5 Phase I Enzyme Inhibition

5.1 Introduction

The previous chapter was mostly aimed at problems associated with drug failure due to enzyme induction. However, when drug clearance is slowed or even stopped for any reason, the consequences are more dangerous and occur much more rapidly compared with enzyme induction. Generally, the pharmacological effects of the drugs will be greatly intensified, leading to a clear manifestation of symptoms in the patient. In drugs with a high therapeutic index, this may not be a problem and the effects of the drug accumulation will be reversible. In narrow therapeutic index drugs, the effects can be lethal in hours. In other cases, a drug may induce a potentially lethal pharmacological effect that is only seen in very high doses, way above the normal range. This effect may or may not have been seen in the initial pre-clinical (animal) toxicity testing of the drug. The following illustrative histories underline the effects of drug accumulation.

History 1

A previously healthy 29-year-old male used terfenadine twice daily for one year to treat allergic rhinitis. The patient drank grapefruit juice two to three times weekly. On the day of his death, he consumed two glasses of juice, took his terfenadine dose, and then mowed his lawn; within one hour he became ill, collapsed and died. Although usually undetectable, post-mortem terfenadine and terfenadine metabolite plasma levels were reported as 35 and 130 ng/mL respectively. These levels are within range of previously noted arrhythmogenic levels of terfenadine. The individual had no evidence of impaired hepatic function.

Analysis

The presence of grapefruit juice appears to have caused unusually high levels of the parent drug to be present in the patient’s plasma. Hence some com-
ponent of the juice prevented the clearance of the parent drug, leading to drug accumulation, which led to a fatal cardiac arrhythmia.

History 2

A 67-year-old male patient stabilized on warfarin began to drink cranberry juice twice daily in response to its reported benefits in recurrent kidney infections. Three days after starting to drink the juice, he suffered a fatal stroke. Post-mortem levels of warfarin were 40 per cent higher than previously sampled in this patient.

Analysis

That the patient had been stabilized on warfarin indicates that his clotting time was within acceptable limits and therefore the drug was being cleared at the same rate it was entering the patient's system. The onset of the consumption of cranberry juice coincided with marked accumulation of warfarin, rendering the patient highly vulnerable to haemorrhage, which occurred within the brain and led to death. It was apparent that the cranberry juice had prevented the clearance of warfarin to inactive metabolites.

History 3

A 64-year-old female with a history of depression was stabilized on amitriptyline, 150 mg/day, but without improvement in mood. Her GP added fluoxetine, 40 mg/day, and within three weeks, the patient's symptoms subsided, although one week later, she collapsed at home and was found in a coma by a relative. The patient recovered consciousness two days later and made a full recovery.

Analysis

The addition of fluoxetine to the regime was associated with accumulation of amitriptyline, which led to unconsciousness and could have led to death had she not been discovered. The fluoxetine must have prevented the clearance of amitriptyline.

History 4

A 44-year-old female epileptic was stabilized on carbamazepine but on the advice of a friend started taking a liquorice preparation for stomach prob-
lems. Over a period of two days, she became gradually more sedated and confused, until she had difficulty standing up. She was admitted to hospital and recovered within three days.

**Analysis**

The liquorice extract was taken in considerable amounts and appears to have interfered with the clearance of carbamazepine, leading to drug accumulation and symptoms of toxicity.

**History 5**

A 55-year-old female stabilized on warfarin suffered from recurrent acid indigestion over the Christmas period and started to self-medicate with over-the-counter cimetidine on the advice of a relative. A few days later, while gardening, the patient noticed that a small cut bled profusely and did not appear to clot for a long period. The patient reported to a hospital accident and emergency room, where her prothrombin time was shown to be excessive. The hospital advised her to use an alternative anti-acid agent and her prothrombin time returned to normal over several days.

**Analysis**

The excessive anticoagulation was due to a reduction in the clearance of warfarin by cimetidine, which could be averted by the use of low-dose (<400 mg) ranitidine or famotidine, which are not usually associated with changes in warfarin pharmacokinetics. An acceptable proton-pump inhibitor would be lansoprazole, but not omeprazole.

**Overall analysis**

In these cases:

- The patient was already stabilized on a particular medicine, which suggests that the dosage and clearance are approximately balanced.
- The addition to the regime prevented clearance of the first drug, leading to accumulation and toxicity.
- The toxicity would be predictable as an intensification of the normal pharmacological response, again indicating that drug accumulation was responsible.
• The toxic responses occurred within hours rather than days, after the addition of the drug.

