11 Pharmacokinetics and related topics

11.1 Pharmacodynamics and pharmacokinetics

In previous chapters, we looked at the molecular targets for drugs and the mechanisms by which drugs interact with these targets. In chapter 13, we shall look at how drugs can be designed to optimize binding interactions with their targets. This is an area of medicinal chemistry known as **pharmacodynamics.** However, the compound with the best binding interactions for a target is not necessarily the best drug to use in medicine. This is because a clinically useful drug has to travel through the body in order to reach its target. There are many barriers and hurdles in its way and, as far as the drug is concerned, it is a long and arduous journey. The study of how a drug reaches it target, and what happens to it during that journey, is known as **pharmacokinetics.** When carrying out a drug design programme, it is important to study pharmacokinetics alongside pharmacodynamics. There is no point perfecting a compound with superb drug–target interactions if it has no chance of reaching its target. The four main topics to consider in pharmacokinetics are absorption, distribution, metabolism and excretion (often abbreviated to ADME).

11.2 Drug absorption

Drug absorption refers to the route or method by which a drug reaches the blood supply. This in turn depends on how the drug is administered. The most common and preferred method of administering drugs is the oral route and so we shall first concentrate on the various barriers and problems associated with oral delivery.

An orally taken drug enters the **gastrointestinal tract** (GIT), which comprises the mouth, throat, stomach, and the upper and lower intestines. A certain amount of the drug may be absorbed through the mucosal membranes of the mouth, but most passes down into the stomach where it encounters gastric juices and hydrochloric acid. These chemicals aid in the digestion of food and will treat drugs in a similar fashion if the drug is susceptible to breakdown. For example, the first penicillin used clinically was broken down in the stomach and had to be administered by injection. Other acid-labile drugs, such as **local anaesthetics** or **insulin**, cannot be given orally. If the drug *does* survive the stomach, it enters the upper intestine where it encounters digestive enzymes that serve to break down food. Assuming the drug survives this attack, it then has to pass through the cells lining the intestinal or gut wall. This means that the drug has to pass through a cell membrane on two occasions, first to enter the cell and then to exit it on the other side. Once the drug has passed through the cells of the gut wall, it can enter the blood supply relatively easily as the cells lining the blood vessels have pores between them through which most drugs can pass. In other words, drugs enter the blood vessels by passing between cells rather than through them.

The drug is now transported in the blood to the body's 'customs office'—the liver. The liver contains enzymes which are ready and waiting to intercept foreign chemicals, and modify them such that they are more easily excreted—a process called drug metabolism (section 11.4).

It can be seen that stringent demands are made on any orally taken drug. The drug must be chemically stable to survive the stomach acids, and metabolically stable to survive the digestive enzymes in the GIT as well as the metabolic enzymes in the liver. It must also have the correct balance of water versus fat solubility. If the drug is too polar (hydrophilic), it will fail to pass through the fatty cell membranes of the gut wall (section 1.2.1). On the other hand, if the drug is too fatty (hydrophobic), it will be poorly soluble in the gut and will dissolve in fat globules. This means that there will be poor surface contact with the gut wall, resulting in poor absorption.

It is noticeable how many drugs contain an amine functional group. There are good reasons for this. Amines are often involved in a drug's binding interactions with its target. However, they are also an answer to the problem of balancing the dual requirements of water and fat solubility. Amines are weak bases, and it is found that many of the most effective drugs are amines having a pK_a value in the range 6–8. In other words, they are partially ionized at blood pH and can easily equilibrate between their ionized and non-ionized forms. This allows them to cross cell membranes in the non-ionized form, while the presence of the ionized form gives the drug good water solubility and permits good binding interactions with its target binding site (Fig. 11.1).

The extent of ionization at a particular pH can be determined by the **Henderson–Hasselbalch equation**:

$$pH = pK_a + \log \frac{[RNH_2]}{[RNH_3^+]}$$

where $[RNH_2]$ is the concentration of the free base and $[RNH_3^+]$ is the concentration of the ionized amine. K_a is the equilibrium constant for the equilibrium shown in Fig. 11.1, and the Henderson–Hasselbalch equation can be derived from the equilibrium constant:

$$K_a = \frac{[H^+][RNH_2]}{[RNH_3^+]}$$

$$\text{Therefore} \quad pK_a = -\log \frac{[H^+][RNH_2]}{[RNH_3^+]}$$

$$= -\log[H^+] - \log \frac{[RNH_2]}{[RNH_3^+]}$$

$$= pH - \log \frac{[RNH_2]}{[RNH_3^+]}$$

$$\text{Therefore} \quad pH = pK_a + \log \frac{[RNH_2]}{[RNH_3^+]}$$

Note that when the concentration of the ionized and unionized amines are identical (i.e. when $[RNH_2] = [RNH_3^+]$), the ratio $([RNH_2]/[RNH_3^+])$ is 1. Since $\log 1 = 0$, the Henderson–Hasselbalch equation will simplify to $pH = pK_a$. In other words, when the amine is 50% ionized, $pH = pK_a$. Therefore, drugs with a pK_a of 6–8 are approximately 50% ionized at blood pH (7.4).

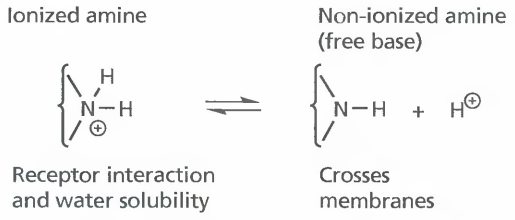


FIGURE 11.1 Equilibrium between the ionized and non-ionized form of an amine.

The hydrophilic–hydrophobic character of the drug is the crucial factor affecting absorption through the gut wall, and the molecular weight of the drug should in theory be irrelevant. For example, **ciclosporin** is successfully absorbed through cell membranes, although it has a molecular weight of about 1200. In practice, however, larger molecules tend to be poorly absorbed, because they are likely to contain a large number of polar functional groups. As a rule of thumb, orally absorbed drugs tend to obey what is known as Lipinski's **rule of five**. The rule of five was derived from an analysis of compounds from the World Drugs Index database, aimed at identifying features that were important in making a drug orally active. It was found that the factors concerned involved numbers that are multiples of 5:

- a molecular weight less than 500
- no more than 5 hydrogen bond donor groups
- no more than 10 hydrogen bond acceptor groups
- a calculated log *P* value less than +5 (log *P* is a measure of a drug's hydrophobicity—section 14.1).

The rule of five has been an extremely useful rule of thumb for many years, but it is neither quantitative nor foolproof. For example, orally active drugs such as **atorvastatin**, **rosuvastatin**, **ciclosporin** and **vinorelbine** do not obey the rule of five. It has also been demonstrated that a high molecular weight does not in itself cause poor oral bioavailability. One of the reasons that the molecular weight appears to be important is that larger molecules invariably have too many functional groups capable of forming hydrogen bonds. Therefore, further research has been carried out to find guidelines that are independent of molecular weight.

Work carried out by Veber *et al.* in 2002, demonstrated the rather surprising finding that molecular flexibility (as measured by the number of freely rotatable bonds present in the structure) plays an important role in oral bioavailability. The more flexible the molecule, the less likely it is to be orally active. Less surprisingly, the analysis showed that the polar surface area of the molecule could be used as a factor instead of the number of hydrogen bonding groups. These findings led to the following parameters for acceptable oral activity. Either:

- a polar surface area $\leq$ 140 Å and $\leq$ 10 rotatable bonds, or
- $\leq$ 12 hydrogen bond donors and acceptors in total and $\leq$ 10 rotatable bonds.

Some researchers set the limit of rotatable bonds to ≤ 7 since the analysis shows a marked improvement in oral bioavailability for such molecules.

These rules are independent of molecular weight and open the way to studying larger structures that have

been 'shelved' up to now. Unfortunately, structures having a molecular weight larger than 500 are quite likely to have more than 10 rotatable bonds. However, the new rules suggest that rigidifying the structures to reduce the number of rotatable bonds would be beneficial. Rigidification tactics are described in section 13.3.9 as a strategy to improve a drug's pharmacodynamic properties, but these same tactics could also be used to improve pharmacokinetic properties.

Polar drugs that break the above rules are usually poorly absorbed and have to be administered by injection. Nevertheless, some highly polar drugs can be absorbed from the digestive system. For example, there are polar drugs that can 'hijack' specific **transport proteins** in the cell membrane (sections 2.7.2 and 10.1). Transport proteins are essential to a cell's survival, as they transport the highly polar building blocks required for various biosynthetic pathways (e.g. amino acids and nucleic acid bases). If the drug bears a structural resemblance to one of these building blocks, then it too may be smuggled into the cell. For example, **levodopa** is transported by the transport protein for the amino acid phenylalanine, and **fluorouracil** is transported by transport proteins for the nucleic acid bases thymine and uracil. The antihypertensive agent **lisinopril** is transported by transport proteins for dipeptides, and the anticancer agent **methotrexate** and the antibiotic **erythromycin** are also absorbed by means of transport proteins.

Other highly polar drugs can be absorbed into the blood supply if they have a low molecular weight (less than 200), as they can then pass through small pores between the cells lining the gut wall.

Occasionally, polar drugs with high molecular weight can cross the cells of the gut wall without actually passing through the membrane. This involves a process known as **pinocytosis** where the drug is engulfed by the cell membrane and a membrane-bound vesicle is pinched off to carry the drug across the cell (Fig. 11.2). The vesicle then fuses with the membrane to release the drug on the other side of the cell.

Sometimes, drugs are deliberately designed to be highly polar so that they are not absorbed from the GIT. These are usually antibacterial agents targeted against gut infections. Making them highly polar ensures that the drug reaches the site of infection in higher concentration (Box 19.2).

11.3 Drug distribution

Once a drug has been absorbed, it is rapidly distributed around the blood supply, then more slowly distributed to the various tissues and organs. The rate and extent of distribution depends on various factors, including the physical properties of the drug itself.

11.3.1 Distribution round the blood supply

The vessels carrying blood round the body are called **arteries**, **veins**, and **capillaries**. The heart is the pump that drives the blood through these vessels. The major artery carrying blood from the heart is called the **aorta**, and as it moves further from the heart, it divides into smaller and smaller arteries—similar to the limbs and branches radiating from the trunk of a tree. Eventually, the blood vessels divide to such an extent that they become extremely narrow—equivalent to the twigs of a tree. These blood vessels are called capillaries, and it is from them that oxygen, nutrients, and drugs can escape in order to reach the tissues and organs of the body. At the same time, waste products such as cell breakdown products and carbon dioxide are transferred from the tissues into the capillaries to be carried away and disposed of. The capillaries now start uniting into bigger and bigger vessels, resulting in the formation of veins which return the blood to the heart.

Once a drug has been absorbed into the blood supply, it is rapidly and evenly distributed throughout the blood supply within a minute—the time taken for the blood volume to complete one circulation. However, this does not mean that the drug is evenly distributed around the body, because the blood supply is richer to some areas of the body than to others.

11.3.2 Distribution to tissues

Drugs do not stay confined to the blood supply. If they did, they would be of little use, since their targets are the cells of various organs and tissues. The drug has to leave the blood supply in order to reach those targets. The body has an estimated 10 billion capillaries with a total surface area of 200 m^2. They probe every part of the body, such that no cell is more than 20–30 μm away from a capillary. Each capillary is very narrow, not much wider than the

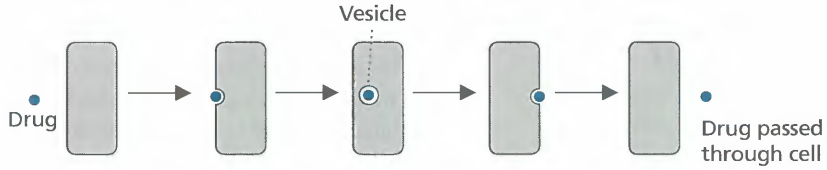


FIGURE 11.2 Pinocytosis.

red blood cells that pass through it. Its walls are made up of a thin single layer of cells packed tightly together. However, there are pores between the cells which are 90–150 Å in diameter—large enough to allow most drug-sized molecules to escape, but not large enough to allow the **plasma proteins** present in blood to escape. Therefore, drugs do not have to cross cell membranes in order to leave the blood system and can be freely and rapidly distributed into the aqueous fluid surrounding the various tissues and organs of the body. Having said that, some drugs are found to bind to plasma proteins in the blood. Since the plasma proteins cannot leave the capillaries, the proportion of drug bound to these proteins is also confined to the capillaries and cannot reach its target.

11.3.3 Distribution to cells

Once a drug has reached the tissues, it can immediately be effective if its target site is a receptor situated in a cell membrane. However, there are many drugs that have to enter the individual cells of tissues in order to reach their target. These include local anaesthetics, enzyme inhibitors, and drugs which act on nucleic acids or intracellular receptors. Such drugs must be hydrophobic enough to pass through the cell membrane, unless they are smuggled through by carrier proteins or taken in by pinocytosis.

11.3.4 Other distribution factors

The concentration levels of free drug circulating in the blood supply rapidly fall away after administration as a result of the distribution patterns described above, but there are other factors at work. Drugs that are excessively hydrophobic are often absorbed into fatty tissues and removed from the blood supply. This fat solubility can lead to problems. For example, obese patients undergoing surgery require a larger than normal volume of general anaesthetic because the gases used are particularly fat soluble. Unfortunately, once surgery is over and the patient has regained consciousness, the anaesthetics stored in the fat tissues will be released and may render the patient unconscious again. **Barbiturates** were once seen as potential intravenous anaesthetics which could replace the anaesthetic gases. Unfortunately, they too are fat soluble and as a result it is extremely difficult to estimate a sustained safe dosage. The initial dose can be estimated to allow for the amount of barbiturate taken up by fat cells, but further doses eventually lead to saturation of the fat depot, and result in a sudden and perhaps fatal increase of barbiturate levels in the blood supply.

Ionized drugs may be bound to various macromolecules and also removed from the blood supply. Drugs may also be bound reversibly to blood plasma proteins such as **albumin**, thus lowering the level of free drug. Therefore, only a small proportion of the drug that has been administered may actually reach the desired target.

11.3.5 Blood–brain barrier

The blood–brain barrier is an important barrier that drugs have to negotiate if they are to enter the brain. The blood capillaries feeding the brain are lined with tight-fitting cells which do not contain pores (unlike capillaries elsewhere in the body). Moreover, the capillaries are coated with a fatty layer formed from nearby cells, providing an extra fatty barrier through which drugs have to cross. Therefore, drugs entering the brain have to dissolve through the cell membranes of the capillaries and also through the fatty cells coating the capillaries. As a result, polar drugs such as **penicillin** do not easily enter the brain.

The existence of the blood–brain barrier makes it possible to design drugs which will act at various parts of the body (e.g. the heart) and have no activity in the brain, thus reducing any central nervous system (CNS) side effects. This is done by increasing the polarity of the drug such that it does not cross the blood–brain barrier. On the other hand, drugs that are intended to act in the brain must be designed such that they *are* able to cross the blood–brain barrier. This means that they must have a minimum number of polar groups, or have these groups temporarily masked (see prodrugs; section 14.6). Having said that, some polar drugs can cross the blood–brain barrier with the aid of carrier proteins, and others (e.g. **insulin**) can cross by the process of pinocytosis previously described. The ability to cross the blood–brain barrier has an important bearing on the analgesic activity of opioids (section 24.5).

11.3.6 Placental barrier

The placental membranes separate a mother's blood from the blood of her fetus. The mother's blood provides the fetus with essential nutrients and carries away waste products, but these chemicals must pass through the placental barrier. Since food and waste products can pass through the placental barrier, it is perfectly feasible for drugs to pass through as well. Drugs such as **alcohol**, **nicotine**, and **cocaine** can all pass into the fetal blood supply. Fat-soluble drugs will cross the barrier most easily, and drugs such as **barbiturates** will reach the same levels in fetal blood as in maternal blood. Such levels may have unpredictable effects on fetal development. They may also prove hazardous once the baby is born. Drugs and other toxins can be removed from fetal blood by the maternal blood and detoxified. Once the baby is born, it may have the same levels of drugs in its blood as the mother, but it does not have the same ability to detoxify

or eliminate them. As a result, drugs will have a longer lifetime and may have fatal effects.

11.3.7 Drug–drug interactions

Drugs such as **warfarin** and **methotrexate** are bound to albumin and plasma proteins in the blood and are unavailable to interact with their targets. When another drug is taken which can compete for plasma protein binding (e.g. sulfonamides), then a certain percentage of previously bound drug is released, increasing the concentration of the drug and its effect.

