the toxicity; and covalent chemical reactions are involved. Isoniazid therefore invokes mechanisms 1 and 4 of the classification listed above (Section 18.2).

18.5.2 Paracetamol and phenacetin

The story of these two drugs and related toxic chemicals epitomizes just about the entire scope of drug toxicity studies. Phenacetin (acetophenetidin) had been in use for some 50 years before Brodie and Axelrod discovered a metabolite, paracetamol (acetaminophen), of even greater potential value as an analgaesic. Phenacetin was notorious for its propensity to cause renal damage, and paracetamol provided hope that there could be a related compound that did not cause this toxicity. Paracetamol has since become the most widely used pain killer worldwide, and also, coincidentally, the most important single cause of fulminant liver, but not renal, failure in Western countries. These two compounds, together with related molecules, provide a fascinating case study in mechanisms of toxicity, the role of metabolic and other pharmacokinetic processes, and the application of plasma concentration measurement as an aid to the diagnosis and treatment of drug toxicity.

Phenacetin is chemically related to acetanilide, which is metabolized to aniline and then to phenylhydroxylamine (Figure 18.3). *In vivo*, acetanilide causes haemolysis and methaemoglobinaemia. Phenylhydroxylamine, is highly toxic if administered directly.

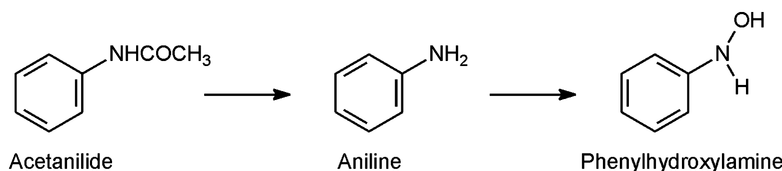


Figure 18.3 Metabolism of acetanilide.

Haemoglobin is a tetramer, each unit containing haem, a porphyrin that has an iron atom at its centre. The protein is bound via a histidine nitrogen, leaving the sixth coordination position free for oxygen binding. Studies have shown that the oxidation state of the iron when oxygen is bound is $\sim$3.2. When oxygen binds it oxidizes the iron, removing an electron and forming superoxide (Figure 18.4). Fe^{3+} being smaller than Fe^{2+} moves closer to the centre of the porphyrin ring causing the allosteric change that has been observed. This results in co-operative binding of O_2 to the other three haem groups. Because molecular oxygen can only bind to haemoglobin, the Fe^{3+} in methaemoglobin must be reduced back to Fe^{2+}. Several enzymes are involved, the chief one being cytochrome b_5-reductase [reduced nicotinamide adenine dinucleotide

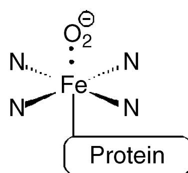


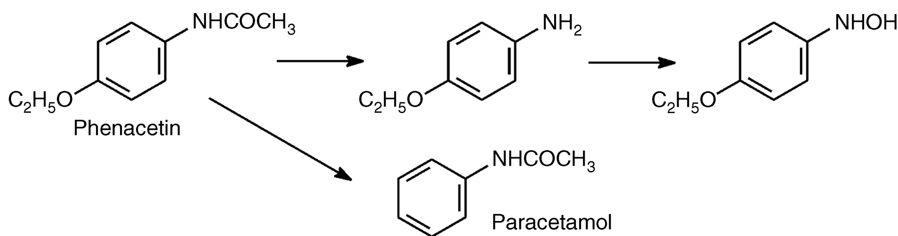
Figure 18.4 Simple representation of oxyhaemoglobin. The porphyrin nitrogens are depicted and the globular protein binds via a histidine nitrogen. Oxyhaemoglobin is diamagnetic, i.e. there are no unpaired electrons, so it is postulated that one electron from iron (giving Fe^{3+}) is paired with one from oxygen, giving superoxide.

(NADH), others being NADPH-methaemoglobin reductase and the involvement of ascorbate and reduced glutathione (GSH).

Thus, oxidizing agents including oxidized metabolites, for example phenylhydroxylamine which can be oxidized to a nitroso derivative and reduced back to aniline, can promote the formation of methaemoglobin from haemoglobin, either by direct oxidation or by depleting the cofactors required for the reduction pathways referred to above.

Haemolysis caused by aromatic amines, such as aniline, has been traced to the same chemistry, but with variations. The demonstration that haemolysis only occurs *in vivo* provides evidence that metabolic activation is needed. The *N*-acetylated precursors of amines (e.g. acetanilide) cause haemolysis to a lesser degree than do their respective amines, and this toxicity is prevented by inhibitors of deacetylation. These inhibitors have no effect on amine toxicity as such. Thus it is apparent that the anilides require metabolic transformation in two steps in order to cause haemolysis, the two steps being deacetylation followed by *N*-hydroxylation (a *C*-hydroxylation analogue is possible). In spite of this, the two toxicities, haemolysis and methaemoblobinaemia are differently affected by phenobarbital pretreatment, which increases methaemoglobinaemia but decreases haemolysis. Thus, although the same basic chemistry is involved in the two reactions, it seems likely that different active intermediates are involved. In fact, the existence of a reactive intermediate in the sense of a free radical alkylating agent is not needed to explain the methaemoblobinaemia, whereas such an intermediate is postulated for the haemolysis reaction.