• The toxicity manifests so quickly that death can occur before even the patient realizes what is happening.

• The toxic effects were rapidly reversible once the inhibiting drug was withdrawn.

• The effects can occur in response to the patient’s decision to either self-medicate or change their diet routine, without consultation with medical staff, or the effect can occur after medical staff fail to be aware of the potential reaction.

5.2 Inhibition of metabolism – general aspects

In complete contrast to enzyme induction, drug inhibition is not usually a process where a logical adaptive response can be made by the patient’s metabolism. The fact that some inhibitors can impair CYP operation for as long as they are administered indicates that the patient’s homeostatic systems are not equipped to detect the inhibition effect and cannot quickly respond to the change in the situation within the timescale – it is rather like suddenly blocking the exhaust pipe of a running engine – it will cough and then simply stop. Sometimes, another CYP or metabolizing system may be capable of clearing some of the accumulating drug at higher concentrations. The kidneys may also eliminate some unchanged drug. That the lung can clear some volatile chemicals such as alcohols is exploited in road safety in the detection of drunk drivers. However, if the drug’s main route of clearance is a particular CYP in the liver and clearance is mostly dependent on the liver, the resultant accumulation will occur relatively rapidly and toxicity or even death can result.

Essentially, inhibition-based drug reactions are much more potentially clinically serious than induction effects, due to this short timescale and the speed that the patient’s clinical situation can change, leading to irreversible damage (such as a stroke or heart attack) within hours of consuming the inhibitor. This is especially problematic in the light of the increasing prevalence of ‘polypharmacy’, where patients may be taking several pharmacologically active compounds at once.

Another factor is that the inhibitor may arise from a decision the patients make themselves, through the desire to ‘self-help’, without informing their doctor. It is also possible that a mistake by a medical practitioner could lead to a potent inhibitor reducing the clearance of a potentially toxic drug.
Tissue homeostatic mechanisms in the liver and other tissues can respond to inhibition in certain circumstances, i.e. some form of adaptation to the situation can occur to restore clearance of the usual substrate. This depends on the type of inhibitor and the frequency of dosage and will be discussed later.

5.3 Mechanisms of inhibition

General aspects of inhibition

Enzymes and tissue/cell receptors share similar features. A receptor binds a molecule that then acts like a switch to trigger a cascade of molecules to instruct the cell to perform a function. The molecule must fit the receptor precisely and then trigger the cascade, like a key, which first enters a lock, then is successfully turned to open it. A key that fits and enters the lock, but does not turn it, not only fails to open the door but also prevents the correct key from being fitted. The lock is essentially ‘inhibited’.

Although they are highly specialized, CYPs are enzymes like any other in the body and they are inhibited according to the same general principles as other enzymes. How tightly a chemical interacts with a CYP isoform is based on how powerful is the mutual attraction (affinity) between the chemical and the active site of the enzyme.

In the case of CYPs and any enzyme, affinity must be strong enough to ensure the substrate is bound for sufficient time to process it to a product. The quicker this process occurs, the faster the ‘turnover’ of the enzyme and the more efficient it is. It is useful to try to visualize a CYP isoform, or any other human enzyme for that matter, as a three-dimensional machine tool, or a spot welding machine. The enzyme cycles hundreds of times a second. If any single aspect of substrate binding or processing (oxidation or reduction), followed by product release is prevented, the sequential nature of these events means that the enzyme stops functioning. Another analogy might be an automatic paper stapler in a photocopier. Whatever analogy you might use, it is useful to try to visualize enzymes as dynamic micro machines. Broadly, inhibitors of CYPs may frustrate the enzymes’ operating processes in two main ways, with varying impact on drug clearance and the individual enzyme ‘health’ and survival. At high concentrations, many inhibitors might block several CYP subfamilies, but at lower concentrations, they show more selectivity and their potency in blocking individual isoforms can be measured. Inhibition can occur through four main processes: competitive, non-competitive, uncompetitive and mechanism-based. Which type of inhibition occurs with various drugs can depend on many factors, such as drug concentration and the characteristics of a particular CYP isoform. Many drugs can act as competitive inhibitors with one CYP and non-competitive with others. Many studies with inhibitors of drug metabolism are carried out in
vitro with human CYPs, either in human liver or in expressed enzyme systems (see Appendix A). These studies do not always reflect what will happen when the drugs are used in patients, but are a reasonable starting point to predict whether a new drug might interfere with the metabolism of another.