KEY POINTS

- Pharmacodynamics is the study of how drugs interact with a molecular target, whereas pharmacokinetics is the study of how a drug reaches its target in the body and how it is affected on that journey.
- The four main issues in pharmacokinetics are absorption, distribution, metabolism, and excretion.
- Orally taken drugs have to be chemically stable to survive the acidic conditions of the stomach, and metabolically stable to survive digestive and metabolic enzymes.
- Orally taken drugs must be sufficiently polar to dissolve in the GIT and blood supply, but sufficiently fatty to pass through cell membranes.
- Most orally taken drugs obey Lipinski's rule of five and have no more than seven rotatable bonds.
- Highly polar drugs can be orally active if they are small enough to pass between the cells of the gut wall, are recognized by carrier proteins, or are taken across the gut wall by pinocytosis.
- Distribution round the blood supply is rapid. Distribution to the interstitial fluid surrounding tissues and organs is rapid if the drug is not bound to plasma proteins.
- Some drugs have to enter cells in order to reach their target.
- A certain percentage of a drug may be absorbed into fatty tissue and/or bound to macromolecules.
- Drugs entering the CNS have to cross the blood–brain barrier. Polar drugs are unable to cross this barrier unless they make use of carrier proteins or are taken across by pinocytosis.
- Some drugs cross the placental barrier into the fetus and may harm development or prove toxic in newborn babies.

11.4 Drug metabolism

When drugs enter the body, they are subject to attack from a range of metabolic enzymes. The role of these enzymes is to degrade or modify the foreign structure, such that it can be more easily excreted. As a result, most drugs undergo some form of metabolic reaction resulting in structures known as metabolites. Very often these metabolites lose the activity of the original drug, but in some cases they may retain a certain level of activity. Some metabolites may possess a different activity from the parent drugs, resulting in side effects or toxicity. A knowledge of drug metabolism and its possible consequences can aid the medicinal chemist in designing new drugs which do not form unacceptable metabolites. Equally, it is possible to take advantage of drug metabolism to activate drugs in the body. This is known as a prodrug strategy (see section 14.6). It is now a requirement to identify all the metabolites of a new drug before it can be approved. The structure and stereochemistry of each metabolite has to be determined and the metabolite must be tested for biological activity (section 15.1.2).

11.4.1 Phase I and phase II metabolism

Drugs are foreign substances as far as the body is concerned, and the body has its own method of getting rid of such chemical invaders. If the drug is polar, it will be quickly excreted by the kidneys (section 11.5). However, non-polar drugs are not easily excreted and the purpose of drug metabolism is to convert such compounds into more polar molecules that *can* be easily excreted.

Non-specific enzymes (particularly **cytochrome P450 enzymes** in the liver) are able to add polar functional groups to a wide variety of drugs. Once the polar functional group has been added, the overall drug is more polar and water soluble, and is more likely to be excreted when it passes through the kidneys. An alternative set of enzymatic reactions can reveal masked polar functional groups which might already be present in a drug. For example, there are enzymes which can demethylate a methyl ether to reveal a more polar hydroxyl group. Once again, the more polar product (metabolite) is excreted more efficiently.

These reactions are classed as phase I reactions and generally involve oxidation, reduction, and hydrolysis (see Figs. 11.3–11.9). Most of these reactions occur in the liver but some (such as the hydrolysis of esters and amides) can also occur in the gut wall, blood plasma, and other tissues. Some of the structures most prone to oxidation are *N*-methyl groups, aromatic rings, the terminal positions of alkyl chains, and the least hindered positions of alicyclic rings. Nitro, azo, and carbonyl groups are prone to reduction by reductases, while amides and esters are prone to hydrolysis by esterases. For some drugs, two or more metabolic reactions might occur, resulting in different metabolites; other drugs may not be metabolized at all. A knowledge of the metabolic reactions that are possible for different functional groups allows

the medicinal chemist to predict the likely metabolic products for any given drug, but only drug metabolism studies will establish whether these metabolites are really formed.

Drug metabolism has important implications when it comes to using chiral drugs, especially if the drug is to be used as a racemate. The enzymes involved in catalysing metabolic reactions will often distinguish between the two enantiomers of a chiral drug, such that one enantiomer undergoes different metabolic reactions from the other. As a result, both enantiomers of a chiral drug have to be tested separately to see what metabolites are formed. In practice, it is usually preferable to use a single enantiomer in medicine, or design the drug such that it is not asymmetric (section 13.3.8).

A series of metabolic reactions classed as phase II reactions also occur, mainly in the liver (see Figs. 11.10–11.16). Most of these reactions are **conjugation reactions**, whereby a polar molecule is attached to a suitable polar 'handle' that is already present on the drug or has been introduced by a phase I reaction. The resulting conjugate has greatly increased polarity, thus increasing its excretion rate in urine or bile even further.

Both phase I and phase II reactions can be species specific, which has implications for *in vivo* metabolic studies. In other words, the metabolites formed in an experimental animal may not necessarily be those formed in humans. A good knowledge of how metabolic reactions differ from species to species is important in determining which test animals are relevant for drug metabolism tests. Both sets of reactions can also be regioselective and stereoselective. This means that metabolic enzymes can distinguish between identical functional groups or alkyl groups located at different parts of the molecule (regioselectivity) as well as between different stereoisomers of chiral molecules (stereoselectivity).

11.4.2 Phase I transformations catalysed by cytochrome P450 enzymes

The enzymes that constitute the cytochrome P450 family are the most important metabolic enzymes and are located in liver cells. They are **haemoproteins** (containing haem and iron) and they catalyse a reaction that splits molecular oxygen, such that one of the oxygen atoms is introduced into the drug and the other ends up in water (Fig. 11.3). As a result they belong to a general class of enzymes called the **monooxygenases.**

There are at least 33 different cytochrome P450 enzymes, grouped into four main families CYP1-CYP4. Within each family there are various subfamilies designated by a letter, and each enzyme within that subfamily is designated by a number. For example, CYP3A4 is enzyme 4 in the subfamily A of the main family 3. Most drugs in current use are metabolized by five primary CYP enzymes (CYP3A, CYP2D6, CYP2C9, CYP1A2 and CYP2E1). The isozyme CYP3A4 is particularly important in drug metabolism, and is responsible for the metabolism of most drugs. The reactions catalysed by cytochrome P450 enzymes are shown in Figs. 11.4 and 11.5 and can involve the oxidation of carbon, nitrogen, phosphorus, sulfur, and other atoms.

Oxidation of carbon atoms can occur if the carbon atom is either exposed (i.e. easily accessible to the enzyme) or activated (Fig. 11.4). For example, methyl substituents on the carbon skeleton of a drug are often easily accessible and are oxidized to form alcohols, which may be oxidized further to carboxylic acids. In the case of longer-chain substituents, the terminal carbon and the penultimate carbon are the most exposed carbons in the chain and are both susceptible to oxidation. If an aliphatic ring is present, the most exposed region is the part most likely to be oxidized.

Activated carbon atoms next to an sp^2 carbon centre (i.e. allylic or benzylic positions) or an sp carbon centre (i.e. a propynylic position) are more likely to be oxidized than exposed carbon atoms (Fig. 11.4). Carbon atoms which are alpha to a heteroatom are also activated and prone to oxidation. In this case, hydroxylation results in an unstable metabolite that is immediately hydrolysed resulting in the dealkylation of amines, ethers, and thioethers or the dehalogenation of alkyl halides. The aldehydes which are formed from these reactions generally undergo further oxidation to carboxylic acids by aldehyde dehydrogenases (section 11.4.4). Tertiary amines are found to be more reactive to oxidative dealkylation than secondary amines because of their greater basicity, while *O*-demethylation of aromatic ethers is faster than *O*-dealkylation of larger alkyl groups. *O*-Demethylation is important to the analgesic activity of codeine (section 24.5).

Cytochrome P450 enzymes can catalyse the oxidation of unsaturated sp^2 and sp carbon centres present in alkenes, alkynes, and aromatic rings (Fig. 11.5). In the case of alkenes, a reactive epoxide is formed which is deactivated by the enzyme **epoxide hydrolase** to form a diol. In some cases, the epoxide may evade the enzyme.

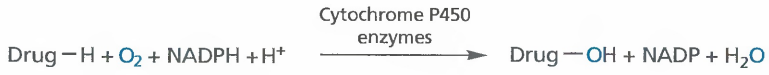


FIGURE 11.3 Oxidation by cytochrome P450 enzymes.

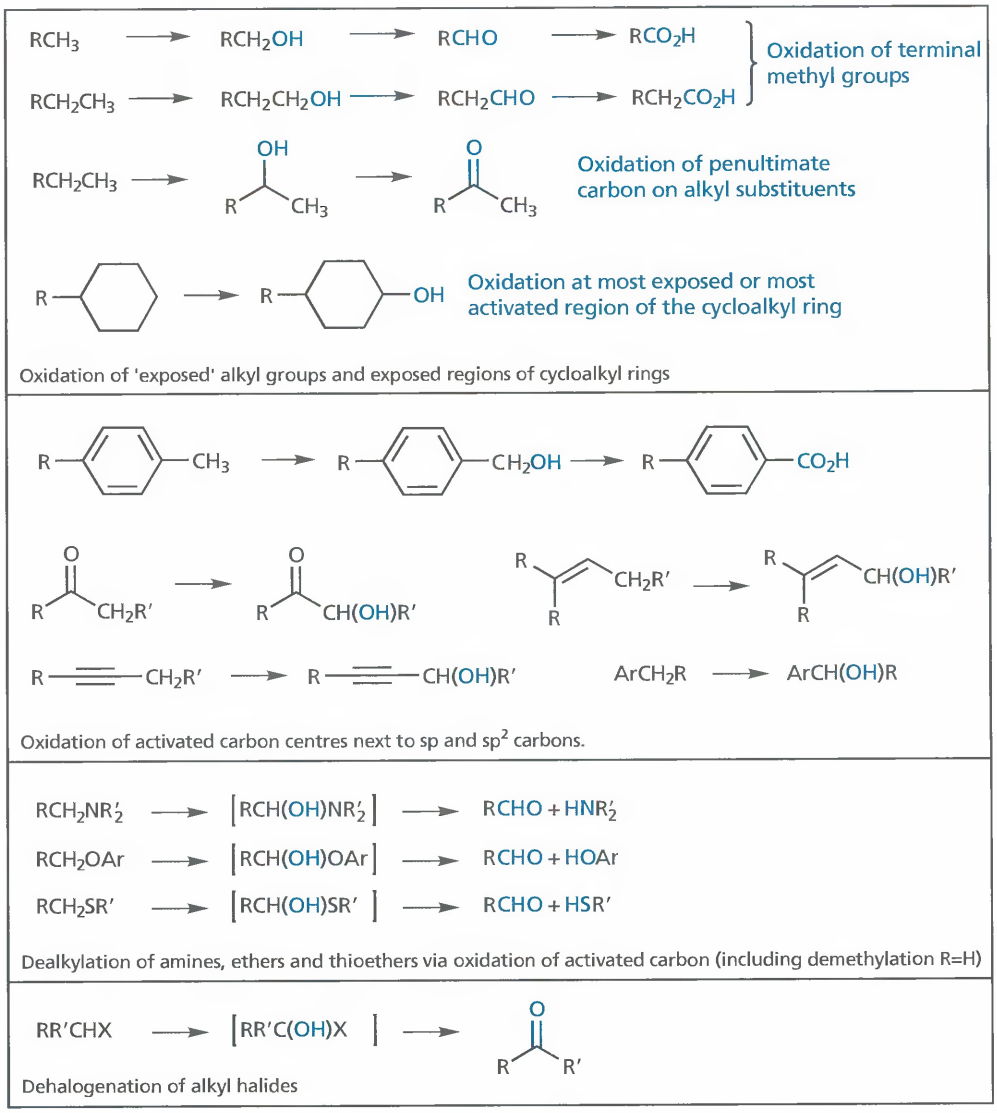


FIGURE 11.4 Oxidative reactions catalysed by cytochrome P450 enzymes on saturated carbon centres.

If this happens, it can act as an alkylating agent and react with nucleophilic groups present in proteins or nucleic acids, leading to toxicity. The oxidation of an aromatic ring results in a similarly reactive epoxide intermediate which can have several possible fates. It may undergo a rearrangement reaction involving a hydride transfer to form a phenol, normally at the *para* position. Alternatively, it may be deactivated by epoxide hydrolase to form a diol or react with **glutathione *S*-transferase** to form a conjugate (section 11.4.5). If the epoxide intermediate evades these enzymes, it may act as an alkylating agent and prove toxic. Electron-rich aromatic rings are likely to be epoxidized more quickly than those with electron-withdrawing substituents, and this has consequences for drug design (e.g. Case Study 6).

Tertiary amines are oxidized to *N*-oxides as long as the alkyl groups are not sterically demanding. Primary and secondary amines are also oxidized to *N*-oxides, but these are rapidly converted to hydroxylamines and beyond. Aromatic primary amines are also oxidized in stages to aromatic nitro groups—a process which is related to the toxicity of aromatic amines since highly electrophilic intermediates are formed which can alkylate proteins or nucleic acids. Aromatic primary amines can also be methylated in a phase II reaction (section 11.4.5) to a secondary amine which can then undergo phase I oxidation to produce formaldehyde and primary hydroxylamines. Primary and secondary amides can be oxidized to hydroxylamides. These functional groups have also been linked with toxicity and carcinogenicity. Thiols can

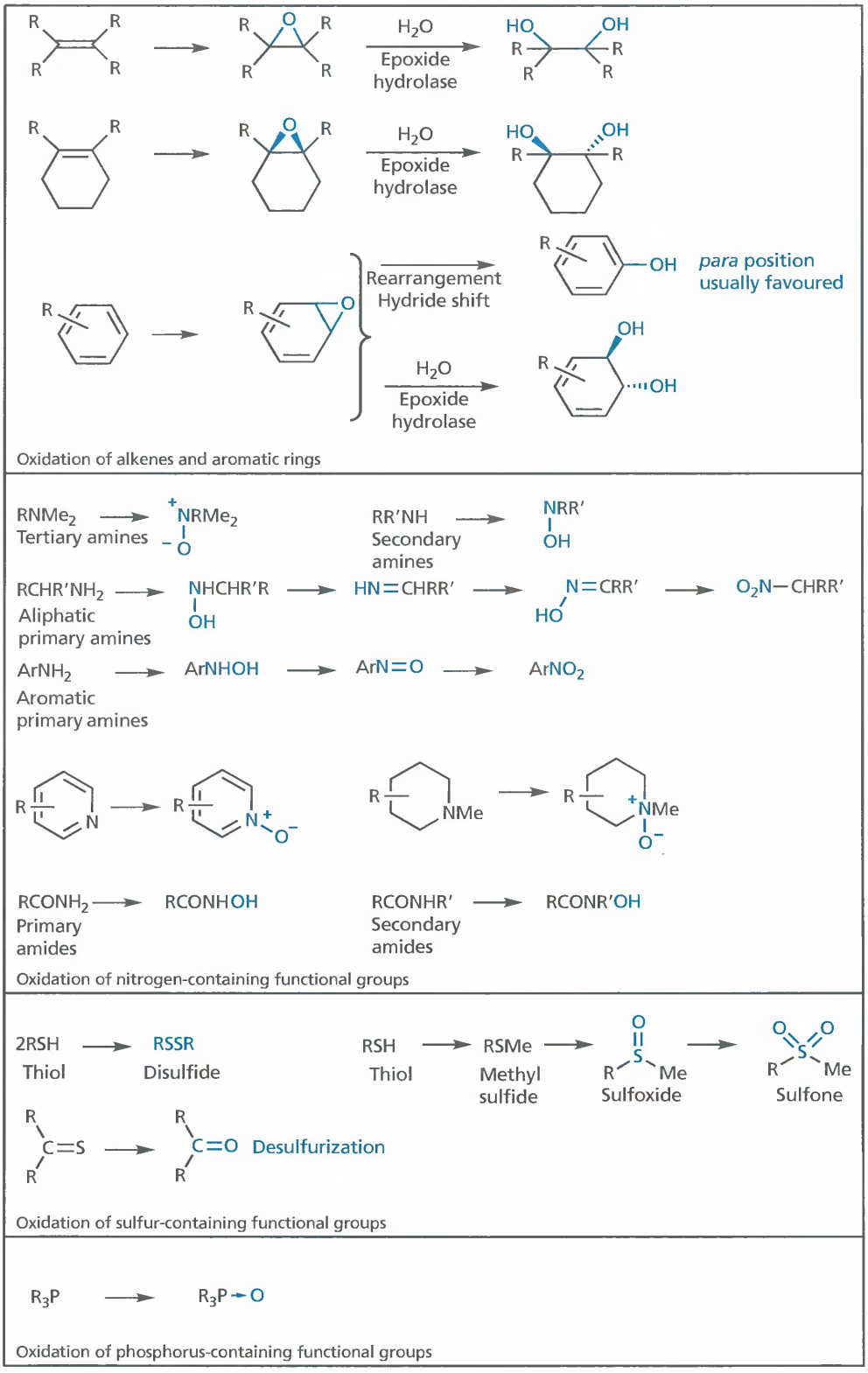


FIGURE 11.5 Oxidative reactions catalysed by cytochrome P450 enzymes on heteroatoms and unsaturated carbon centres.

be oxidized to disulfides. There is evidence that thiols can be methylated to methyl sulfides, which are then oxidized to sulfides and sulfones.