The metabolic reactions of phenacetin can include hydroxylamine production.



However, phenacetin is not particularly a cause of blood problems, but it has certainly been implicated in renal papillary necrosis, and probably caused renal pelvic carcinoma when widely used. Large doses also caused hepatic necrosis in animals, especially in species forming large quantities of *N*-hydroxylated metabolites. Paracetamol is particularly able to cause hepatic necrosis in such species, and in humans taking acute high doses. With phenacetin, covalent binding of the compound, or of radioactivity derived from it paralleled hepatic necrosis. This necrosis is associated with GSH depletion, and when caused by paracetamol in humans it can be successfully treated with glutathione precursors, or SH-donors,

including *N*-acetylcysteine, L-cysteine, cysteamine, L-methionine, D-penicillamine, and dimercaprol. *N*-Acetylcysteine has emerged as the treatment of choice. Glutathione itself fails to penetrate hepatocytes. These compounds do not affect paracetamol metabolism as such. With phenacetin, the intact molecule is involved in the covalent binding, shown by the use of ring-labelled and acetyl-labelled derivatives which bind equally. However, elucidating a definitive mechanism is complicated by the fact these drugs may be metabolically deacetylated and reacetylated (Nicholls *et al.*, 2006).

In the search for the understanding of mechanisms, it was originally suggested that an additional hydroxylated metabolite of phenacetin (*N*-hydroxy-4-ethoxyacetanilide) might be involved, first losing its ethyl group non-enzymatically, forming a reactive imidoquinone [*N*-acetyl-4-benzoimidoquinone or *N*-acetyl-*p*-benzoquinone imine (NAPQI)]. This product is now known to be formed from paracetamol, without the requirement for the desethylation step that is needed with phenacetin. The imidoquinone can undergo nucleophilic addition at the 3-position of its benzene ring, reacting with functional –SH groups in cells. This explains the finding that the glutathione conjugate of phenacetin appears in the urine as a mercapturic acid conjugate of a 3-acetyl-cysteine-4-hydroxy derivative (see Figure 3.16). The reactive imidoquinone is capable of mediating both liver and kidney toxicity, but only if it is formed in quantities great enough to exceed the glutathione availability, as the toxic metabolite only reacts with key tissue constituents once the glutathione pool has been depleted by ~30%. The formation of a mercapturic acid conjugate shows that at some stage the unstable arylating intermediate acts as an electrophilic reactant, having a fractional positive charge (indicated in Figure 18.5 as δ^+). This will react with nucleophilic cell macromolecules.

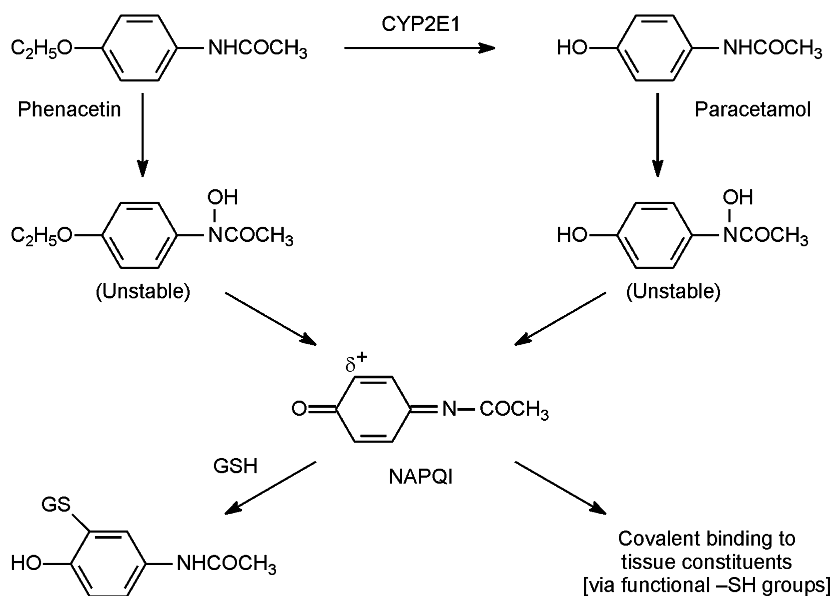


Figure 18.5 Proposed scheme for production of toxic metabolites of phenacetin and paracetamol.