**Competitive inhibition**

This is the simplest form of inhibition, where the substrate (drug) and the inhibitor are very similar in structure and have similar affinities for the same place, i.e. the CYP active site (Figure 5.1). A CYP substrate is normally processed to a different molecule, that is, a metabolite, which then has a much reduced affinity for an active site and is more water-soluble, so it diffuses elsewhere. A competitive inhibitor of a CYP isoform is usually not a substrate and acts like a similar key to the correct key for a doorlock; it may enter and leave the lock freely but does not operate it. As it is not processed into a product, it does not leave the vicinity of the CYP and binds and detaches continually. The CYP might be unable to metabolize the inhibitor, due to particular features of the molecule that might prevent oxidation, but promote binding to the active site. This form of inhibition is common in CYPs and is governed by the law of mass action, which states that the rate of a
reaction (in this case enzyme binding) is governed by the concentration of the participants. So for CYP metabolism, whichever agent, drug or inhibitor, is in the greatest concentration, then this will occupy the active site. At low inhibitor concentrations more drug can be added to overcome the inhibitory effects. However, as drug levels must be increased to overcome the inhibitor, this effectively means that the drug’s affinity falls for the site ($K_m$ increases) in the presence of the inhibitor. Enzymes are often subject to this process of competitive inhibition because it is usually part of the endogenous feedback control mechanism on product formation. This generally involves enzymes that use cellular energy, or are at the junction of several biosynthetic pathways. When high levels of product are formed, these inhibit the substrate, so limiting the enzyme’s ‘turnover’, i.e. when the desired product level is reached. This is rather like a thermostat in a heating system, which automatically maintains a preset temperature irrespective of outside temperatures. This is seen in the regulation of vital endogenous molecules like NADPH and glutathione (GSH) and the process avoids unnecessary use of cellular energy. Although the enzyme is temporarily disabled, it is undamaged and has not cycled or used any reducing power. Mathematically, if a Lineweaver–Burk double reciprocal plot is made of competitive inhibition, the $K_m$ (inverse of the affinity) changes, but the $V_{max}$ does not; in other words, the enzyme will still run at a maximum rate if enough substrate is used, but affinity falls off.

A new drug might be evaluated as a possible inhibitor of a given CYP isoform; if the inhibition of the known CYP substrate yields a Lineweaver–Burk plot as described above, then the new drug is a competitive inhibitor of that CYP and it is likely that the inhibitor is binding the CYP at its active site. There are several examples of competitive inhibitors of CYP isoforms. Indeed, if two drugs of similar affinities are cleared by the same isoform, then competitive inhibition can occur. The major clinically relevant group of competitive inhibitors are the azole antifungal agents.

**Azoles**

It is not surprising that these agents are potent human P450 inhibitors, as a great deal of money, time and effort was put into designing them as inhibitors of fungal CYPs. They prevent the fungal synthesis of ergosterol, by blocking lanosterol alpha-C14-demethylase, so causing the substrates (14-alpha-methylsterols) to accumulate and this disrupts fungal membranes. Unfortunately, as mentioned in Chapter 2, since all living system CYPs originate from a common bacterial source, inhibition of azole compounds also occurs in human CYPs. Interestingly, this is relatively specific; ketoconazole was initially the most commonly used azole agent and this is a potent competitive inhibitor of CYP3A4, as well as a number of other sex steroid-handling CYPs. This meant that the drug was quite toxic, as it caused a significant fall in
testosterone levels in blood, which could lead to feminization of males. This could be seen as the appearance of breasts (gynaecomastia), loss of spermatogenesis production and impotence. The female menstrual cycle was also disrupted. These effects, coupled with other toxicity, such as GI tract irritation, nausea, vomiting and occasional severe liver toxicity, propelled the continuing development of these agents to less toxic azoles, which would be more potent therapeutically, but with less human CYP impact. These appeared in the 1990s, in the form of itraconazol and fluconazole, which were followed by the third-generation triazoles, such as voriconazole and posaconazole, which are hundreds of times more potent than ketoconazole as antifungals in vitro, but much less inhibitory on human CYPs, although they do still inhibit CYPs 3A4, 2C9 and 2C19. Indeed, the clearance of the anti-rejection drug tacrolimus (CYP3A4 substrate) is probably inhibited by voriconazole. All azoles, including others such as miconazole and clotrimazole, are generally purely competitive inhibitors, due to their lone pair of electrons on theazole nitrogen, which temporarily binds to the haem groups of several CYPs.