11.4.3 Phase I transformations catalysed by flavin-containing monooxygenases

Another group of metabolic enzymes present in the endoplasmic reticulum of liver cells is the flavin-containing monooxygenases. These enzymes are chiefly responsible for metabolic reactions involving oxidation at nucleophilic nitrogen, sulfur, and phosphorus atoms rather than at carbon atoms. Several examples are given in Fig. 11.6. Many of these reactions are also catalysed by cytochrome P450 enzymes.

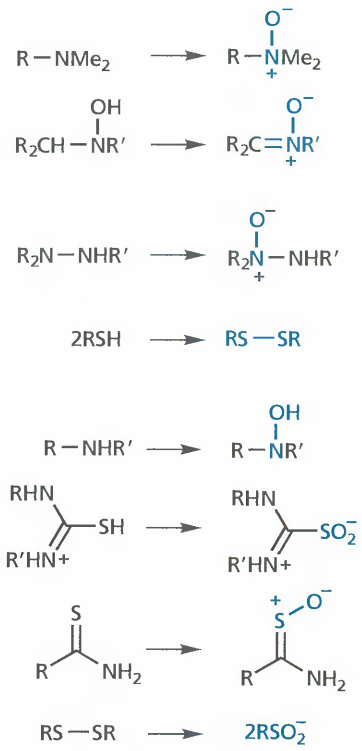


FIGURE 11.6 Phase I reactions catalysed by flavin monooxygenases.

11.4.4 Phase I transformations catalysed by other enzymes

There are several oxidative enzymes in various tissues around the body that are involved in the metabolism of endogenous compounds, but can also play a role in drug metabolism (Fig. 11.7). For example, **monoamine oxidases** are involved in the deamination of catecholamines (section 23.5), but have been observed to oxidize some drugs. Other important oxidative enzymes include alcohol dehydrogenases and aldehyde dehydrogenases. The aldehydes formed by the action of alcohol dehydrogenases on primary alcohols are usually not observed, as they are converted to carboxylic acids by aldehyde dehydrogenases.

Reductive phase I reactions are less common than oxidative reactions, but reductions of aldehyde, ketone, azo, and nitro functional groups have been observed in specific drugs (Fig. 11.8). Many of the oxidation reactions described for heteroatoms in Figs. 11.5–11.7 are reversible, and are catalysed by reductase enzymes. Cytochrome P450 enzymes are involved in catalysing some of these reactions. Remember that enzymes can catalyse a reaction in both directions, depending on the nature of the substrate, and so although cytochrome P450 enzymes are predominantly oxidative enzymes, it is possible for them to catalyse some reductions.

The hydrolysis of esters and amides is a common metabolic reaction, catalysed by esterases and peptidases respectively (Fig. 11.9). These enzymes are present in various organs of the body including the liver. Amides tend to be hydrolysed more slowly than esters. The presence of electron-withdrawing groups can increase the susceptibility of both amides and esters to hydrolysis.

11.4.5 Phase II transformations

Most phase II reactions are **conjugation reactions** catalysed by transferase enzymes. The resulting conjugates are usually inactive, but there are exceptions to this rule. Glucuronic acid conjugation is the most common of these reactions. Phenols, alcohols, hydroxylamines and carboxylic acids form ***O*-glucuronides** by reaction with

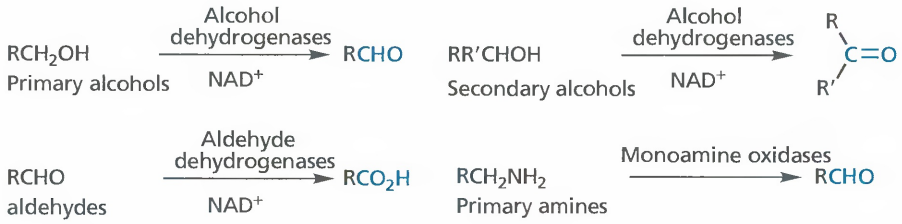


FIGURE 11.7 Phase I oxidative reactions catalysed by miscellaneous enzymes.

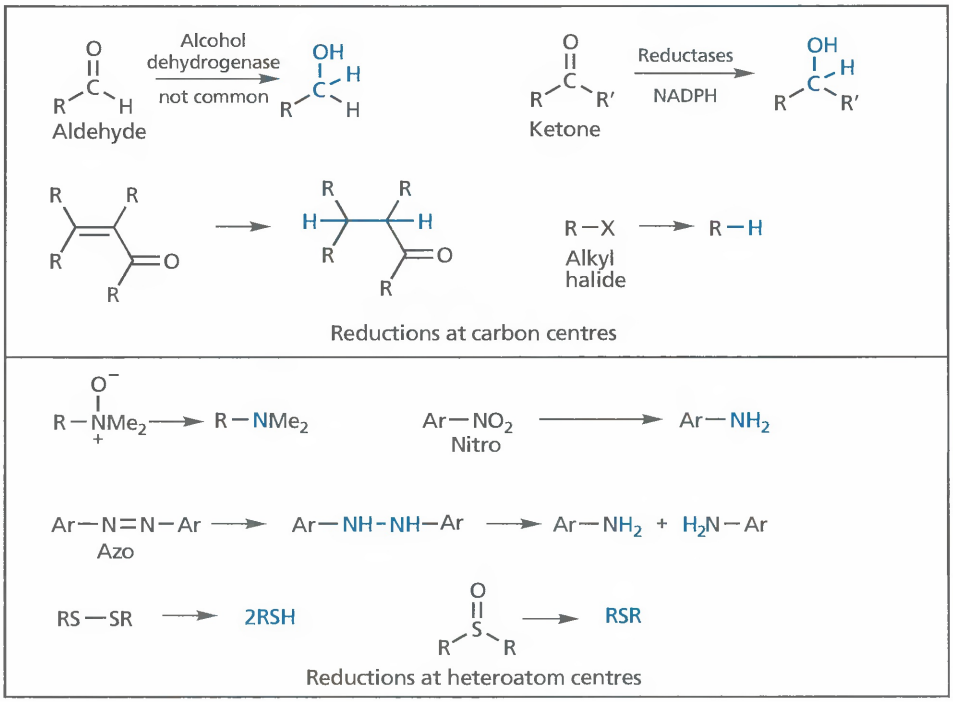


FIGURE 11.8 Phase I reductive reactions.

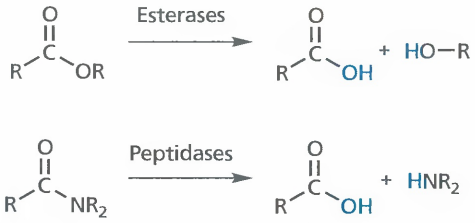


FIGURE 11.9 Hydrolysis of esters and amides.

UDFP-glucuronate such that a highly polar glucuronic acid molecule is attached to the drug (Fig. 11.10). The resulting conjugate is excreted in the urine, but may also be excreted in the bile if the molecular weight is over 300.

A variety of other functional groups such as sulfonamides, amides, amines, and thiols (Fig. 11.11) can react to form *N*- or *S*-glucuronides. *C*-Glucuronides are also possible in situations where there is an activated carbon centre next to carbonyl groups.

Another form of conjugation is sulfate conjugation (Fig. 11.12). This is less common than glucuronation and is restricted mainly to phenols, alcohols, arylamines, and *N*-hydroxy compounds. The reaction is catalysed by **sulfotransferases** using the cofactor 3′-phosphoadenosine 5′-phosphosulfate as the sulfate source. Primary amines, secondary amines, secondary alcohols, and phenols form stable conjugates, whereas primary alcohols form reactive sulfates which can act as toxic alkylating agents. Aromatic hydroxylamines and hydroxylamides also form unstable sulfate conjugates that can be toxic.

Drugs bearing a carboxylic acid group can become conjugated to amino acids by the formation of a peptide link. In most animals, glycine conjugates are generally formed, but L-glutamine is the most common amino acid used for conjugation in primates. The carboxylic acid present in the drug

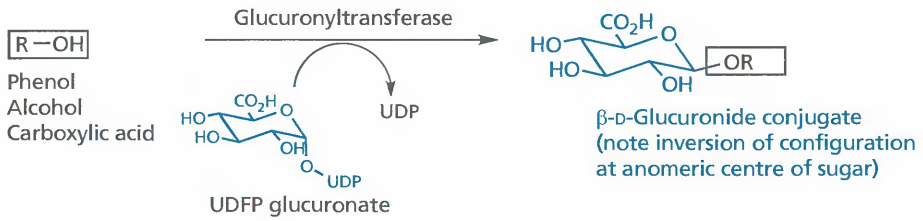


FIGURE 11.10 Glucuronidation of alcohols, phenols and carboxylic acids.

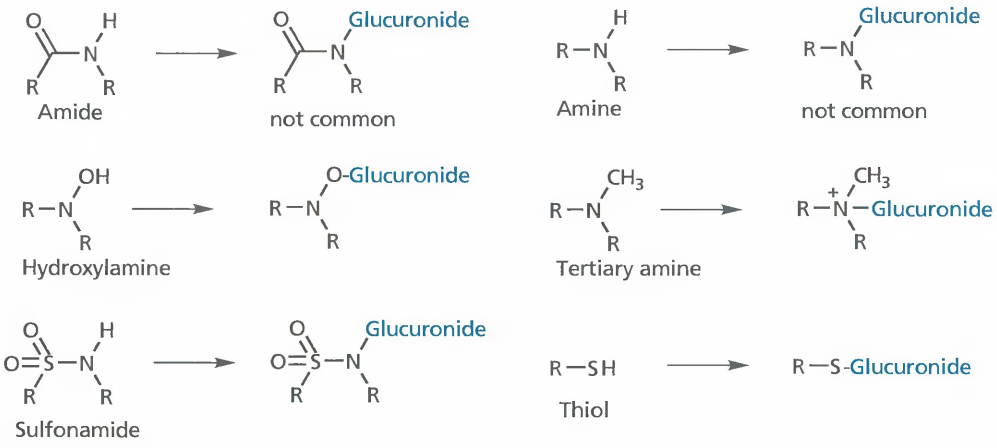


FIGURE 11.11 Glucuronidation of miscellaneous functional groups.

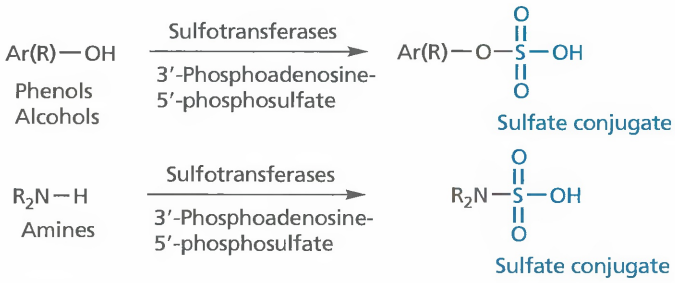


FIGURE 11.12 Examples of sulfoconjugation phase II reactions.

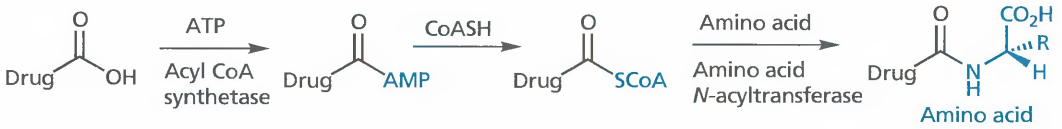


FIGURE 11.13 Formation of amino acid conjugates.

is first activated by formation of a coenzyme A thioester which is then linked to the amino acid (Fig. 11.13).

Electrophilic functional groups such as epoxides, alkyl halides, sulfonates, disulfides, and radical species can react with the nucleophilic thiol group of the tripeptide **glutathione** to give glutathione conjugates which can be subsequently transformed to **mercapturic acids** (Fig. 11.14). The glutathione conjugation reaction can take place in most cells, especially those in the liver and kidney, and is catalysed by **glutathione transferase**. This conjugation reaction is important in detoxifying potentially dangerous environmental toxins or electrophilic alkylating agents formed by phase I reactions (Fig. 11.15). Glutathione conjugates are often excreted in the bile but are more usually converted to mercapturic acid conjugates before excretion.

Not all phase II reactions result in increased polarity. Methylation and acetylation are important phase II reactions which usually *decrease* the polarity of the drug (Fig. 11.16). An important exception is the methylation of pyridine rings, which leads to polar quaternary salts.

The functional groups that are susceptible to methylation are phenols, amines, and thiols. Primary amines are also susceptible to acetylation. The enzyme cofactors involved in contributing the methyl group or acetyl group are ***S*-adenosyl methionine** and **acetyl SCoA** respectively. Several methyltransferase enzymes are involved in the methylation reactions. The most important enzyme for *O*-methylations is catechol *O*-methyltransferase, which preferentially methylates the *meta* position of catechols (section 23.5). It should be pointed out, however, that methylation occurs less frequently than other conjugation reactions and is more important in biosynthetic pathways or the metabolism of endogenous compounds.

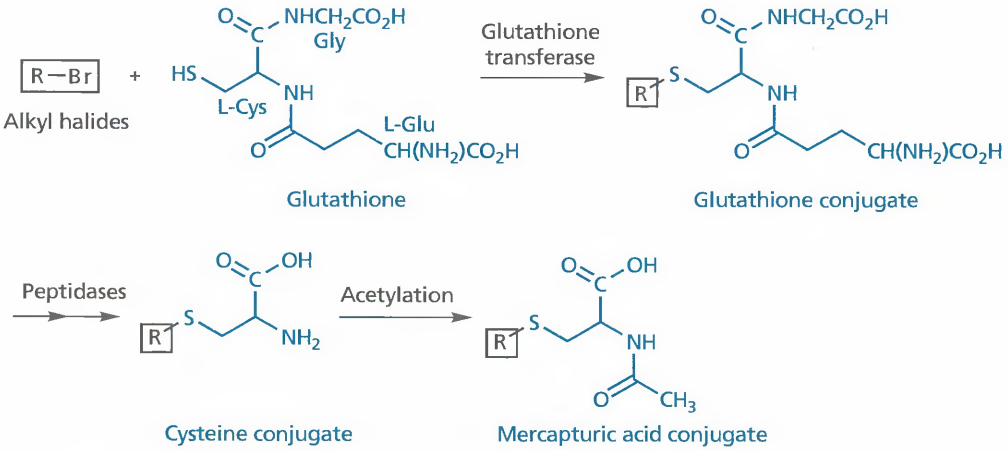


FIGURE 11.14 Formation of glutathione and mercapturic acid conjugates from an alkyl halide.

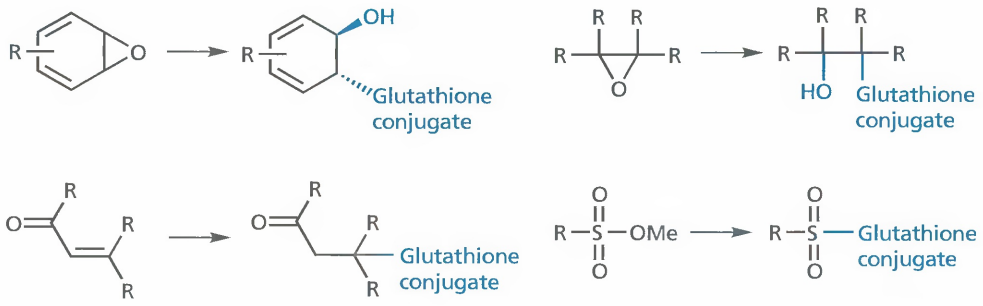


FIGURE 11.15 Formation of glutathione conjugates (Glu–Cys–Gly) with electrophilic groups.