The question arises as to why phenacetin and paracetamol, with such similar chemistry, show different patterns of toxicity. They differ in polarity of course, and therefore in affinity as substrates for P-450, which is responsible for *N*-hydroxylation and presumably for conversion of phenacetin to paracetamol. The *N*-oxidation can occur in both liver and kidney, and can be induced by phenobarbital pretreatment, and reduced by inhibitors of drug metabolism. It may occur to an increased proportional extent in one tissue at high doses,

or after prolonged exposure, the former affecting paracetamol, the latter phenacetin. So the quantities and location of the active material can easily differ between the two compounds. It has been suggested that paracetamol fails to produce acute renal effects because it is not metabolized by kidney cells at a rate sufficient to exceed the glutathione capacity for conjugation. Because the formation of the reactive product from the *N*-oxides is promoted by acidic conditions, which occur more readily in the kidney, this could affect phenacetin more than paracetamol in some way. It has also been suggested that the *N*-hydroxylated phenacetin but not the *N*-hydroxylated paracetamol might be sufficiently stable after formation in the liver to reach the kidney in sufficient quantities to be toxic. Finally, phenacetin might affect the renal papilla and cause pelvic carcinoma, but not affect the proximal tubule, because of some drug concentration effect, or some difference in local concentrations of P-450. It is remarkable that two compounds could be so similar yet so different, and that one should have been rejected clinically and the other adopted with such enthusiasm as a substitute.

It is now established that the initial step in paracetamol toxicity is metabolism to NAPQI, which leads to depletion of GSH and covalent adduct formation of 'acetaminophen-cysteine adducts'. Immunochemical studies have shown that the cellular sites of covalent binding correlate with the toxicity. It has been shown that nitrated tyrosine is formed in hepatic centrilobular cells, and these adducts colocalize in cells containing the acetaminophen-cysteine adducts. Peroxynitrite, which is a highly reactive nitrating and oxidizing species formed by rapid reaction of nitric oxide and superoxide, produces nitrated tyrosine. Normally, GSH detoxifies peroxynitrite, but after GSH depletion by NAPQI, peroxynitrite nitrates protein tyrosine. It has also been hypothesized that lipid peroxidation may play a role. Thus nitration of tyrosine correlates with necrosis (Figure 18.6).

Paracetamol is rapidly absorbed from the small intestine, with its C_{max} occurring within 1–2 hours of tablet dosing. Normal C_{max} values are around $20\,mg\,L^{-1}$. There is 20% presystemic metabolism by sulfation in the gut wall. Further metabolism occurs in the liver and the half-life in plasma is 1.5–3 hours. About 90% is metabolized to inactive sulfate and glucuronide conjugates that are excreted in urine, but the remainder is metabolized to the highly reactive intermediate NAPQI, which is immediately bound by intracellular glutathione and eliminated as mercapturic acid adducts in the urine. High doses of paracetamol produce

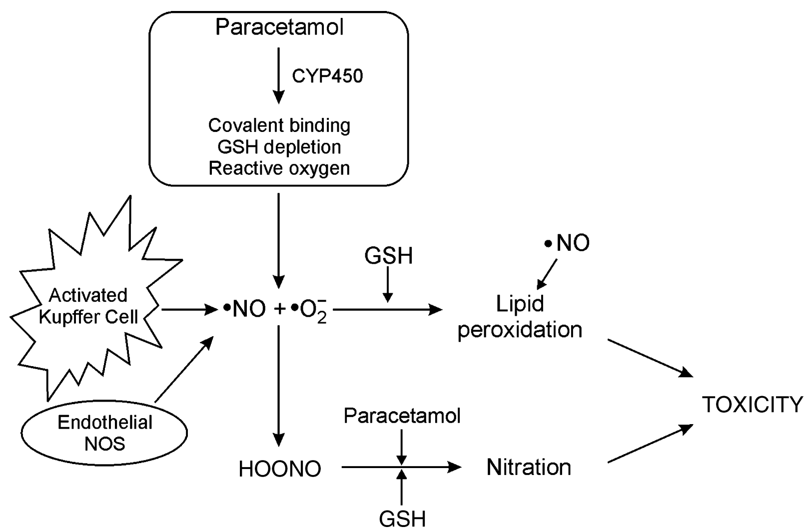


Figure 18.6 Postulated mechanisms of paracetamol-induced hepatotoxicity. Hepatocytes, Kupffer cells and endothelial cells participate in production of reactive nitrogen and oxygen species. The relative levels of nitric oxide (•NO) and superoxide ($\bullet O^{-}{}_{2}$) determine whether mechanism of hepatic necrosis is dependent on protein nitrosylation or lipid peroxidation. (Redrawn from Jaeschke *et al.*, 2002.)

greater quantities of NAPQI and cause toxic depletion of glutathione stores, and also binds to other proteins causing damage to liver cells. The hepatotoxic dose is generally thought to be about 150 mg kg^{-1} but there is considerable variation between individuals.