Clinically, even the newer azoles can make a marked impact on the clearance of CYP3A4 substrates. In one clinical study the peak plasma concentration of the 3A4 substrate felodipine was increased eightfold and the area under the curve sixfold by the presence of itraconazole. Fluconazole is a much weaker inhibitor of CYP3A4, but can be potent a inhibitor of other CYPs such as 2C19. Fluconazole has been shown to increase the half-life of omeprazole by threefold and its area under the curve (AUC) by a similar value. Other 3A4 substrates, such as midazolam, terfenadine and lovastatin, show similar effects with this azole. Clearly, the impact of the inhibition on the pharmacological effects of these drugs is very strong, with significant potentiation of their particular effects. It is interesting that although the inhibitory action of these drugs is simple and reversible, the clinical effect of this process on other drug effects can potentially be extremely serious. However, in a matter of hours after the withdrawal of theazole, the inhibiting effect is lost and substrate clearance resumes. There appears to be no way that the liver CYP nuclear ‘management system’ which is seen operating so successfully with enzyme inducers, can overcome the effects of a drug such as ketoconazole when the agent is taken for a long period of time. The serious disruption of steroid metabolism (gynaecomastia again) testifies to this problem. The inhibition appears to be stable for as long as the drug is administered. This has led to attempts to use inhibitors such as ketoconazole and cimetidine to deliberately block the clearance of certain drugs, of which more later.

Non-competitive inhibition

Non-competitive inhibition does not involve the inhibitor and substrate competing for the same active site (Figure 5.1). In non-competitive inhibition,
there is another site, known as the allosteric site, which is distant from the active site. Once a ligand binds this allosteric site, the conformation of the active site is automatically changed and it becomes less likely to bind the substrate and product formation tails off. This process of allosteric binding is another example of the endogenous control of product formation, perhaps by another product/substrate from a related or similar pathway. The net result is to slow or even halt product formation at the main site, depending on how much allosteric binding occurs. The Lineweaver–Burk plot will show a fall-off in $V_{\text{max}}$ (enzyme cannot run at maximal rate) but $K_m$ does not change, that is, the affinity of the substrate for the site is unchanged.

It has been demonstrated experimentally that many drugs are non-competitive inhibitors of CYP isoforms. This means that the inhibitor is not binding at the active site and must exert some allosteric effect elsewhere. As knowledge of the active site of CYPs is still incomplete, we are still not fully aware as to exactly where these allosteric sites are and where they figure in the control of CYPs. In Chapter 3, it was discussed that CYP3A4 had more than one site available for binding and that various substrates could influence the binding of other substrates, probably connected with hormone metabolism. This potentially provides an hour-by-hour modulation of CYP activity, which is of course necessary during steroidal control of reproductive processes. It is likely that non-competitive inhibition is a result of drugs fitting these allosteric sites within most CYP isoforms and influencing binding of substrates to the main catalytic site. There are several examples of non-competitive inhibitors of CYPs. St John’s wort extract (hyperforin) is a potent inhibitor of CYP2D6 in vitro, although it is not known if this occurs significantly in vivo. Omeprazole and lansoprazole are non-competitive CYP3A4 inhibitors in vitro.

**Uncompetitive inhibition**

This is an unusual form of inhibition, where the inhibitor binds only to the enzyme/substrate complex (Figure 5.1). This has the effect of stimulating enzyme/substrate complex formation so increasing affinity (fall in $K_m$), although the enzyme/substrate/inhibitor complex is non-functional, so the $V_{\text{max}}$ falls. This appears to be a relatively rare form of inhibition of human CYPs by therapeutic drugs, although some dietary agents such as the flavonoid tangeretin, found in citrus fruits, is an uncompetitive inhibitor of CYP3A4 in human liver microsomes.