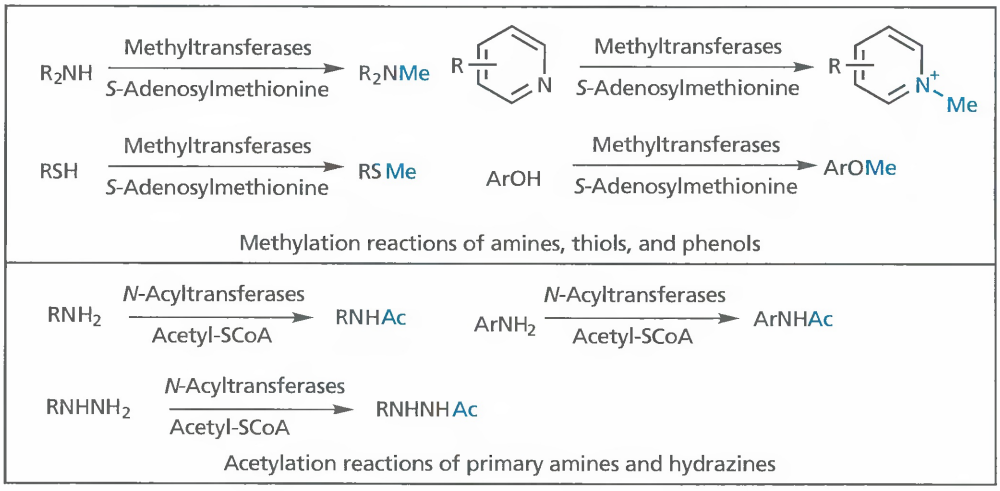


FIGURE 11.16 Methylation and acetylation.

It is possible for drugs bearing carboxylic acids to become conjugated with cholesterol. Cholesterol conjugates can also be formed with drugs bearing an ester group by means of a transesterification reaction. Some drugs with an alcohol functional group form conjugates with fatty acids by means of an ester link.

11.4.6 Metabolic stability

Ideally, a drug should be resistant to drug metabolism because the production of metabolites complicates drug therapy (Box 11.1). For example, the metabolites formed will usually have different properties from the

BOX 11.1 Metabolism of an antiviral agent

Indinavir is an antiviral agent used in the treatment of HIV that is prone to metabolism, resulting in seven different metabolites (Fig. 1). Studies have shown that the CYP3A subfamily of cytochrome P450 enzymes is responsible for six of these metabolites. The metabolites concerned arise from *N*-dealkylation of the piperazine ring, *N*-oxidation of the pyridine ring, *para*-hydroxylation of the phenyl ring, and hydroxylation of the indane ring. The seventh metabolite is a glucuronide conjugate of the pyridine ring. All these reactions occur individually to produce five separate metabolites. The remaining two metabolites arise from two or more metabolic reactions taking place on the same molecule.

The major metabolites are those resulting from dealkylation. As a result, research has been carried out to try and design indinavir analogues that are resistant to this reaction. For example, structures having two methyl substituents on the activated carbon next to pyridine have been effective in blocking dealkylation (Fig. 2).

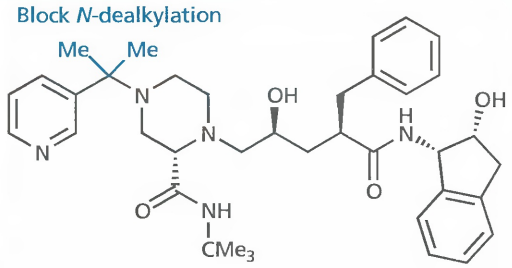


FIGURE 2 Analogue of indinavir resistant to *N*-dealkylation.

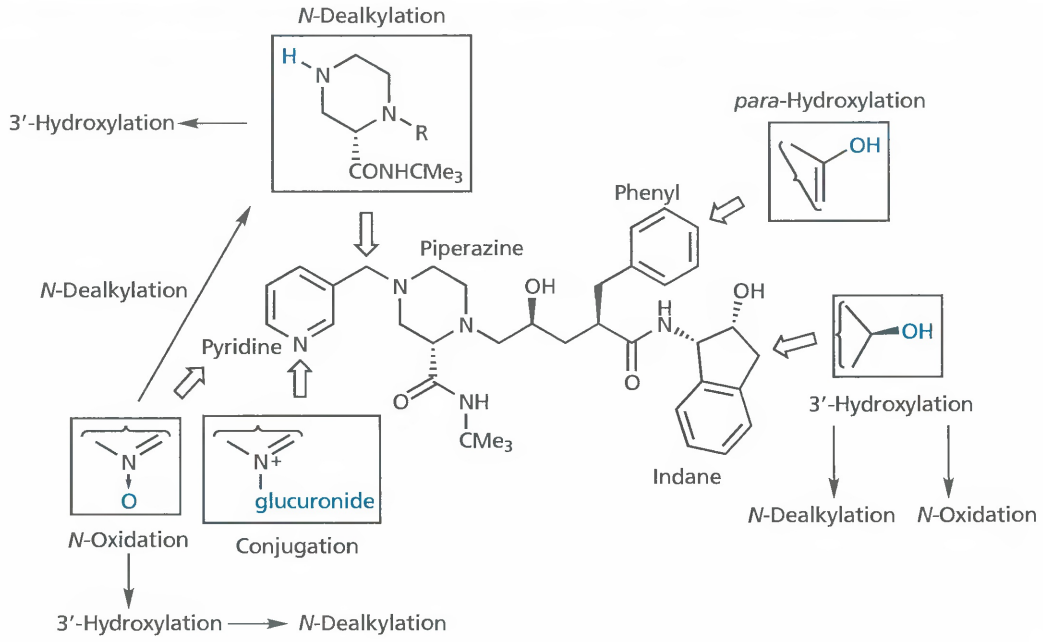


FIGURE 1 Metabolism of indinavir.

original drug. In some cases, activity may be lost. In others, the metabolite may prove to be toxic. For example, the metabolites of **paracetamol** cause liver toxicity, and the carcinogenic properties of some polycyclic hydrocarbons are due to the formation of epoxides.

Another problem arises from the fact that the activity of metabolic enzymes varies from individual to individual. This is especially true of the cytochrome P450 enzymes with at least a 10-fold variability for the most important isoform CYP3A4. Individuals may even lack

particular isoforms. For example, 8% of Americans lack the CYP2D6 isoform, which means that drugs normally metabolized by this enzyme can rise to toxic levels. Examples of drugs that are normally metabolized by this isozyme are **desipramine**, **haloperidol** and **tramadol**. Some prodrugs require metabolism by CYP2D6 in order to be effective. For example, the analgesic effects of **codeine** are due to its metabolism by CYP2D6 to morphine. Therefore, codeine is ineffective in patients lacking this isozyme. The profile of these enzymes in different patients can vary resulting in a difference in the way a drug is metabolized. As a result, the amount of drug that can be safely administered also varies.

Differences across populations can be quite significant resulting in different countries having different recommended dose levels for particular drugs. For example, the rate at which the antibacterial agent **isoniazid** is acetylated and deactivated varies amongst populations. Asian populations acylate the drug at a fast rate whereas 45–65% of Europeans and North Americans have a slow rate of acylation. **Pharmacogenomics** is the study of genetic variations between individuals and how these affect individual responses to drugs. In the future, it is possible that 'fingerprints' of an individual's genome may allow better prediction of which drugs would be suitable for that individual, and which drugs might produce unacceptable side effects. This in turn may avoid drugs having to be withdrawn from the market as a result of rare toxic side effects.

Another complication involving drug metabolism and drug therapy relates to the fact that cytochrome P450 activity can be affected by other chemicals. For example, certain foods have an influence. Brussels sprouts and cigarette smoke enhance activity, whereas grapefruit juice inhibits it. This can have a significant effect on the activity of drugs metabolized by cytochrome P450 enzymes. For example, the immunosuppressant drug **ciclosporin** and the dihydropyridine hypotensive agents are more efficient when taken with grapefruit juice, as their metabolism is reduced. However, serious toxic effects can arise if the antihistamine agent **terfenadine** is taken with grapefruit juice. Terfenadine is actually inactive, but it is metabolized to an active form called **fexofenadine** (Fig. 11.17). If metabolism is inhibited by grapefruit juice, terfenadine persists in the body and can cause serious cardiac toxicity. As a result, fexofenadine itself is now favoured over terfenadine and is marketed as **Allegra**.

Certain drugs are also capable of inhibiting or promoting cytochrome P450 enzymes, leading to a phenomenon known as **drug–drug interaction**, where the presence of one drug affects the activity of another. For example, several antibiotics can act as cytochrome P450 inhibitors and will slow the metabolism of drugs metabolized by these enzymes. Other examples are the drug–drug interactions that occur between the anticoagulant **warfarin** and the barbiturate **phenobarbital** (Fig. 11.17), or between warfarin and the anti-ulcer drug **cimetidine** (section 25.2.7.3).

Phenobarbital stimulates cytochrome P450 enzymes and accelerates the metabolism of warfarin, making it less effective. On the other hand, cimetidine inhibits cytochrome P450 enzymes, thus slowing the metabolism of warfarin. Such drug–drug interactions affect the plasma levels of warfarin and could cause serious problems if the levels move outwith the normal therapeutic range.

Herbal medicine is not immune from this problem either. **St John's wort** is a popular remedy used for mild to moderate depression. However, it promotes the activity of cytochrome P450 enzymes and decreases the effectiveness of contraceptives and warfarin.

Because of the problems caused by cytochrome P450 activation or inhibition, new drugs are usually tested to check whether they have any effect on cytochrome P450 activity or are metabolized themselves by these enzymes.

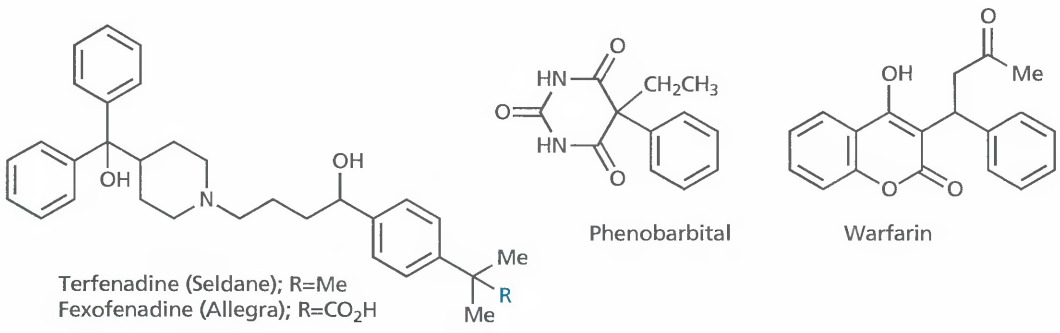


FIG 11.17 Drugs which are metabolized by cytochrome P450 enzymes or affect the activity of cytochrome P450 enzymes.

Indeed, an important goal in many projects is to ensure that such properties are lacking.

Drugs can be defined as hard or soft with respect to their metabolic susceptibility. In this context, **hard drugs** are those that are resistant to metabolism and remain unchanged in the body. **Soft drugs** are designed to have a predictable, controlled metabolism where they are inactivated to non-toxic metabolites and excreted. A group is normally incorporated which is susceptible to metabolism, but will ensure that the drug survives sufficiently long to achieve what it is meant to do before it is metabolized and excreted. Drugs such as these are also called **antedrugs**.

11.4.7 The first pass effect

Drugs that are taken orally pass directly to the liver once they enter the blood supply. Here, they are exposed to drug metabolism before they are distributed around the rest of the body, and so a certain percentage of the drug is transformed before it has the chance to reach its target. This is known as the first pass effect. Drugs that are administered in a different fashion (e.g. injection or inhalation) avoid the first pass effect and are distributed around the body before reaching the liver. Indeed, a certain proportion of the drug may not pass through the liver at all, but may be taken up in other tissues and organs en route.

11.5 Drug excretion

Drugs and their metabolites can be excreted from the body by a number of routes. Volatile or gaseous drugs are excreted through the lungs. Such drugs pass out of the capillaries that line the air sacs (alveoli) of the lungs, then diffuse through the cell membranes of the alveoli into the air sacs, from where they are exhaled. Gaseous **general anaesthetics** are excreted in this way and move down a concentration gradient from the blood supply into the lungs. They are also administered through the lungs, in which case the concentration gradient is in the opposite direction and the gas moves from the lungs to the blood supply.

The **bile duct** travels from the liver to the intestines and carries a greenish fluid called **bile** which contains bile acids and salts that are important to the digestion process. A small number of drugs are diverted from the blood supply back into the intestines by this route. Since this happens from the liver, any drug eliminated in this way has not been distributed round the body. Therefore, the amount of drug distributed is less than that absorbed. However, once the drug has entered the intestine, it can be reabsorbed so it has another chance.

It is possible for as much as 10–15% of a drug to be lost through the skin in **sweat**. Drugs can also be excreted through saliva and breast milk, but these are minor excretion routes compared with the kidneys. There are concerns, however, that mothers may be passing on drugs such as **nicotine** to their baby through breast milk.

The **kidneys** are the principal route by which drugs and their metabolites are excreted (Fig. 11.18). The kidneys filter the blood of waste chemicals and these chemicals are subsequently removed in the urine. Drugs and their metabolites are excreted by the same mechanism.

Blood enters the kidneys by means of the **renal artery**. This divides into a large number of capillaries, each one of which forms a knotted structure called a **glomerulus** that fits into the opening of a duct called a **nephron**. The blood entering these glomeruli is under pressure, and so plasma is forced through the pores in the capillary walls into the nephron, carrying with it any drugs and metabolites that might be present. Any compounds that are too big to pass through the pores, such as plasma proteins and red blood cells, remain in the capillaries with the remaining plasma. Note that this is a filtration process, so it does not matter whether the drug is polar or hydrophobic: all drugs and drug metabolites will be passed equally efficiently into the nephron. However, this does not mean that every compound will be *excreted* equally efficiently, because there is more to the process than simple filtration.

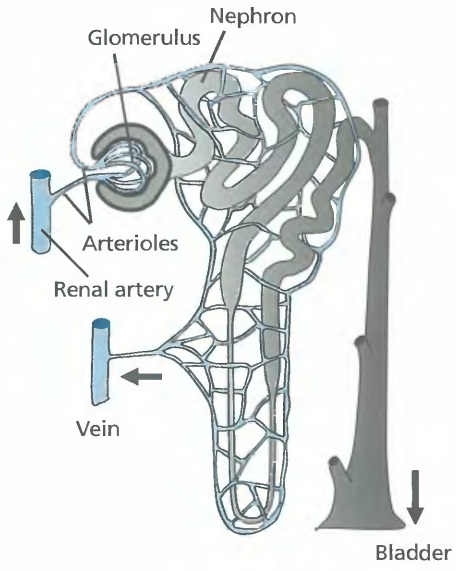


FIGURE 11.18 Excretion by the kidneys.

The filtered plasma and chemicals now pass through the nephron on their route to the bladder. However, only a small proportion of what starts that journey actually finishes it. This is because the nephron is surrounded by a rich network of blood vessels carrying the filtered blood away from the glomerulus, permitting much of the contents of the nephron to be reabsorbed into the blood supply. Most of the water that was filtered into the nephron is quickly reabsorbed through pores in the nephron cell membrane which are specific for water molecules, and bar the passage of ions or other molecules. These pores are made up of protein molecules called **aquaporins**. As water is reabsorbed, drugs and other agents are concentrated in the nephron and a concentration gradient is set up. There is now a driving force for compounds to move back into the blood supply down the concentration gradient. However, this can only happen if the drug is sufficiently hydrophobic to pass through the cell membranes of the nephron. This means that hydrophobic compounds are efficiently reabsorbed back into the blood, whereas polar compounds are not and are excreted. This process of excretion explains the importance of drug metabolism to drug excretion. Drug metabolism makes a drug more polar so that it is less likely to be reabsorbed from the nephrons.

Some drugs are actively transported from blood vessels into the nephrons. This process is called **facilitated transport**, and is important in the excretion of penicillins (section 19.5.1.9).