The treatment of paracetamol overdose is guided either by dose consumed (when ingestion is staggered over a period of time) or by plasma concentrations of the drug (when the dose was in one single event at an identifiable time). The plasma concentration approach uses various nomograms linking points on a semilogarithmic graph of concentration between four hours post-overdose to 11–20 hours later. The early versions of these nomograms were devised by Prescott (see Prescott, 1996) and Rumack and Matthew (1975). Thus, if the plasma concentration is less than a point on a line between 200 mg L^{-1} at the 4 hour point and 30 mg L^{-1} at the 15 hour point, little more than supportive treatment is needed. This can include the use of ipecacuanha to induce vomiting, gastric washout (lavage) of unabsorbed drug, and activated charcoal to reduce any further absorption, but note that charcoal will inhibit the absorption of orally administered antidotes such as methionine. The nomogram should not be confused with the decline of the plasma concentration of the paracetamol – it reflects experience with various approaches to treatment and the probability of significant quantities of the toxic metabolite being present, and the fact that the need for the antidote relates to the dose consumed and the time since consumption. This nomogram line has been revised lower over the years, with one group of authors favouring use of a 24-hour data point, and others favouring a 25% reduction in the threshold concentrations to comply with US Food and Drug Administration guidelines designed to provide a better safety margin, albeit increasing the use of *N*-acetylcysteine which is not without complications. A further lowering is favoured by some physicians in vulnerable ('high-risk') populations such as those on enzyme inducing drugs, chronic alcohol abusers, fasting patients, and dehydrated patients, who, at least in some cases, are likely to produce greater proportional conversion to the toxic product.

Figure 18.7 illustrates the clinical approach to paracetamol poisoning. This figure relates plasma paracetamol concentration at diagnosis to time post-ingestion. It shows the three nomogram lines, normal, US and high-risk, and the time course of plasma paracetamol in three different reports. In all three cases, proactive treatment with *N*-acetylcysteine was warranted because the concentrations at one time or

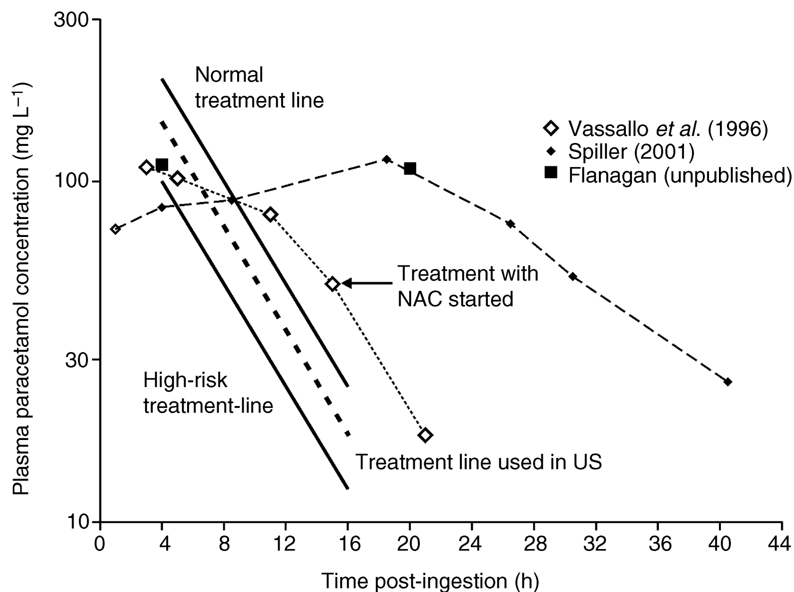


Figure 18.7 Paracetamol concentrations after paracetamol self-poisoning. (From Flanagan *et al.*, 2008.)

another were to the right of the nomograms. Note that in the 'Spiller' case, absorption of the paracetamol was delayed such that a single early plasma paracetamol concentration would have indicated no need for antidote dosing, and even in the other two cases the initial plasma concentration could have been misleading.

Finally, perhaps the single biggest risk factor is that there are patients treated with combination products of paracetamol plus narcotic analgaesics (such as 'Darvocet') who then add over the counter paracetamol to their dosing regimen without realizing that they are exposing themselves to potentially lethal doses of the paracetamol in the process.

18.5.3 Toxicity associated with prolonged exposure to therapeutic doses

Some of the most serious toxic reactions are associated with the normal pharmacological properties of drugs, experienced over a prolonged period of time, presumably at steady-state concentrations, and possibly involving long-term exposure to metabolites of the drugs concerned. No particular pharmacokinetic observations have been made in association with such problems, except perhaps, the general guideline that patients should receive the lowest possible dosage over the shortest possible time consistent with a useful therapeutic effect.