**Mechanism-based inhibitors**

This type of inhibition is outside the normal classification as outlined with competitive, non-competitive and uncompetitive inhibitions. Mechanism
based inhibition generally involves the same initial steps as a competitive inhibitor, but then the CYP catalytic cycle proceeds, reducing power is consumed and a metabolite is formed, which then occupies the P450 active site for a far longer period than the usual substrate would (Figure 5.2). Mechanism-based inhibitors could occupy an allosteric site in a CYP and thus act as non-competitive inhibitors; macrolide antibiotics are sometimes classed as non-competitive inhibitors even though they are mechanism-based. The nearest mechanical analogy to a mechanism-based inhibitor would be the incorrect key turning fully in the lock and not opening, followed by difficult extraction of the key, or even the key breaking off in the lock. This form of inhibition can range from delayed product release, all the way to a violently reactive species-mediated damage to the enzyme, leading to the destruction of the active site. There are degrees of mechanism-based inhibition and moderately potent inhibitors such as the macrolides (like erythromycin) are eventually removed from the CYP active site, but do not usually damage the enzyme. However, highly potent mechanism-based inhibitors such as the contents of grapefruit juice, damage the enzyme to a degree that it is non-functional. This latter process is often termed ‘suicide’ inhibition. Clinically, a competitive inhibitor should wear off after just one or two half-lives, i.e. a few hours to a day or so, depending on a number of factors (inhibitor and substrate dosage, etc.). The most extreme form of mechanism-based inhibition, such as grapefruit juice, norfluoxetine or MDMA-mediated ‘suicide’ inhibition, destroys the enzyme from one dose of inhibitor and this takes several days to resolve.
The effects of mechanism-based inhibition can be shown very clearly in vitro, where the potency of the inhibition is much greater when the CYP enzymes are incubated with NADPH and the compound prior to the addition of the usual substrate. This enables the enzymes to use the reducing power to run the catalytic cycle, which forms the reactive metabolite, which effectively disables the enzyme. The longer this process goes on, the more enzyme is disabled, so the inhibition becomes more potent over time. $V_{\text{max}}$ falls and affinity decreases and obviously, no matter how much substrate is added, the inhibition continues, as the law of mass action cannot apply as the inhibitor may well be already covalently bound to most of the CYP sites. If the substrate is present in reasonably high concentrations prior to the appearance of the inhibitor, the substrate can protect the enzyme, although if the inhibitor continues to be present in adequate concentration, this protective effect will eventually be lost. In vivo, mechanism-dependent inhibition lasts for days as previously mentioned, although the inhibition is clinically reversible, but as far as the individual enzyme is concerned, irreversible. This is because the clinical effect is consistent with the time taken for more P450 enzyme to be resynthesized to replace the inactivated enzyme. Obviously this will only be clinically reversible if the inhibitor was only dosed once, or over a short period. Mechanism-based inhibition is often summarized as follows:

- The inhibition becomes stronger over time;
- Inhibition does not progress without co-factors (NADPH);
- Presence of substrate slows the rate of inhibition by protecting the CYP;
- After inhibition, intact enzyme cannot be detected by analytical techniques (irreversibly inactivated).

There are many clinically important non-competitive and mechanism-based ‘suicide’ inhibitors, which vary in the intensity of their inhibition. These include the macrolides (erythromycin, clarithromycin, oleandomycin), HIV protease inhibitors (ritonavir, indinavir, saquinavir, nelfinavir), the SSRIs (e.g. fluoxetine) and finally grapefruit juices. Unfortunately, all these compounds are metabolized by, and eventually inhibit, our major CYP3A4, whilst the illicit amphetamine derivative MDMA irreversibly blocks CYP2D6 (Appendix B).

**Grapefruit juice**

Although patients have been heroically consuming grapefruit juice for their health for decades, it took until the late 1980s before effects on drug clearance were noted and several more years before it was realized that there could be a major problem with drug interactions (History 1). This led to the regu-
latory authorities to remove terfenadine from the list of OTC medicines in the UK. The most noteworthy feature of the effect of grapefruit juice is its potency from a single ‘dose’ which coincides with a typical single breakfast intake of the juice, say around 200–300ml. Studies with CYP3A substrates such as midazolam have shown that it can take up to three days before the effects wear off, which is consistent with the synthesis of new enzyme. The most interesting aspect is that grapefruit juice was thought not to inhibit hepatic CYP3A4, but gut CYP3A. It is useful to clarify this point. In the mid 1990’s it was shown that grapefruit juice did not appear to inhibit the clearance of intravenously dosed drugs, although it would inhibit when the drug was orally dosed. More recently it has been established that in vitro, there is no difference in the inhibitory effects of azoles (fluconazole and ketoconazole) on CYP3A4 from human gut or liver. So grapefruit juice should be technically capable of blocking hepatic CYP3A4. However, when modest amounts 200–300mls of average strength juice are consumed, the combination of its irreversible CYP binding and a high gut CYP3A expression, means that virtually none of the inhibitor physically reaches the liver. In 2003, human volunteer studies showed that if the dosage of grapefruit juice is very high, enough inhibitor escapes the gut CYP binding to block hepatic CYP3A4.