KEY POINTS

- Drugs are exposed to enzyme-catalysed reactions which modify their structure. This is called drug metabolism and can take place in various tissues. However, most reactions occur in the liver.
- Orally taken drugs are subject to the first pass effect.
- Drugs administered by methods other than the oral route avoid the first pass effect.
- Phase I metabolic reactions typically involve the addition or exposure of a polar functional group. Cytochrome P450 enzymes present in the liver carry out important phase I oxidation reactions. The types of cytochrome P450 enzymes present vary between individuals, leading to varying rates of drug metabolism.
- The activity of cytochrome P450 enzymes can be affected by food, chemicals, and drugs, resulting in drug–drug interactions and possible side effects.
- Phase II metabolic reactions involve the addition of a highly polar molecule to a functional group. The resulting conjugates are more easily excreted.
- Drug excretion can take place through sweat, exhaled air or bile, but most excretion takes place through the kidneys.
- The kidneys filter blood such that drugs and their metabolites enter nephrons. Non-polar substances are reabsorbed into the blood supply, but polar substances are retained in the nephrons and excreted in the urine.

11.6 Drug administration

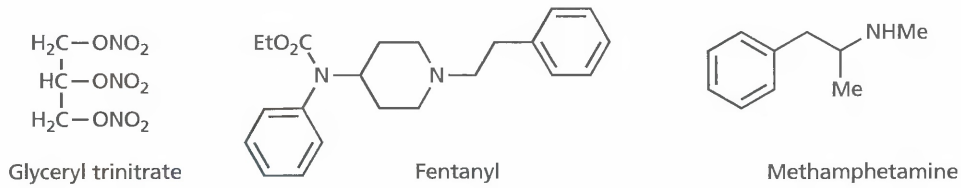
There is a large variety of ways in which drugs can be administered, and many of these avoid some of the problems associated with oral administration. The main routes are: oral, sublingual, rectal, epithelial, inhalation, and injection. The method chosen will depend on the target organ and the pharmacokinetics of the drug.

11.6.1 Oral administration

Orally administered drugs are taken by mouth. This is the preferred option for most patients, so there is more chance that the patient will comply with the drug regimen and complete the course. However, the oral route places the greatest demands on the chemical and physical properties of the drug as described above.

Drugs given orally can be taken as pills, capsules, or solutions. Drugs taken in solution are absorbed more quickly and a certain percentage may even be absorbed through the stomach wall. For example, approximately 25–33% of **alcohol** is absorbed into the blood supply from the stomach; the rest is absorbed from the upper intestine. Drugs taken as pills or capsules are mostly absorbed in the upper intestine. The rate of absorption is partly determined by the rate at which the pills and capsules dissolve. This in turn depends on such factors as particle size and crystal form. In general, about 75% of an orally administered drug is absorbed into the body within 1–3 hours. Specially designed pills and capsules can remain intact in the stomach to help protect acid-labile drugs from stomach acids. The containers then degrade once they reach the intestine.

Care has to be taken if drugs interact with food. For example, **tetracycline** binds strongly to calcium ions, which inhibits absorption, so foods such as milk should be avoided. Some drugs bind other drugs and prevent absorption. For example, **colestyramine** (used to lower cholesterol levels) binds to **warfarin** and also to the thyroid drug **levothyroxine sodium**, so these drugs should be taken separately.



Glyceryl trinitrate Fentanyl Methamphetamine

FIGURE 11.19 Glyceryl trinitrate, fentanyl and methamphetamine.

11.6.2 Absorption through mucous membranes

Some drugs can be absorbed through the mucous membranes of the mouth or nose, thus avoiding the digestive and metabolic enzymes encountered during oral administration. For example, heart patients take **glyceryl trinitrate** (Fig. 11.19) by placing it under the tongue (sublingual administration). The opiate analgesic **fentanyl** (Fig. 11.19) has been given to children in the form of a lollipop, and is absorbed through the mucous membranes of the mouth. The Incas absorbed **cocaine** sublingually by chewing coca leaves.

Nasal decongestants are absorbed through the mucous membranes of the nose. Cocaine powder is absorbed in this way when it is sniffed, as is **nicotine** in the form of snuff. Nasal sprays have been used to administer analogues of peptide hormones such as **anti-diuretic hormone**. These drugs would be quickly degraded if taken orally.

Eye drops are used to administer drugs directly to the eye and thus reduce the possibility of side effects elsewhere in the body. For example, the eye condition known as glaucoma is treated in this way. Nevertheless, some absorption into the blood supply can still occur and some asthmatic patients suffer bronchospasms when taking **timolol** eye drops.

11.6.3 Rectal administration

Some drugs are administered rectally as **suppositories**, especially if the patient is unconscious, vomiting, or unable to swallow. However, there are several problems associated with rectal administration: the patient may suffer membrane irritation, and although the extent of drug absorption is efficient, it can be unpredictable. It is not the most popular of methods with patients either!

11.6.4 Topical administration

Topical drugs are those which are applied to the skin. For example, steroids are applied topically to treat local skin irritations. It is also possible for some of the drug to be absorbed through the skin (**transdermal absorption**) and to enter the blood supply, especially if the drug is lipophilic. **Nicotine patches** work in this fashion, as do hormone replacement therapies for **oestrogen**. Drugs are absorbed by this method at a steady rate, and avoid the acidity of the stomach, or the enzymes in the gut or gut wall. Other drugs that have been applied in this way include the analgesic **fentanyl**, and the antihypertensive agent **clonidine**. Once applied, the drug is slowly released from the patch and absorbed through the skin into the blood supply over several days. As a result, the level of drug remains relatively constant over that period.

A technique known as **iontophoresis** is being investigated as a means of topical administration. Two miniature electrode patches are applied to the skin and linked to a reservoir of the drug. A painless pulse of electricity is applied, which has the effect of making the skin more permeable to drug absorption. By timing the electrical pulses correctly, the drug can be administered such that fluctuations in blood levels are kept to a minimum. Similar devices are being investigated which use ultrasound to increase skin permeability.

11.6.5 Inhalation

Drugs administered by inhalation avoid the digestive and metabolic enzymes of the GIT or liver. Once inhaled, the drugs are absorbed through the cell linings of the respiratory tract into the blood supply. Assuming the drug is able to pass through the hydrophobic cell membranes, absorption is rapid and efficient because the blood supply is in close contact with the cell membranes of the lungs. For example, **general anaesthetic gases** are small, highly lipid-soluble molecules which are absorbed almost as fast as they are inhaled.

Non-gaseous drugs can be administered as **aerosols**. This is how antiasthmatic drugs are administered, and it allows them to be delivered to the lungs in far greater quantities than if they were given orally or by injection. In the case of anti-asthmatics, the drug is made sufficiently polar that it is poorly absorbed into the bloodstream. This localizes it in the airways, and lowers the possibility of side effects elsewhere in the body (e.g. action on the heart). However, a certain percentage of an inhaled drug is inevitably swallowed and can reach

the blood supply by the oral route. This may lead to side effects. For example, tremor is a side effect of the anti-asthmatic **salbutamol** as a result of the drug reaching the blood supply.

Several drugs of abuse are absorbed through inhalation or smoking (e.g. **nicotine**, **cocaine**, **marijuana**, **methamphetamine** (Fig. 11.19), and **heroin**). Smoking is a particularly hazardous method of taking drugs. A normal cigarette is like a mini-furnace producing a complex mixture of potentially carcinogenic compounds, especially from the tars present in tobacco. These are not absorbed into the blood supply, but coat the lung tissue, leading to long-term problems such as lung cancer. The tars in cannabis are considerably more dangerous than those in tobacco. If cannabis is to be used in medicine, safer methods of administration are desirable (i.e. inhalers).

11.6.6 Injection

Drugs can be introduced into the body by intravenous, intramuscular, subcutaneous, or intrathecal injection. Injection of a drug produces a much faster response than oral administration because the drug reaches the blood supply more quickly. The levels of drug administered are also more accurate, because absorption by the oral route has a level of unpredictability due to the first pass effect. Injecting a drug, however, is potentially more hazardous. For example, some patients may have an unexpected reaction to a drug, and there is little that can be done to reduce the levels once the drug has been injected. Such side effects would be more gradual and treatable if the drug was given orally. Secondly, sterile techniques are essential when giving injections, to avoid the risks of bacterial infection or of transmitting hepatitis or AIDS from a previous patient. Finally, there is a greater risk of receiving an overdose when injecting a drug.

The **intravenous** route involves injecting a solution of the drug directly into a vein. This method of administration is not particularly popular with patients, but it is a highly effective method of administering drugs in accurate doses and it is the fastest of the injection methods. However, it is also the most hazardous method of injection. Since its effects are rapid, the onset of any serious side effects or allergies is also rapid. It is important, therefore, to administer the drug as slowly as possible and to monitor the patient closely. An intravenous drip allows the drug to be administered in a controlled manner such that there is a steady level of drug in the system. The local anaesthetic **lidocaine** is given by intravenous injection. Drugs that are dissolved in oily liquids cannot be given by intravenous injection as this may result in the formation of blood clots.

The **intramuscular** route involves injecting drugs directly into muscle, usually in the arm, thigh, or buttocks. Drugs administered in this way do not pass round the body as rapidly as they would if given by intravenous injection, but they are still absorbed faster than by oral administration. The rate of absorption depends on various factors such as the diffusion of the drug, blood supply to the muscle, the solubility of the drug, and the volume of the injection. Local blood flow can be reduced by adding adrenaline to constrict blood vessels. Diffusion can be slowed by using a poorly absorbed salt, ester, or complex of the drug (section 14.6.2). The advantage of slowing down absorption is in prolonging activity. For example, oily suspensions of steroid hormone esters are used to slow absorption. Drugs are often administered by intramuscular injection when they are unsuitable for intravenous injection, and so it is important to avoid injecting into a vein.

Subcutaneous injection involves injecting the drug under the surface of the skin. Absorption depends on factors such as how fast the drug diffuses, the level of blood supply to the skin, and the ability of the drug to enter the blood vessels. Absorption can be slowed by the same methods described for intramuscular injection. Drugs which can act as irritants should not be administered in this way as they can cause severe pain and may damage local tissues.

Intrathecal injection means that the drug is injected into the spinal cord. Antibacterial agents that do not normally cross the blood–brain barrier are often administered in this way. Intrathecal injections are also used to administer **methotrexate** in the treatment of childhood leukaemia in order to prevent relapse in the CNS.

Intraperitoneal injection involves injecting drugs directly into the abdominal cavity. This is very rarely used in medicine, but it is a method of injecting drugs into animals during preclinical tests.

11.6.7 Implants

Continuous osmotically driven minipumps for **insulin** have been developed which are implanted under the skin. The pumps monitor the level of insulin in the blood and release the hormone as required to keep levels constant. This avoids the problem of large fluctuations in insulin levels associated with regular injections.

Gliadel is a wafer that has been implanted into the brain to administer anticancer drugs direct to brain tumours, thus avoiding the blood–brain barrier.

Polymer-coated, drug-releasing stents have been used to keep blood vessels open after a clot clearing procedure called angioplasty.

Investigations are underway into the use of implantable microchips which could detect chemical signals in the body and release drugs in response to these signals.

KEY POINTS

- Oral administration is the preferred method of administering drugs, but it is also the most demanding on the drug.
- Drugs administered by methods other than the oral route avoid the first pass effect.
- Drugs can be administered such that they are absorbed through the mucous membranes of the mouth, nose, or eyes.
- Some drugs are administered rectally as suppositories.
- Topically administered drugs are applied to the skin. Some drugs are absorbed through the skin into the blood supply.
- Inhaled drugs are administered as gases or aerosols to act directly on the respiratory system. Some inhaled drugs are absorbed into the blood supply to act systemically.
- Polar drugs, which are unable to cross cell membranes, are given by injection.
- Injection is the most efficient method of administering a drug but it is also the most hazardous. Injection can be intravenous, intramuscular, subcutaneous, or intrathecal.
- Implants have been useful in providing controlled drug release such that blood concentrations of the drug remain as level as possible.

11.7 Drug dosing

Because of the number of pharmacokinetic variables involved, it can be difficult to estimate the correct dose regimen for a drug (i.e. the amount of drug used for each dose and the frequency of administration). There are other issues to consider as well. Ideally, the blood levels of any drug should be constant and controlled, but this would require a continuous, intravenous drip which is clearly impractical for most drugs. Therefore, drugs are usually taken at regular time intervals, and the doses taken are designed to keep the blood levels of drug within a maximum and minimum level such that they are not too high to be toxic, yet not too low to be ineffective. In general, the concentration of free drug in the blood (i.e. not bound to plasma protein) is a good indication of the availability of that drug at its target site. This does not mean that blood concentration levels are the same as the concentration levels at the target site. However, any variations in blood concentration will result in similar fluctuations at the target site. Thus, blood concentration levels can be used to determine therapeutic and safe dosing levels for a drug.

Figure 11.20 shows two dose regimens. Dose regimen A quickly reaches the therapeutic level but continues to rise to a steady state which is toxic. Dose regimen B involves half the amount of drug provided with the same frequency. The time taken to reach the therapeutic level is certainly longer, but the steady state levels of the drug remain between the therapeutic and toxic levels—the **therapeutic window**.

Dose regimens involving regular administration of a drug work well in most cases, especially if the size of each dose is less than 200 mg and doses are taken once or twice a day. However, there are certain situations where timed doses are not suitable. The treatment of diabetes with **insulin** is a case in point. Insulin is normally secreted continuously by the pancreas, so the injection of insulin at timed intervals is unnatural and can lead to a whole range of physiological complications.

Other dosing complications include differences of age, sex, and race. Diet, environment, and altitude also have an influence. Obese people present a particular problem, as it can be very difficult to estimate how much of a drug will be stored in fat tissue and how much will remain free in the blood supply. The precise time when drugs

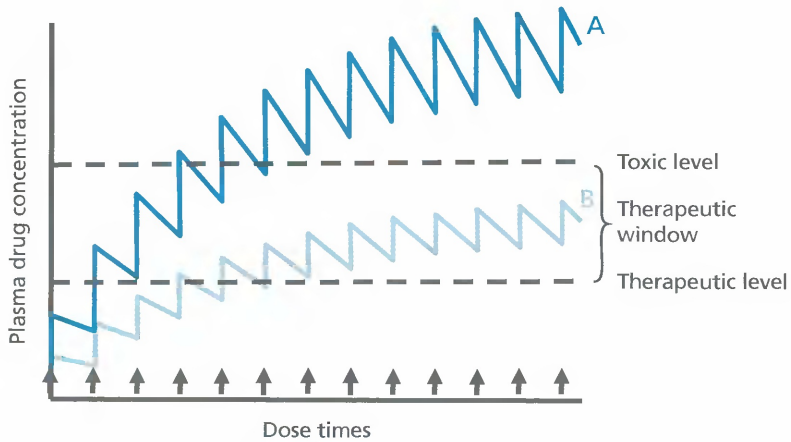


FIGURE 11.20 Dose regimens.

TABLE 11.1 Fluctuation of drug concentration levels on regular dosing

Time of dosing (hour)	0	4	8	12	16	20	24
Max level (μg/ml)	1.0	1.5	1.75	1.87	1.94	1.97	1.98
Min level (μg/ml)	0.5	0.75	0.87	0.94	0.97	0.98	0.99

are taken may be important, because metabolic reaction rates can vary throughout the day.

Drugs can interact with other drugs. For example, some drugs used for diabetes are bound by plasma protein in the blood supply and are therefore not free to react with their targets. However, drugs such as **aspirin** may displace them from plasma protein, leading to a drug overdose. Aspirin has this same effect on anticoagulants.

Problems can also occur if a drug inhibits a metabolic reaction and is taken with a drug normally metabolized by that reaction. The latter is more slowly metabolized than normal, increasing the risk of an overdose. For example, the antidepressant drug **phenelzine** inhibits the metabolism of amines and should not be taken with drugs such as **amphetamines** or **pethidine**. Even amine-rich foods can lead to adverse effects, implying that cheese and wine parties are hardly the way to cheer the victim of depression. Other examples were described in section 11.4.6.

When one considers all these complications, it is hardly surprising that individual variability to drugs can vary by as much as a factor of 10.

11.7.1 Drug half-life

The half-life ($t_{1/2}$) of a drug is the time taken for the concentration of the drug in blood to fall by half. The removal or elimination of a drug takes place through both excretion and drug metabolism, and is not linear with time. Therefore, drugs can linger in the body for a significant period of time. For example, if a drug has a half-life of 1 hour, then there is 50% of it left after 1 hour. After 2 hours, there is 25% of the original dose left, and after 3 hours, 12.5% remains. It takes 7 hours for the level to fall below 1% of the original dose. Some drugs, such as the opioid analgesic **fentanyl**, have short half-lives (45 minutes), whereas others such as **diazepam** (Valium) have a half-life measured in days. In the latter case, recovery from the drug may take a week or more.