One such problem is 'serotonin syndrome'. This is associated with prolonged use of selective serotonin reuptake inhibitors (SSRIs) and, to lesser extent, with tricyclic antidepressants (TCAs) which increase the concentrations of serotonin in the brain. The clinical picture involves confusion, autonomic instability, and neuromuscular abnormalities. It is most often seen in patients taking two or more drugs at therapeutic doses that increase CNS serotoninergic activity by different mechanisms, such as monoamine oxidase inhibitors or pethidine, with SSRIs or TCAs. The combination of monoamine oxidase inhibitors and SSRIs or TCAs is discouraged but not unknown. The serotonin syndrome can be precipitated by single doses, but usually at single doses above the therapeutic range. Additionally, at least with amitriptyline, there can be a life-threatening combination of hypotension and heart block (produced by peripheral anti-adrenergic effects on the heart and blood vessels), parasympathetic block (rendering ineffective the normal reflex responses to reduction in heart rate, and/or the occurrence of anticholinergic effects of the amitryptiline on the heart) and CNS depression associated with respiratory depression.

A complex situation occurs with prolonged opioid exposure (opioid syndrome), in which the clinical manifestations are again bradycardia, CNS depression, hypotension and respiratory depression, plus, with some opioids, miosis and decreased gastrointestinal motility, complicated, of course, by addiction. Again, acute overdose can be life-threatening, more, in this case, from respiratory depression than from peripheral nervous system effects.

One of the oldest of these syndromes is the 'neuroleptic syndrome' caused by chlorpromazine and other phenothiazine antipsychotic drugs. Chlorpromazine caused such a revolution in psychiatry that prescribers became overenthusiastic in their use of the drug, following a practice then used with digoxin (Section 14.2.6.2), in first dosing to a limit set by the appearance of unwanted effects, then reducing the dose sufficiently for the unwanted effects to disappear. In this way, it was presumed, the probability of trouble-free therapy being achieved was optimized. This practice arose before any pharmacokinetic data had been obtained with these drugs. In time, it was realized that, with acute dosage, chlorpromazine, like amitriptyline, can cause nerve block, with CNS, cardiac, sympathetic and parasympathetic effects combining to produce life-threatening sedation, low blood pressure, and slowing of the heart all at the same time. After acute doses, recovery is complete and rapid. However, long term dosing at dose levels just below the threshold for unwanted effects caused a combination of slowly reversible nerve block, plus, 'tardive dyskinesia', a seemingly irreversible movement disorder similar to Parkinson's disease. The word 'tardive' indicates 'late in onset'. This syndrome was at first thought to be a characteristic of the schizophrenia that was being treated, and in a number of cases this led to an *increase* in dosing. When it became possible to measure

chlorpromazine plasma concentrations it was found that reducing the dose and thus plasma concentrations, to the minimum consistent with useful therapeutic effects, the risk of developing of neuroleptic syndrome and tardive dyskinesia was greatly reduced. This work did not lead to monitoring of plasma concentrations in the way now common with drugs with narrow therapeutic indices (Chapter 19), but it did have a considerable impact on the treatment of schizophrenia, which evolved from being a long-term problem treated with high doses of drugs over periods of years in psychiatric hospitals, to an acute condition treated with smaller doses in the context of outpatient care.

As already stated, there is no unifying pharmacokinetic theory for these syndromes, characterized by the occurrence of a combination of normal and abnormal pharmacology with, mostly, long-term exposure to drugs and their metabolites. All can involve 'normal' doses, and/or both acute and chronic overdosing, especially, when long-term, possibly with the metabolites of the drugs involved. Treatment will almost always involve sampling for identification and quantitative assessment of the concentrations of the causative agents in the blood. Treatment of acute toxicity will involve reduction in exposure, by means of gastric lavage, dosing with activated charcoal to adsorb unabsorbed drug, and observation while the normal elimination processes take effect. Sometimes proactive means such as adjustment of urinary pH, or plasmapheresis, will be used to reduce body content. Thus, while drug concentrations will be especially useful in diagnosis of problems in asymptomatic patients at risk, it is rare for drug concentrations to dictate patient management decisions.

18.5.4 Salicylate

Poisoning by salicylate and other non-steroidal anti-inflammatory drugs is a common problem in emergency medicine. This syndrome is characterized by haematemesis, tachypnoea, hyperpnoea, dyspnoea, tinnitus, deafness, lethargy, seizures and confusion. Clinical toxicity is primarily a function of the degree and duration of acid–base disturbance, resulting from the depression of respiratory centre and consequent carbon dioxide exchange. Aspirin toxicity can result from both acute and chronic overexposure, and the pharmacology of the toxicity can be related to or unrelated to the mechanism of analgesia, anti-inflammation, and antipyresis. At therapeutic doses aspirin can cause gastric irritation, as well as nausea and vomiting. Occasionally, the effect of aspirin on blood clotting through its inhibition of platelet aggregation can cause bruising. Very rarely, there can be an allergic response exhibited as urticaria or anaphylactic shock.