11.7.2 Steady-state concentration

Drugs are metabolized and eliminated as soon as they are administered, so it is necessary to provide regular doses in order to maintain therapeutic levels in the body. It is important, therefore, to know the half-life of the drug in order to calculate the frequency of dosing required to reach and maintain these levels. In general, the time

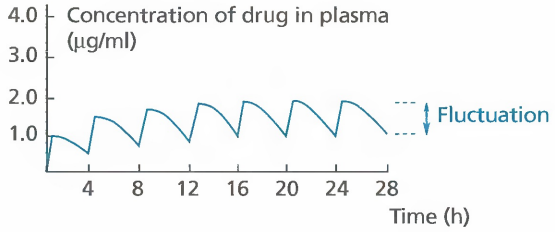


FIGURE 11.21 Graphical representation of fluctuation of drug concentration levels on regular dosing.

taken to reach a **steady-state concentration** is six times the drug's half-life. For example, the concentration levels of a drug with a half-life of 4 hours, supplied at 4-hourly intervals, is shown in Table 11.1 and Figure 11.21.

Note that there is a fluctuation in level in the period between each dose. The level is at a maximum after each dose, and falls to a minimum before the next dose is provided. It is important to ensure that the level does not drop below the therapeutic level, but does not rise to such a level that side effects are induced. The time taken to reach steady state concentration is not dependent on the size of the dose, but the blood level achieved at steady state is. Therefore, the levels of drug present at steady state concentration depend on the size of each dose given, as well as the frequency of dosing. During clinical trials, blood samples are taken from patients at regular time intervals to determine the concentration of the drug in the blood. This helps determine the proper dosing regime in order to get the ideal blood levels.

The area under the plasma drug concentration curve (AUC) represents the total amount of drug that is available in the blood supply during the dosing regime.

11.7.3 Drug tolerance

With certain drugs, it is found that the effect of the drug diminishes after repeated doses, and it is necessary to increase the size of the dose in order to achieve the same results. This is known as drug tolerance. There are several mechanisms by which drug tolerance can occur. For example, the drug can induce the synthesis of metabolic enzymes which result in increased metabolism of the drug. **Pentobarbital** (Fig. 11.22) is a barbiturate sedative which induces enzymes in this fashion.

Alternatively, the target may adapt to the presence of a drug. Occupancy of a target receptor by an antagonist

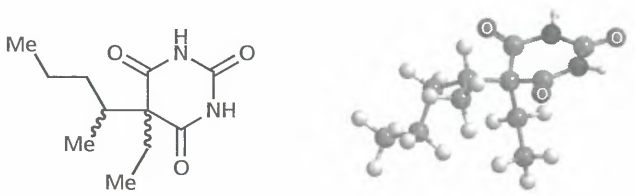


FIGURE 11.22 Pentobarbital.

may induce cellular effects which result in the synthesis of more receptor (section 8.7). As a result, more drug will be needed in the next dose to antagonize all the receptors.

Physical dependence is usually associated with drug tolerance. Physical dependence is a state in which a patient becomes dependent on the drug in order to feel normal. If the drug is withdrawn, uncomfortable **withdrawal symptoms** may arise which can only be alleviated by re-taking the drug. These effects can be explained in part by the effects which lead to drug tolerance. For example, if cells have synthesized more receptors to counteract the presence of an antagonist, the removal of the antagonist means that the body will have too many receptors. This results in a 'kickback' effect where the cell becomes oversensitive to the normal neurotransmitter or hormone, and this is what produces withdrawal symptoms. These will continue until the excess receptors have been broken down by normal cellular mechanisms—a process that may take several days or weeks (see also sections 8.6–8.7).

11.7.4 Bioavailability

Bioavailability refers to how quickly and how much of a particular drug reaches the blood supply once all the problems associated with absorption, distribution, metabolism, and excretion have been taken into account. Oral bioavailability (F) is the fraction of the ingested dose that survives to reach the blood supply. This is an important property when it comes to designing new drugs and should be considered alongside the pharmacodynamics of the drug (i.e. how effectively the drug interacts with its target).

11.8 Formulation

The way a drug is formulated can avoid some of the problems associated with oral administration. Drugs are normally taken orally as tablets or capsules. A tablet is usually a compressed preparation that contains 5–10% of the drug, 80% of fillers, disintegrants, lubricants, glidants, and binders, and 10% of compounds which ensure easy disintegration, disaggregation, and dissolution of the tablet in the stomach or the intestine. The disintegration time can be modified for a rapid effect or for sustained release. Special coatings can make the tablet resistant to the stomach acids such that it only disintegrates in the duodenum as a result of enzyme action or alkaline pH. Pills can also be coated with sugar, varnish, or wax to disguise taste. Some tablets are designed with an osmotically active core, surrounded by an impermeable membrane with a pore in it. This allows the drug to percolate out from the tablet at a constant rate as the tablet moves through the digestive tract.

A capsule is a gelatinous envelope enclosing the active substance. Capsules can be designed to remain intact for some hours after ingestion in order to delay absorption. They may also contain a mixture of slow-release and fast-release particles to produce rapid and sustained absorption in the same dose.

The drug itself needs to dissolve in aqueous solution at a controlled rate. Such factors as particle size and crystal form can significantly affect dissolution. Fast dissolution is not always ideal. For example, slow dissolution rates can prolong the duration of action or avoid initial high plasma levels.

11.9 Drug delivery

The various aspects of drug delivery could fill a textbook in itself, so any attempt to cover the topic in a single section is merely tickling the surface, let alone scratching it! However, it is worth appreciating that there are various methods by which drugs can be physically protected from degradation and/or targeted to treat particular diseases such as cancer and inflammation. One approach is to use a prodrug strategy (section 14.6), which involves chemical modifications to the drug. Another approach covered in this section is the use of water-soluble macromolecules to help the drug reach its target. The macromolecules concerned are many and varied and include synthetic polymers, proteins, liposomes, and antibodies. The drug itself may be covalently linked to the macromolecule or encapsulated within it. The following are some illustrations of drug delivery systems.

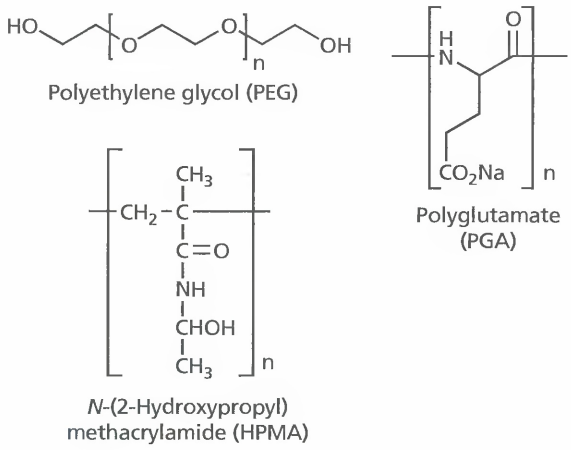


FIGURE 11.23 Synthetic polymers used for polymer–drug conjugates.

Antibodies were described in section 10.7.2 and have long been seen as a method of targeting drugs to cancer cells. Methods have been devised of linking anticancer drugs to antibodies to form **antibody–drug conjugates** that remain stable on their journey through the body but release the drug at the target cell. A lot of research has been carried out on these conjugates, and this is discussed in detail in section 21.9.2. However, there are problems associated with antibodies. The amount of drug that can be linked to the protein is quite limited and there is the risk of an immune reaction where the body identifies the antibody as foreign and tries to reject it.

A similar approach is to link the drugs to synthetic polymers such as polyethylene glycol (PEG), polyglutamate or *N*-(2-hydroxypropyl) methacrylamide (HPMA) to form polymer-drug conjugates (Fig. 11.23). Again the amount of drug that can be linked is limited, but a variety of anticancer-polymer conjugates are currently undergoing clinical trials. Such conjugates help to protect the lifetime of the drug by decreasing the rates of metabolism and excretion. **Pegaptanib** is a preparation that was approved for treating a vascular disease in the eye and consists of an oligonucleotide drug linked to PEG (section 10.5).

Protein-based polymers are being developed as drug delivery systems for the controlled release of ionized drugs. For example, the cationic drugs **Leu-enkephalin** or **naltrexone** could be delivered using polymers with anionic carboxylate groups. Ionic interactions between the drug and the protein result in folding and assembly of the protein polymer to form a protein–drug complex and the drug is then released at a slow and constant rate. The amount of drug carried could be predetermined by the density of carboxylate binding sites present and the accessible surface area of the vehicle. The rate of release could be controlled by varying the number of hydrophobic amino acids present. The greater the number of hydrophobic amino acids present, the weaker the affinity between the carboxylate binding groups and the drug. Once the drug is released, the protein carrier would be metabolized like any normal protein.

A physical method of protecting drugs from metabolic enzymes in the bloodstream and allowing a steady slow release of the drug is to encapsulate the drug within small vesicles called **liposomes** and then inject them into the blood supply (Fig. 11.24). These vesicles or globules consist of a bilayer of fatty phospholipid molecules (similar to a cell membrane) and will travel round the circulation, slowly leaking their contents. Liposomes are known to be concentrated in malignant tumours and this provides a possible method of delivering antitumour drugs to these cells. It is also found that liposomes can fuse with the plasma membranes of a variety of cells, allowing the delivery of drugs or DNA into these cells. As a result, they may be useful for gene therapy. The liposomes can be formed by sonicating a suspension of a phospholipid (e.g. phosphatidylcholine) in an aqueous solution of the drug.

Another future possibility for targeting liposomes is to incorporate antibodies into the liposome surface such that specific tissue antigens are recognized. Liposomes have a high drug-carrying capacity, but it can prove difficult to control the release of drug at the required rate and the slow leakage is a problem if the liposome is carrying a toxic anticancer drug such as **doxorubicin**. The liposomes can also be trapped by the reticuloendothelial

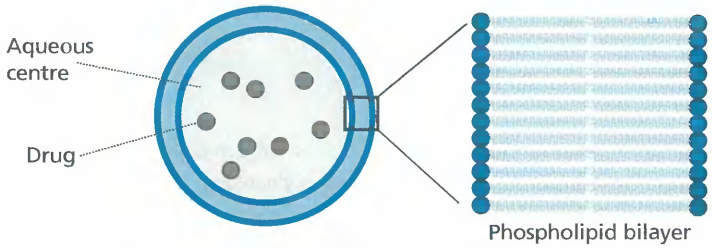


FIGURE 11.24 Liposome containing drug.

system (RES) and removed from the blood supply. The RES is a network of cells which can be viewed as a kind of filter. One solution to this problem has been to PEGylate the liposome by attaching PEG polymers to the liposome (see also section 14.8.2). The tails of the PEG polymers project out from the liposome surface and act as a polar outer shell which both protects and shields the liposome from destructive enzymes and the reticuloendothelial system, increasing its lifetime significantly and reducing leakage of its passenger drug. **DOXIL** is a PEGylated liposome containing doxorubicin which is used successfully in anticancer therapy as a once-monthly infusion.

The use of injectable **microspheres** has been approved for the delivery of human growth hormone. The microspheres containing the drug are made up of a biologically degradable polymer and slowly release the hormone over a 4-week period.

A large number of important drugs have to be administered by injection because they are either susceptible to digestive enzymes or cannot cross the gut wall. This includes the ever-growing number of therapeutically useful peptides and proteins being generated by biotechnology companies using recombinant DNA technology. Drug delivery systems which could deliver these drugs orally would prove a huge step forward in medicine. For example, liposomes are currently being studied as possible oral delivery systems. Another approach currently being investigated is to link a therapeutic protein to a hydrophobic polymer such that it is more likely to be absorbed. However, it is important that the conjugate breaks up before the drug enters the blood supply or else it would have to be treated as a new drug and undergo expensive preclinical and clinical trials. **Hexyl-insulin monoconjugate 2** consists of a polymer linked to a lysine residue of insulin. It is currently being investigated as an oral delivery system for **insulin**.

Biologically erodable microspheres have also been designed to stick to the gut wall such that absorption of the drug within the sphere through the gut wall is increased. This has still to be used clinically, but has proved effective in enhancing the absorption of insulin and **plasmid DNA** in test animals. In a similar vein, drugs have been coated with bioadhesive polymers designed to adhere to the gut wall so that the drug has more chance of being absorbed. The use of anhydride polymers has the added advantage that these polymers are capable of crossing the gut wall and enter the bloodstream, taking their passenger drug with them. Emisphere Technologies Inc. have developed derivatives of amino acids and shown that they can enhance the absorption of specific proteins. It is thought that the amino acid derivatives interact with the protein and make it more lipophilic so that it can cross cell membranes directly.

Drug delivery systems are being investigated which will carry oligonucleotides such as DNA, antisense molecules and siRNAs (section 9.7.2). For example, nucleic acid–lipid particles are being investigated as a means of delivering oligonucleotides into liver cells. Such particles are designed to have a positive charge on their exterior since this encourages adsorption to the negatively charged cell membranes of target cells. Another method of carrying and delivering oligonucleotides into cells is to incorporate them into viruses that are capable of infecting cells. However, there are risks associated with this approach and there have been instances of fatalities during clinical trials. Therefore, nanotechnology is being used to construct artificial viruses which will do the job more safely.

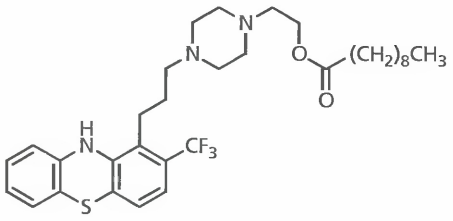
KEY POINTS

- Drugs should be administered at the correct dose levels and frequency to ensure that blood concentrations remain within the therapeutic window.
- The half-life of a drug is the time taken for the blood concentration of the drug to fall by half. A knowledge of the half-life is required to calculate how frequently doses should be given to ensure a steady-state concentration.
- Drug tolerance is where the effect of a drug diminishes after repeated doses. In physical dependence a patient becomes dependent on a drug and suffers withdrawal symptoms on stopping the treatment.
- Formulation refers to the method by which drugs are prepared for administration, whether by solution, pill, capsule, liposome, or microsphere. Suitable formulations can protect drugs from particular pharmacokinetic problems.

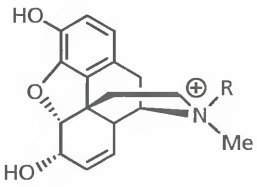
QUESTIONS

1. Benzene used to be a common solvent in organic chemistry but is no longer used because it is a suspect carcinogen. Benzene undergoes metabolic oxidation by cytochrome P450 enzymes to form an electrophilic epoxide, which can alkylate proteins and DNA. Toluene is now used as a solvent in place of benzene. Toluene is also oxidized by cytochrome P450 enzymes, but the metabolite is less toxic and is rapidly excreted. Suggest what the metabolite might be, and why the metabolism of toluene is different from that of benzene.

2. The antipsychotic drug **fluphenazine** has a prolonged period of action when it is given by intramuscular injection, but not when it is given by intravenous injection. Suggest why this is the case.



Fluphenazine



Morphine; R=H
Quaternary salt; R=Me

3. Morphine binds strongly to opioid receptors in the brain to produce analgesia. *In vitro* studies on opioid receptors show that the quaternary salt of morphine also binds strongly. However, the compound is inactive *in vivo* when injected intravenously. Explain this apparent contradiction.

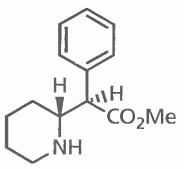
4. The phenol group of morphine is important in binding morphine to opioid receptors and causing analgesia. Codeine has the same structure as morphine but the phenol group is masked as a methyl ether. As a result, codeine binds poorly to opioid receptors and should show no analgesic activity. However, when it is taken *in vivo* it shows useful analgesic properties. Explain how this might occur.

5. The pK_a of histamine is 5.74. What is the ratio of ionized to unionized histamine (**a**) at pH 5.74 (**b**) at pH 7.4?

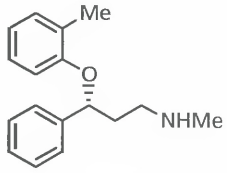
6. A drug contains an ionized carboxylate group and shows good activity against its target in *in vitro* tests. When *in vivo* tests were carried out, the drug showed poor activity when it was administered orally but good activity when it was administered by intravenous injection. The same drug was converted to an ester, but proved inactive *in vitro*. Despite that, it proved to be active *in vivo* when it was administered orally. Explain these observations.

7. Atomoxetine and methylphenidate are used in the treatment of attention deficit hyperactivity disorder. Suggest possible metabolites for these structures.

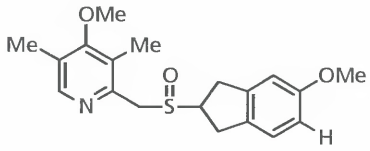
8. Suggest metabolites for the proton pump inhibitor—omeprazole.



Methylphenidate



Atomoxetine



Omeprazole

9. A drug has a half-life of 4 hours. How much of the drug remains after 24 hours?

FURTHER READING

Duncan, R. (2003) The dawning era of polymer therapeutics. *Nature Reviews Drug Discovery*, **2**, 347–360.

Goldberg, M., and Gomez-Orellana, I. (2003) Challenges for the oral delivery of macromolecules. *Nature Reviews Drug Discovery*, **2**, 257–258.

Guengerich, F. P. (2002) Cytochrome P450 enzymes in the generation of commercial products. *Nature Reviews Drug Discovery*, **1**, 359–366.

Langer, R. (2003) Where a pill won't reach. *Scientific American*, April, 32–39.

LaVan, D. A., Lynn, D. M., and Langer, R. (2002) Moving smaller in drug discovery and delivery. *Nature Reviews Drug Discovery*, **1**, 77–84.

Lindpaintner, K. (2002) The impact of pharmacogenetics and pharmacogenomics on drug discovery. *Nature Reviews Drug Discovery*, **1**, 463–469.

Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeney, P. J. (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, **23**, 3–25 (rule of five).

Mastrobattista, E., *et al.* (2006) Artifical viruses: a nanotechnological approach to gene delivery. *Nature Reviews Drug Discovery*, **5**, 115–121.

Nicholson, J. K., and Wilson, I. D. (2003) Understanding global systems biology: metabonomics and the continuum of metabolism. *Nature Reviews Drug Discovery*, **2**, 668–676.

Pardridge, W. M. (2002) Drug and gene targeting to the brain with molecular Trojan horses. *Nature Reviews Drug Discovery*, **1**, 131–139.

Roden, D. M., and George, A. L. (2002) The genetic basis of variability in drug responses. *Nature Reviews Drug Discovery*, **1**, 37–44.

Roses, A. D. (2002) Genome-based pharmacogenetics and the pharmaceutical industry. *Nature Reviews Drug Discovery*, **1**, 541–549.

Rowland, M., and Tozer, T. N. (1980) *Clinical pharmacokinetics*. Lea and Febiger, Philadelphia.

Saltzman, W. M., and Olbricht, W. L. (2002) Building drug delivery into tissue engineering. *Nature Reviews Drug Discovery*, **1**, 177–186.

Sam, A. P., and Tokkens, J. G. (eds.) (1996) *Innovations in drug delivery: impact on pharmacotherapy*. Anselmus Foundation, Hauten, The Netherlands.

Stevenson, R. (2003) Going with the flow. *Chemistry in Britain*, November, 18–20 (aquaporins).

van de Waterbeemd, H., Testa, B., and Folkers, G. (eds.) (1997) *Computer-assisted lead finding and optimisation*. Wiley-VCH, New York.

Veber, D. F., *et al.* (2002) Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry*, **45**, 2615–2623.

Willson, T. M., and Kliewer, S. A. (2002) PXR, CAR and drug metabolism. *Nature Reviews Drug Discovery*, **1**, 259–266.

Titles for general further reading are listed on p. 725.

CASE STUDY 1
Statins

Statins are an important group of cholesterol-lowering drugs that act as enzyme inhibitors. The market for cholesterol-lowering drugs is the largest in the pharmaceutical sector and is dominated by the statins, with substantial rewards for the companies that produce them. In 2002, **atorvastatin** and **simvastatin** recorded revenues of about 7 billion and 5.3 billion dollars respectively. In this case study, we shall look at how these drugs were discovered, and how they interact with their target at the molecular level. First, we shall consider the role of cholesterol in coronary heart disease and how the inhibition of an enzyme can lower cholesterol levels.

Cholesterol and coronary heart disease

Cholesterol is an important constituent of cell membranes and is also the biosynthetic precursor for steroid hormones. It is vital, therefore, to the normal, healthy functioning of cells, and can be synthesized in cells or obtained from the diet. Problems arise if too much cholesterol is present in the diet since this can lead to cardiovascular disease.

Since cholesterol is a fatty molecule, it cannot dissolve in blood and so it has to be transported round the body by particles known as **low-density lipoprotein (LDL)** or **high-density lipoprotein (HDL)**. LDLs are particles about 22 nm in diameter having a mass of 3 million Daltons. Each particle contains a lipoprotein of 4536 amino acid residues that encircles a variety of fatty acids, keeping them soluble in the aqueous environment of the blood supply. The particle also contains a polyunsaturated fatty acid called linoleate, several phospholipids and a large number of cholesterol molecules. LDL serves to transport cholesterol and triglycerides from the liver to the peripheral tissues. When a cell requires cholesterol, it produces LDL receptors which are placed in the cell membrane. LDL binds to these receptors and is then endocytosed into the cell where it releases cholesterol into the cytoplasm.

HDLs are lipoprotein particles about 8–11 nm in diameter that carry fatty acids and cholesterol from tissues back to the liver, where they are removed from the blood supply. They are called high density lipoproteins because they contain a higher proportion of protein than the LDLs. As they travel round the body, they steadily increase in size as they pick up cholesterol from the tissues.

Mortality from coronary heart disease has been shown to be associated with high levels of LDL or low levels of HDL. Inevitably, LDLs transport cholesterol to the arteries, and if cholesterol is retained there it can lead to the formation of fatty plaques which narrow the arteries, resulting in an increased risk of atherosclerosis. If a clot forms and blocks an artery supplying blood to heart muscle it leads to a heart attack. If the clot blocks an artery serving the brain, a stroke results. Thus, lowering the levels of LDL and/or increasing HDL should reduce the risk of heart attacks and strokes. When the statins were first designed, the aim was to lower the levels of cholesterol that were synthesized in the body. The statins certainly do this, but as we shall see later, it is the subsequent effects on LDL plasma levels that are more important in their protective actions against cardiovascular disease.

Target enzyme

Cholesterol is synthesized within cells, and one way of lowering cholesterol levels in the blood is to block this synthesis. This can be achieved by finding a drug which will inhibit one of the enzymes involved in cholesterol biosynthesis. However, there are more than 30 enzymes involved in the biosynthetic pathway, so how does one decide which of these enzymes is the best target? In fact, the choice can be narrowed down by targeting the enzyme that catalyses the rate limiting step for the overall process, since this provides the most effective inhibition of the biosynthetic pathway. The enzyme catalysing the rate limiting step is a reductase enzyme called **3-hydroxy-3-methylglutaryl-coenzyme A reductase** (HMGR or HMG-CoA reductase). The reaction involved is the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate with the aid of NADPH as a cofactor (section 3.5.4) (Fig. CS1.1).

HMGR is thought to consist of four protein subunits and is one of the most highly regulated enzymes known.

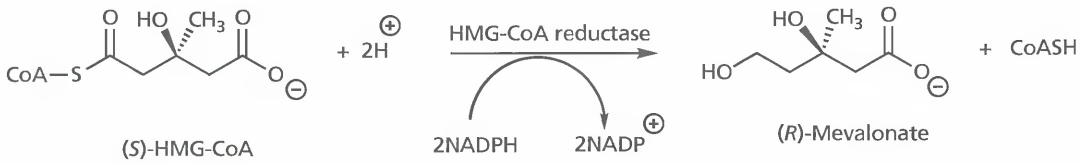


FIGURE CS1.1 Reaction catalysed by 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR or HMG-CoA reductase).

The enzyme's activity can be decreased in a number of ways if too much cholesterol is produced. First, high levels of cholesterol within the cell trigger a signal transduction process which activates a protein kinase responsible for phosphorylating HMGR and inactivating it. Second, the rate at which the enzyme is synthesized through transcription and translation is controlled by the intracellular levels of cholesterol. Finally, the rate at which the enzyme is degraded appears to be influenced by cholesterol levels.

There are two active sites present in the tetrameric structure, and each one is located between two of the monomers. The portion of the active site which binds the substrate HMG-CoA is predominantly on one monomer, while the portion that binds the cofactor NADPH is situated on a neighbouring monomer.

As far as the reaction pathway is concerned, it involves a reductive cleavage involving two hydride transfers (Fig. CS1.2). The hydride is provided by the cofactor NADPH, and so two NADPH molecules are required for each reaction.

The HMGR enzyme is found to be highly flexible in its three-dimensional structure, and this has an important role to play in the binding and activity of statins, as we shall see.

Different amino acid residues in the active site have important roles to play in the enzyme-catalysed reaction, either in binding the substrate or in the mechanism of the reaction.

As far as binding goes, a positively charged lysine residue (Lys-735) forms an ionic bond with the negatively charged carboxylate group of HMG-CoA. Other residues such as Ser-684 and Asp-690 interact with the alcohol group by hydrogen bonding, while Lys-691 is involved in a hydrogen bonding interaction with the carbonyl group (Fig. CS1.3). The coenzyme A moiety is also bound by different interactions into a narrow hydrophobic slot within the active site.

Other amino acids play an important part in the mechanism of the enzyme-catalysed reaction (Fig CS1.4). A histidine residue (His-866) acts as an acid catalyst and provides the proton required by coenzyme A to depart as a leaving group. A special mention needs to be made regarding the binding role of Lys-691. We have already seen that this forms a hydrogen bond to the substrate, but it also plays a particularly important role in stabilizing

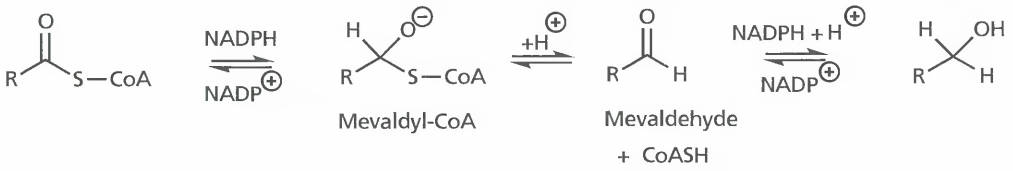


FIGURE CS1.2 Reaction pathway catalysed by 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR or HMG-CoA reductase).

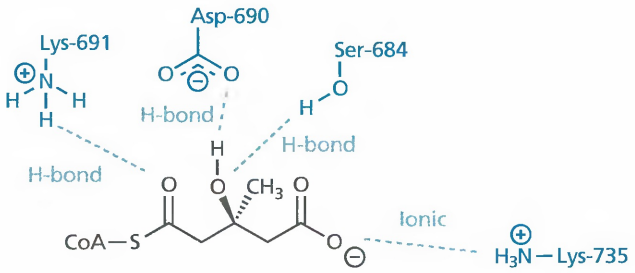


FIGURE CS1.3 Binding interactions for 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA).

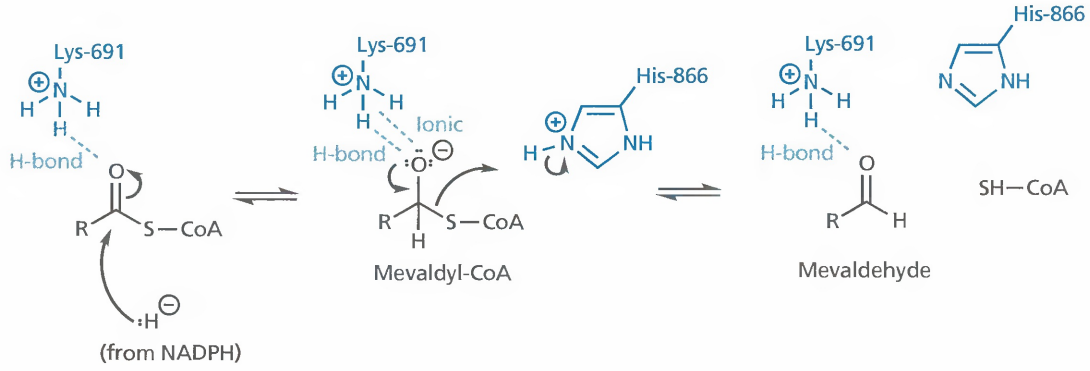


FIGURE CS1.4 Mechanism of the first reduction.

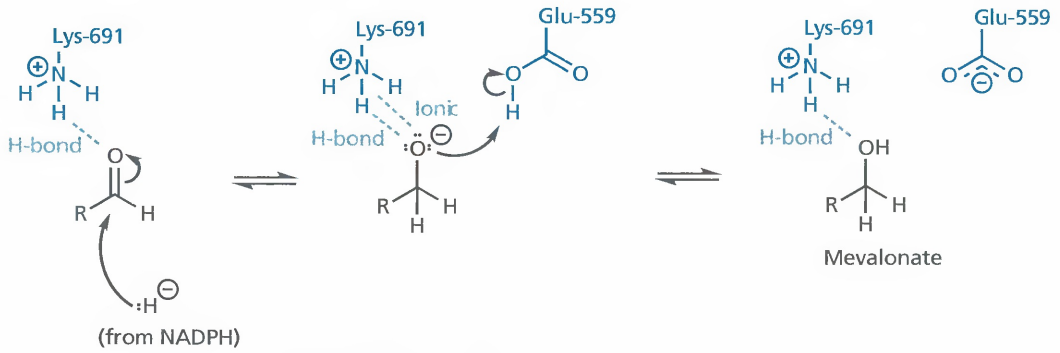


FIGURE CS1.5 Mechanism of the second reduction.

the negatively charged oxygen of mevaldyl-CoA through hydrogen bonding and ionic interactions. This not only helps to stabilize the intermediate but also stabilizes the transition state leading to it. Consequently, the activation energy for the first step in the mechanism is lowered, allowing the reaction to occur more easily.

An uncharged glutamic acid residue (Glu-559) is also involved as an acid catalyst and provides a proton for the final stage where mevaldehyde is reduced to mevalonate (Fig. CS1.5).

It is rather unusual to have an uncharged glutamic acid residue present in an active site. The reason that it is not ionized is that there is a neighbouring aspartate residue (Asp-767) which affects the pK_a of the glutamic acid residue. The aspartate residue also helps to stabilize the ionic form of Lys-691 through the hydrogen bonding network shown in Fig. CS1.6.

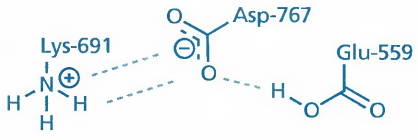


FIGURE CS1.6 Hydrogen bonding network involving Lys-691, Asp-767 and Glu-559.

Discovery of statins

Once the HMGR enzyme was identified as a potential target, researchers set out to find a lead compound that would inhibit it. They started by concentrating their attention on compounds produced by microorganisms. This might appear odd, but the rationale was that microorganisms are constantly involved in chemical warfare with each other, and so a microorganism that produces a chemical that is toxic to another microorganism gains an advantage in the never ending fight for survival (section 12.4.1.2). It seemed likely that microorganisms lacking HMGR might produce HMGR inhibitors which would be toxic to microbes that require HMGR in order to produce important sterols.

Compactin (Mevastatin) (Fig. CS1.7) was the first potent statin to be found that inhibited HMGR, and it can be viewed as the lead compound for this group of drugs. It is a natural product and was isolated from *Penicillium citrinum* in the 1970s. Studies showed that it was a highly potent inhibitor and had a 10 000-fold higher affinity for the enzyme than the natural substrate. Although it entered clinical trials, the drug never reached the market. The reason for this has never been fully revealed, but it

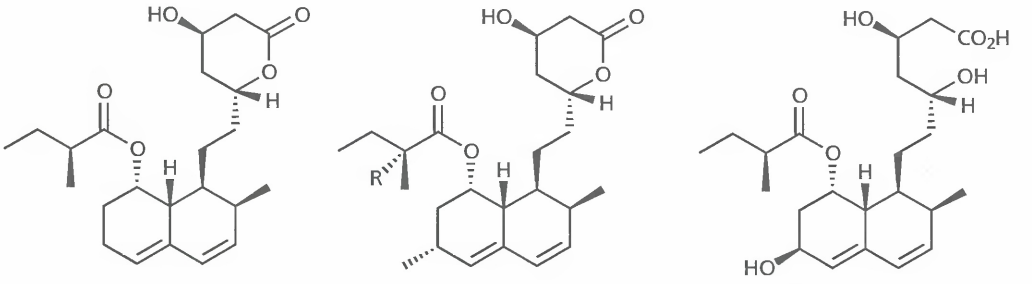


FIGURE CS1.7 Type I statins.

is likely that adverse toxic effects were observed during preclinical trials.