Acute or chronic overexposure causes ringing in the ears and deafness as well as a broad variety of CNS effects ranging from headache to coma. Stimulation by salicylate of the respiratory centre in the medulla causes hyperventilation and hyperpyrexia, which in turn lead to sweating and dehydration. Metabolically there is an increase in oxygen consumption and carbon dioxide production, and thus metabolic acidosis plus respiratory alkalosis. The metabolic acidosis reduces the ionization so that more salicylate enters tissues including the brain, thereby enhancing its CNS toxicity. Also, the kidney excretes more bicarbonate (along with sodium and potassium). The bicarbonate in the urine causes the urine pH to rise, which in fact accelerates the excretion of the salicylate. Thus aspirin in overdose exhibits two of the important pH effects on distribution and elimination discussed in Chapter 2.

Treatment involves whole body cooling, plus cardiovascular and respiratory support, and correction of the acid-base abnormalities. Toxicokinetic intervention involves sampling to identify the toxic chemical(s) involved, administration of activated charcoal to adsorb any unabsorbed aspirin in the gastrointestinal tract, and administration of sodium bicarbonate to alkalinize the urine further in order to speed the elimination of the absorbed dose. The bicarbonate also assists in the reversal of the metabolic acidosis. Forced diuresis along with the bicarbonate is no longer recommended, as it has been found that the additional fluid load involved can complicate the stabilization of the electrolyte balance. Dialysis can be used as a treatment but it is not often needed.

Concentrations of salicylate in plasma are predictive of severity of intoxication and can define treatment. Thus concentrations above 4 mmol L^{-1} dictate that intensive care unit monitoring be employed, while concentrations above 4.45 mmol L^{-1} in chronic intoxication, and above 9.4 mmol L^{-1} in acute ingestion, can indicate a need for the use of haemodialysis.

18.5.5 Toxic chemicals

This group is made up of non-therapeutic toxic chemicals for which there may be or may not be an underlying pharmacokinetic theory in treatment. The toxic alcohols are a case in point.

18.5.5.1 Methanol and ethylene glycol

Methanol and ethylene glycol lead to accumulation of aldehyde and acid metabolites, and from promotion of lactic acid formation due to reduced NAD/NADH ratios. This is assessed as the anion gap:

$$\text{Anion gap} = ([Na^+] + [K^+]) - ([HCO_3^{2-}] + [Cl^-])$$

If the anion gap exceeds 14 mmol L^{-1}, action is required. Ethylene glycol or methanol concentrations of >500 mg L^{-1} are a possible indication for pro-active treatment, with formepizole (4-methylpyrazole, 4MP), or intravenous ethanol, and/or haemodialysis. The use of ethanol as a treatment is based on the fact that ethylene glycol and methanol poisoning are mediated by metabolites. If the formation of these metabolites can be slowed down, then the alternative pathway of renal excretion will dominate the elimination. Ethanol does just that. In being a competing substrate for the enzyme alcohol dehydrogenase, ethanol slows down the metabolism of ethylene glycol and methanol, thus prolonging the treatment (which may seem counterintuitive) but reducing exposure to the toxic metabolites. Ethanol itself is metabolized to less toxic metabolites. The pharmacokinetic data relevant to this approach is summarized in Table 18.1. Formepizole is an inhibitor of alcohol dehydrogenase that achieves a result similar to that achieved with ethanol, without the ethanol effects. However, ethanol is inexpensive and usually readily available in hospitals.

Table 18.1 Mean elimination half-lives in hours of ethylene glycol and methanol (data from Chu *et al.*, 2002)

	Treatment			
	Drug alone	Ethanol	Formepizole	Ethanol + haemodialysis
Ethylene glycol	2.5–4.5[a]	17	19.7	2.6
Methanol	3.01	43	54	3.5

[a]Low level infusions in normal subjects, longer at toxic concentrations.