In 1978, Merck isolated a closely related structure called **mevinolin** from the fermentation broth of *Aspergillus terreus*. This was also a potent inhibitor and clinical trials began in 1980. The drug was marketed in 1987 as **lovastatin** (Fig. CS1.7) and it revolutionized the treatment of hypercholesterolaemia (high cholesterol levels).

Other statins soon followed (Fig. CS1.7). **Simvastatin** is a semi-synthetic structure prepared from lovastatin and was first approved in 1988. **Pravastatin** is derived from compactin by biotransformation and reached the market in 1991.

These statins represent the first generation of statins and have been classified as Type I statins. They are all derived directly or indirectly from fungal metabolites, and share a similar structure which consists of a polar 'head' and a hydrophobic moiety which includes a bicyclic decalin ring (Fig. CS1.8).

Observant readers will immediately object that the structures for lovastatin and simvastatin contain a lactone ring, and not the acyclic polar head shown in Fig. CS1.8. However, the lactone rings observed in these structures

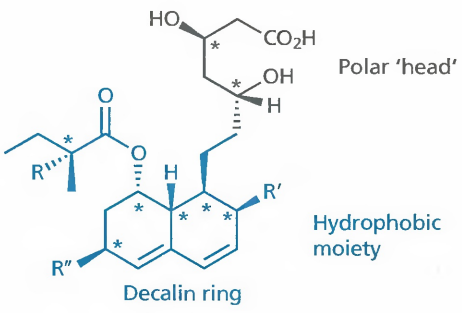


FIGURE CS1.8 General structure for the Type I statins (* represents an asymmetric centre).

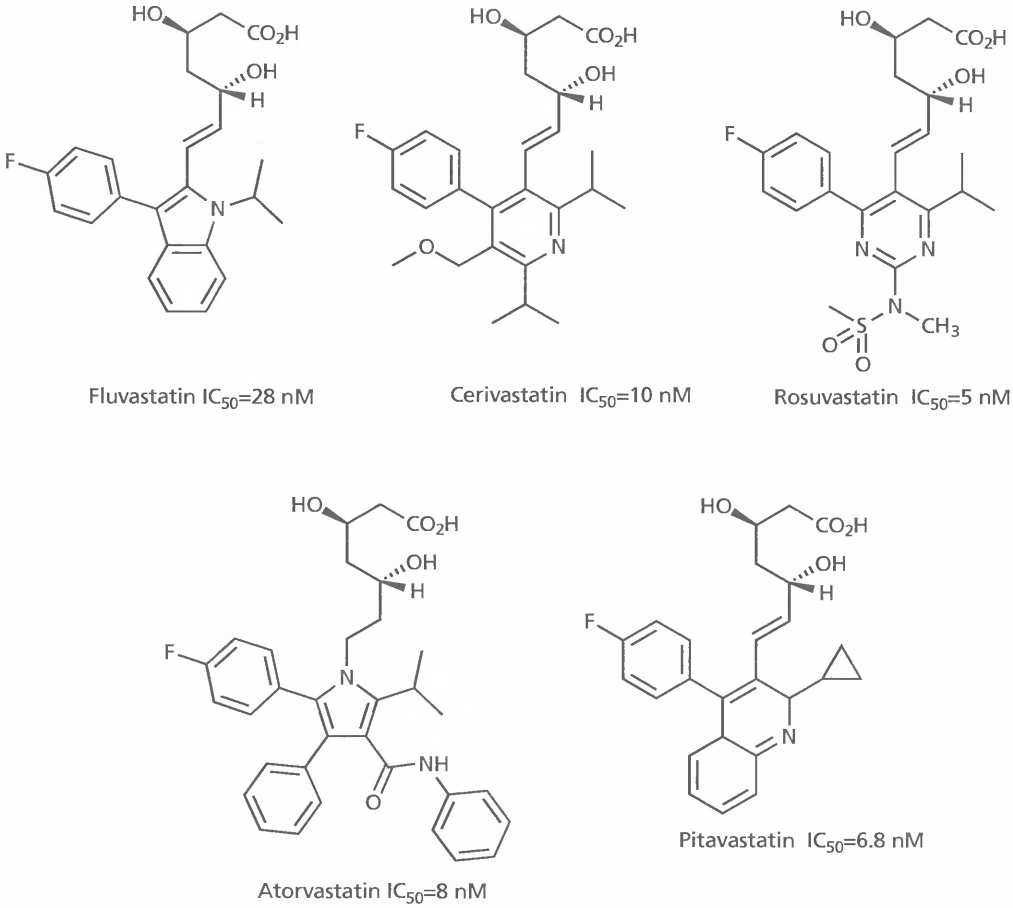
are hydrolysed by enzymes in the body to produce the polar 'head' and it is this structure which is the active drug. Lovastatin and simvastatin are therefore termed as prodrugs (section 14.6).

Although the Type I statins have been extremely effective in lowering cholesterol levels, they do suffer from side effects. They are also difficult to synthesize due to the number of asymmetric centres associated with the decalin ring, and so further work was carried out to find statins with improved activity and reduced side effects, and which would be easier to synthesize. This resulted in a second generation of statins known as the Type II statins.

Type II statins

In contrast to type I statins, type II statins are synthetic structures that contain a different (and larger) hydrophobic moiety from the decalin ring system present in Type I statins (Fig CS1.9). The hydrophobic moieties present in Type II statins may be larger than the decalin system, but they are easier to synthesize, since they contain no asymmetric centres (simplification; section 13.3.8). **Fluvastatin** was marketed in 1994, **atorvastatin** in 1997, **cerivastatin** in 1998 and **rosuvastatin** in 2003. The structures share a number of common structural features, and can be viewed as 'me too' or 'me better' drugs (section 12.4.4.1).

Of these structures, cerivastatin is the most hydrophobic while pravastatin and rosuvastatin are the least hydrophobic. Studies have shown that statins with a lower hydrophobic character are more selective for liver cells where most cholesterol synthesis takes place, and that such statins have fewer side effects. Side effects are thought to be caused by the inhibition of HMGR in other tissues, particularly muscle cells where a condition known as **myalgia** can occur. This is a type of muscle pain or weakness that is particularly



Fluvastatin IC_{50}=28 nM

Cerivastatin IC_{50}=10 nM

Rosuvastatin IC_{50}=5 nM

Atorvastatin IC_{50}=8 nM

Pitavastatin IC_{50}=6.8 nM

FIGURE CS1.9 Type II statins.

prevalent among individuals who take statins and who exercise vigorously. A severe form of muscle toxicity is a condition known as **rhabdomyolysis** which can be fatal. Indeed, cerivastatin was withdrawn in 2001 following a large number of reported cases of rhabdomyolysis, which included 50 fatalities caused by kidney failure.

The most potent statin currently available is rosuvastatin. This structure contains a sulfonamide group which was introduced to lower the drug's hydrophobic character. Coincidentally, the introduction of this group resulted in enhanced binding interactions as described below.

The selectivity associated with less hydrophobic statins is a result of the way they access cells. The less hydrophobic statins do not diffuse easily through cell membranes and require transport proteins to reach effective levels within cells (see also sections 2.7.2 and 14.1). Liver cells possess a transport protein which can carry statins across the cell membrane, whereas muscle cells do not.

Mechanism of action for statins: pharmacodynamics

The statins work by acting as competitive inhibitors (section 7.1.1). They mimic the natural substrate and compete with it in order to bind to the active site. Unlike the natural substrate, they do not undergo an enzyme catalysed reaction and they bind more strongly. How can we explain all of this?

Both the polar head group and the hydrophobic moieties are important to the action of statins. All the statins share the same polar head group and it is this group which mimics the natural substrate (HMG-SCoA). This can be seen more clearly if we redraw the structure of HMG-SCoA as shown in Fig. CS1.10 and compare it with a general structure for the statins. The head group of the statins can therefore mimic the natural substrate and bind to the active site using the same binding interactions. We now need to explain why statins bind more strongly than the natural substrate

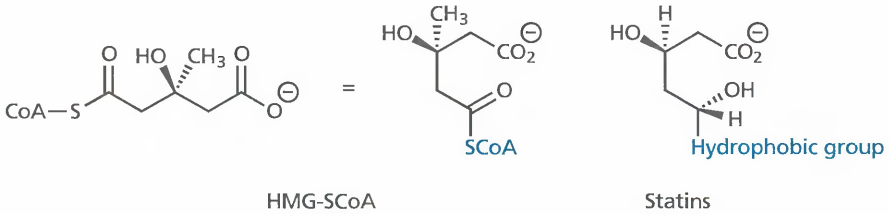


FIGURE CS1.10 Structural comparison of the natural substrate (HMG-SCoA) with statins.

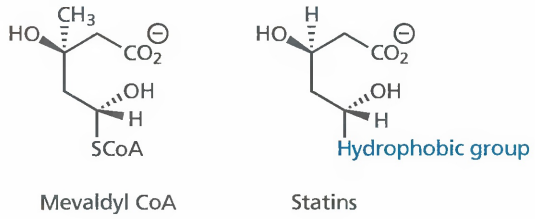


FIGURE CS1.11 Structural comparison of mevaldyl CoA with statins.

and why they are resistant to the enzyme catalysed reaction.

- First of all, the statins contain an extra hydrophobic region that can form additional hydrophobic interactions with a hydrophobic binding region present in the enzyme. This allows the statins to bind more strongly.
- Secondly, the statins are resistant to the enzyme-catalysed reaction, since the coenzyme A moiety in the substrate (which acts as a leaving group) has been replaced with a hydrophobic group that cannot act as a leaving group.

There is one other interesting feature about the statins. They are actually more similar to the first intermediate in the enzyme-catalysed mechanism—mevaldyl CoA—than to the substrate (Fig. CS1.11). Assuming that mevaldyl CoA is less stable than the substrate, this implies that the statins bear some resemblance to the transition state leading to mevaldyl CoA. Consequently, they would be expected to have a stronger binding interaction than the natural substrate and are likely to be acting as transition-state analogues (section 7.4). We shall now look in more detail at the binding interactions of the statins.

Binding interactions of statins

The binding interactions of the substrate with the enzyme has been studied by X-ray crystallography, as has the binding interactions of the statins[1] (section 13.3.11).

[1] These studies were actually carried out using the catalytic portion of the HMGR enzyme, rather than the whole enzyme.

The polar head group of the statins binds in a similar fashion to the substrate, as described previously (Fig. CS1.3). As far as the hydrophobic region is concerned, we might be tempted to think that it would bind to the same region of the active site as coenzyme A. However, studies carried out on the enzyme–substrate complex show that the binding pocket for coenzyme A is narrow and could not possibly accommodate the bulky hydrophobic groups that are present in statins. There is also no other hydrophobic region into which the statins could bind, and so they should really be inactive compounds. The fact that they do bind to the enzyme reflects a marked flexibility that is inherent to the enzyme.

Let us return to look more closely at how the substrate binds to HMGR. When the substrate binds, an alpha-helical section of the protein folds over the active site, shielding it from water and creating a narrow hydrophobic binding region for the coenzyme A portion of the substrate. When a statin binds, the enzyme alters shape in a different manner. Movement of flexible *C*-terminal alpha helices exposes a shallow hydrophobic binding region next to the active site that can accommodate the hydrophobic moiety present in the statin. Thus, the statins are effective inhibitors because they can take advantage of the enzyme's flexibility and essentially create their own binding site.

When the binding interactions of type I and type II statins with the enzyme are compared, it is found that the methylethyl group that is present in most of the type II statins binds to the same part of the shallow hydrophobic region as the decalin ring of type I statins. Type II statins have additional interactions which include van der Waals interactions with the hydrophobic side chains of amino acids such as leucine, valine and alanine. A particularly important interaction involves the fluorophenyl group of type II statins and an arginine residue in the binding region (Fig. CS1.12). First of all, there is a polar interaction between this residue and the fluoro substituent. Secondly, the planar guanidinium group of the residue is stacked over the phenyl ring allowing additional interactions.

Atorvastatin and rosuvastatin can form an extra hydrogen bonding interaction with the enzyme that does

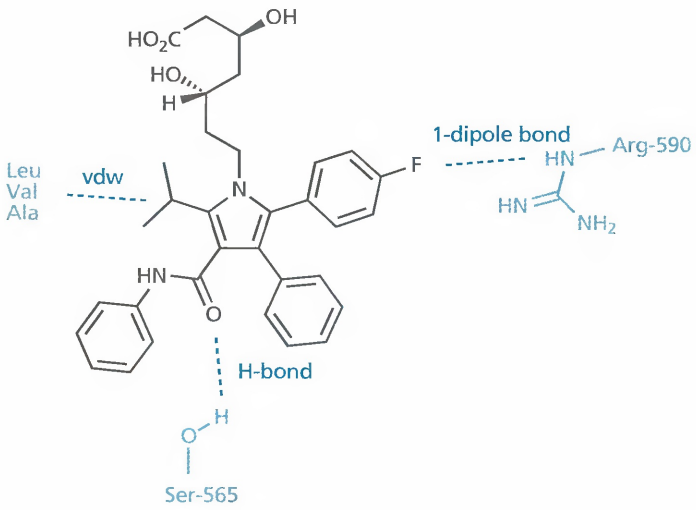


FIGURE CS1.12 Binding interactions for the hydrophobic moiety of atorvastatin with HMGR (3-hydroxy-3-methylglutaryl-coenzyme A reductase).

not occur with other statins. This involves a serine residue which acts as hydrogen bond donor to the carbonyl oxygen atom of atorvastatin (Fig. CS1.12) or to the sulfone oxygen of rosuvastatin.

Rosuvastatin is unique among the statins in having an extra binding interaction between the sulfone group of the drug and Arg-568, making it the most strongly bound statin.

Other mechanisms of action for statins

The action of statins is not purely down to inhibition of HMGR. Inhibition certainly causes a decrease in the levels of mevalonate and cholesterol, but this in turn leads to an upregulation in the transcription and translation process leading to new HMGR which should counteract the inhibition. The fact that statins are still effective is due to other factors. In particular, the lowering of cholesterol levels in liver cells causes an increase in the synthesis of hepatic LDL receptors which are then incorporated into the cell membrane. These receptors are responsible for clearing LDL-cholesterol from the plasma and it is this that is crucial to the effectiveness of the statins.

Other targets for cholesterol lowering drugs

We mentioned earlier that over 30 enzymes are involved in the biosynthesis of cholesterol. Early attempts to find cholesterol-lowering drugs studied the inhibition of enzymes catalysing the later steps in the biosynthetic pathway. There is sense in this since inhibiting an enzyme late on in a biosynthesis is likely to be more selective in its action. In other words, levels of the final product are lowered without affecting the biosynthesis of other compounds which share part of the same biosynthetic pathway. Although the inhibitors were effective, it led to an accumulation of unused substrate which proved insoluble and toxic. When HMG-CoA reductase is inhibited, the substrate is water soluble and easily metabolised. Therefore, it does not build up to toxic levels.

FURTHER READING

Bottorff, M,. and Hansten, P. (2000) Long-term safety of hepatic hydroxymethyl glutaryl coenzyme A reductase inhibitors. *Archives of Internal Medicine*, **160**, 2273–2280.

Jain, K. S., *et al.* (2007) The biology and chemistry of hyperlipidemia. *Bioorganic and Medicinal Chemistry*, **15**, 4674–4699.

Istvan, E. S., *et al.* (2000) Crystal structure of the catalytic portion of human HMG-CoA reductase: insights into regulation of activity and catalysis. *The EMBO Journal*, **19**, 819–830.

Istvan, E. S., and Deseinhofer, J. (2001) Structural mechanisms for statin inhibition of HMG-CoA reductase. *Science,* **292**, 1160–1164.

Istvan, E. (2003) Statin inhibition of HMG-CoA reductase: a 3-dimensional view. *Atherosclerosis Supplements*, **4**, 3–8.

Tobert, J. A. (2003) Lovastatin and beyond: The history of the HMG-CoA reductase inhibitors. *Nature Reviews Drug Discovery*, **2**, 517–526.