18.5.5.2 Halobenzenes

Bromobenzene and related compounds cause hepatic necrosis. Virtually all of the metabolism of bromobenzene is through the formation of 3,4-bromobenzene epoxide, which undergoes further metabolism by a variety of routes depending on the concentration available. At low doses, conjugation with glutathione and eventual excretion as a mercapturic acid conjugate is favoured. At higher doses, the concentrations of the epoxide rise, and formation of 4-bromophenol and arylation of microsomal protein occurs. Another possible route, employing epoxide hydrase, completes the picture without direct involvement in hepatotoxicity (Figure 18.8). Bromobenzene is a model for several widely used drugs, such as phenytoin (diphenylhydantoin) and

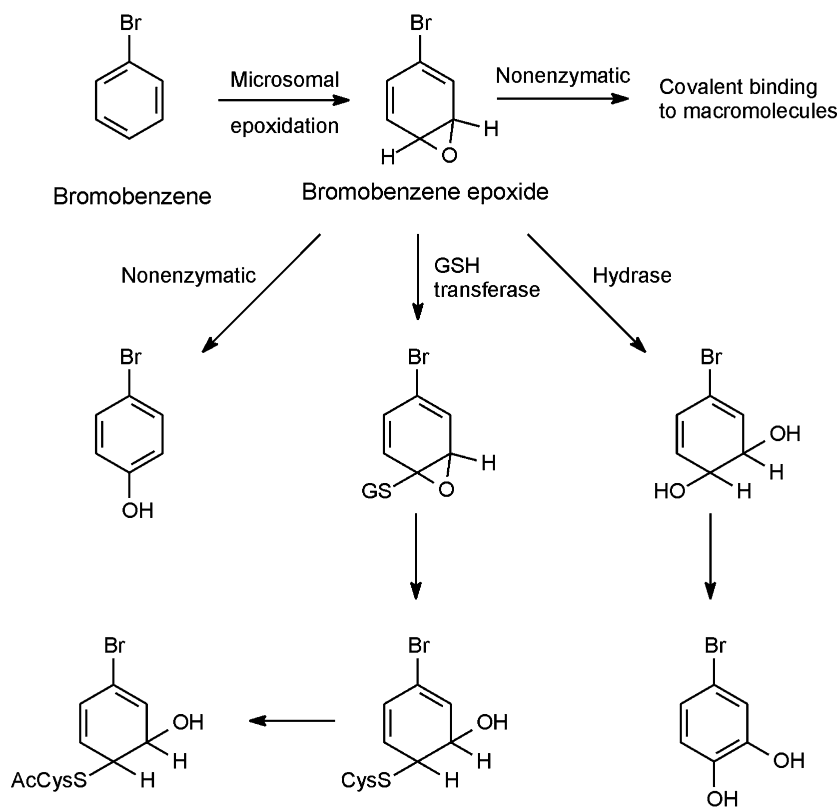


Figure 18.8 Enzymatic and non-enzymatic transformations of bromobenzene.

phenobarbital, which are metabolized to vicinal diol derivatives indicating the formation of epoxide intermediates. However, formation of such intermediates does not necessarily imply that liver toxicity will result.

18.5.5.3 Miscellaneous

Further examples of covalent chemistry involved in toxicology include carcinogenicity (such as with 2-naphthylamine which is an aromatic amine that is converted to a hydroxylamine), chemical damage, teratology (such as with thalidomide) and mutagenicity (such as with mustine hydrochloride, a cancer fighting drug), and with drug allergy (such as with penicillin). Heavy metals provide yet another area of toxicology in which principles of drug disposition and pharmacokinetics are relevant. Thus the world of toxicology provides a fascinating area of application of most, perhaps all, of the principles involved in this book, and, while treatment relies more on history, clinical assessment and interpretation of ancillary investigations, measurement and interpretation of drug concentrations remain critical in providing baseline data, and understanding of mechanisms.

References and further reading

Alapat PM, Zimmerman JL. Toxicology in the critical care unit. *Chest* 2008; 133: 1006–13.

Bertolini A, Ferrari A, Ottani A, Guerzoni S, Tacchi R, Leone S. Paracetamol: new vistas of an old drug. *CNS Drug Rev* 2006; 12: 250–75.

Boger RH. Renal impairment: a challenge for opioid treatment? The role of buprenorphine. *Palliat Med* 2006; 20 Suppl 1: s17–23.
Brodie BB, Axelrod J. The fate of acetophenetidin in man and methods for the estimation of acetophenetidin and its metabolites in biological material. *J Pharmacol Exp Ther* 1949; 97: 58–67.
Brune K. Persistence of NSAIDs at effect sites and rapid disappearance from side-effect compartments contributes to tolerability. *Curr Med Res Opin* 2007; 23: 2985–95.
Chu J, Wang RY, Hill NS. Update in clinical toxicology. *Am J Respir Crit Care Med* 2002; 166: 9–15.
Chyka PA, Erdman AR, Christianson G, Wax PM, Booze LL, Manoguerra AS, *et al.* Salicylate poisoning: an evidence-based consensus guideline for out-of-hospital management. *Clin Toxicol (Phila)* 2007; 45: 95–131.
Clark DW, Layton D, Shakir SA. Do some inhibitors of COX-2 increase the risk of thromboembolic events?: Linking pharmacology with pharmacoepidemiology. *Drug Saf* 2004; 27: 427–56.
Daly FF, Fountain JS, Murray L, Graudins A, Buckley NA. Guidelines for the management of paracetamol poisoning in Australia and New Zealand – explanation and elaboration. A consensus statement from clinical toxicologists consulting to the Australasian poisons information centres. *Med J Aust* 2008; 188: 296–301.
Dargan PI, Jones AL. Acetaminophen poisoning: an update for the intensivist. *Crit Care* 2002; 6: 108–10.
Davies NM, Good RL, Roupe KA, Yanez JA. Cyclooxygenase-3: axiom, dogma, anomaly, enigma or splice error? –Not as easy as 1, 2, 3. *J Pharm Pharm Sci* 2004; 7: 217–26.
Dawson AH, Whyte IM. Therapeutic drug monitoring in drug overdose. *Br J Clin Pharmacol* 1999; 48: 278–83.
Day RO, McLachlan AJ, Graham GG, Williams KM. Pharmacokinetics of nonsteroidal anti-inflammatory drugs in synovial fluid. *Clin Pharmacokinet* 1999; 36: 191–210.
Flanagan RJ. Fatal toxicity of drugs used in psychiatry. *Hum Psychopharmacol* 2008; 23Suppl 1: 43–51.
Flanagan RJ, Taylor A, Watson ID, Whelpton R. *Fundamentals of Analytical Toxicology.* Chichester: John Wiley & Sons, 2008.
Hinz B, Brune K. Cyclooxygenase-2–10 years later. *J Pharmacol Exp Ther* 2002; 300: 367–75.
Isbister GK, Bowe SJ, Dawson A, Whyte IM. Relative toxicity of selective serotonin reuptake inhibitors (SSRIs) in overdose. *J Toxicol Clin Toxicol* 2004; 42: 277–85.
Isbister GK, Buckley NA. The pathophysiology of serotonin toxicity in animals and humans: implications for diagnosis and treatment. *Clin Neuropharmacol* 2005; 28: 205–14.
Isbister GK, Dawson AH, Whyte IM. Comment: serotonin syndrome induced by fluvoxamine and mirtazapine. *Ann Pharmacother* 2001; 35: 1674–5.
Isbister GK, Prior FH, Foy A. Citalopram-induced bradycardia and presyncope. *Ann Pharmacother* 2001; 35: 1552–5.
Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. *Toxicol Sci* 2002; 65: 166–76.
Kaplowitz N. Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov* 2005; 4: 489–99.
Knight TR, Ho YS, Farhood A, Jaeschke H. Peroxynitrite is a critical mediator of acetaminophen hepatotoxicity in murine livers: protection by glutathione. *J Pharmacol Exp Ther* 2002; 303: 468–75.
Maurer HH, Sauer C, Theobald DS. Toxicokinetics of drugs of abuse: current knowledge of the isoenzymes involved in the human metabolism of tetrahydrocannabinol, cocaine, heroin, morphine, and codeine. *Ther Drug Monit* 2006; 28: 447–53.
Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. *J Med Toxicol* 2008; 4: 2–6.
Nicholls AW, Wilson ID, Godejohann M, Nicholson JK, Shockcor JP. Identification of phenacetin metabolites in human urine after administration of phenacetin-C^2H_3: measurement of futile metabolic deacetylation via HPLC/MS-SPE-NMR and HPLC-ToF MS. *Xenobiotica* 2006; 36: 615–29.
Prescott LF. *Paracetamol (Acetaminophen) A critical bibliographic review.* London: Taylor & Francis, 1996.
Rischitelli DG, Karbowicz SH. Safety and efficacy of controlled-release oxycodone: a systematic literature review. *Pharmacotherapy* 2002; 22: 898–904.
Rollason V, Samer C, Piguet V, Dayer P, Desmeules J. Pharmacogenetics of analgesics: toward the individualization of prescription. *Pharmacogenomics* 2008; 9: 905–33.
Rumack BH, Matthew H. Acetaminophen poisoning and toxicity. *Pediatrics* 1975; 55: 871–6.
Spiller HA. Persistently elevated acetaminophen concentrations for two days after an initial four-hour non-toxic concentration. *Vet Hum Toxicol* 2001; 43: 218–9.

Vassallo S, Khan AN, Howland MA. Use of the Rumack-Matthew nomogram in cases of extended-release acetaminophen toxicity. *Ann Intern Med* 1996; 125: 940.
Whyte IM, Dawson AH, Buckley NA. Relative toxicity of venlafaxine and selective serotonin reuptake inhibitors in overdose compared to tricyclic antidepressants. *QJM* 2003; 96: 369–74.
Woolf AD, Erdman AR, Nelson LS, Caravati EM, Cobaugh DJ, Booze LL, *et al.* Tricyclic antidepressant poisoning: an evidence-based consensus guideline for out-of-hospital management. *Clin Toxicol (Phila)* 2007; 45: 203–33.