It might at first appear unusual that an inhibitor of gut wall metabolism would have such a devastating effect on systemic levels of a drug. However, there are a number of drugs that are subject to a very high gut wall component to their ‘first-pass’ metabolism (or pre-systemic metabolism); these include midazolam, terfenadine, lovastatin, simvastatin and astemizole. Their gut CYP clearance is so high that if the juice inhibits it, the concentration reaching the liver can increase six- or sevenfold. If the liver normally only extracts a relatively minor proportion of the parent drug, then plasma levels of the drug increase dramatically towards toxicity levels (see section 1.5). This effect is compounded by grapefruit juice-mediated inhibition of efflux pumps such as P-glycoprotein (see below), which normally retard absorption by constantly pumping xenobiotics out of gut cells almost as soon as they enter them.

As has been mentioned, the inhibitor effects of grapefruit juice in high first-pass drugs is particularly clinically relevant as it can occur after one exposure of the juice. Obviously, the higher the pre-systemic metabolism of a drug (low bioavailability) the greater effect the juice is going to show. One interesting characteristic of the grapefruit juice effect is that the plasma half-lives of the drugs do not change, as the liver carries on metabolizing the drugs as usual, provided the grapefruit juice ‘dose’ is modest.

Summary: drugs that should not be used with grapefruit juice:

- Undergo high pre-systemic (enteric) metabolism;
- Metabolized by CYP3A;
- Pharmacological effects in high dose/high plasma level are life threatening.
Here are some examples of the variable risk of grapefruit juice.

**Drugs that should not be taken with grapefruit juice:**
Terfenadine, statins (simvastatin, cerivastatin (withdrawn, 2001), atorvastatin), amiodarone, astemizole, buspirone, indinavir, sildenafil, pimozide, cilostazol, etoposide, saquinavir

**Drugs that may be problematic with grapefruit juice:**
Benzodiazepines (midazolam, triazolam, diazepam), cyclosporine, nifedipine, nioldipine, synthetic opiates (methadone, dextromethorphan), macrolide antibiotics (erythromycin), carbamazepine, quinine, sertraline,azole antifungals (itraconazole), losartan, steroids (prednisolone)

There are several drugs where others in their chemical class are inhibited by grapefruit juice but they are unaffected:
Fluvastatin, pravastatin, rosuvastatin, loratadine

As to the precise component of grapefruit juice that is responsible for these effects, there are several agents that have been evaluated. The juice contains large numbers of flavonoids, which include naringin, a weak CYP inhibitor, which can be metabolized by gut bacteria to naringenin, which is a more potent inhibitor. However, it is more likely that the bergamottins (6’7’-dihydroxybergamottin and bergamottin itself), a group of furanocoumarins, may be among the most potent CYP inhibitors in the juice. The 6’7’-dihydroxy derivative is much more potent *in vitro* than bergamottin itself. Bergamottins are also found in Seville orange juice, which can exert similar effects to grapefruit juice. It appears that however the juice is prepared, either as concentrate, or canned, as segments or the fruit itself, there is no escape as the inhibitory effects still occur. It has also been found that a further furanocoumarin, epoxybergamottin, which is present in grapefruit peel, is also a CYP3A4 inhibitor. It has been suggested that peel contamination might contribute to the inhibitory effects, or that the epoxybergamottin undergo hydrolysis to the most potent CYP3A4 inhibitor in the juice, 6’-7’-dihydroxybergamottin. A number of other citrous fruits have flavonoid inhibitors also, such as pomolas. The inhibitors in cranberry juice are not known (Case History 2), however further research is likely to uncover more potent inhibitors in various juice extracts.

**Serotonin reuptake inhibitors (SSRIs)**

SSRIs present a particularly difficult therapeutic problem, in that they are the current drugs of choice for depression, but adding them to a regimen of several drugs requires some thought as to the consequences of their considerable inhibitory effects. There are five major SSRIs used in clinical practice: fluoxetine, paroxetine, citalopram, fluvoxamine and sertraline. Although they can be potent *in vitro* inhibitors of CYPs such as 2D6, which oxidizes many
TCAs (History 3) and antipsychotics, their in vivo effects are not necessarily as potent. It is important to realize that not all the SSRIs are potent inhibitors of drug metabolism in vivo, as the doses used for clinical effect are much lower comparatively than those used in in vitro studies that show inhibitory effects. The effects of recommended SSRI dosages for depression have been evaluated in patients on TCAs (History 4) such as desipramine, a 2D6 marker. Fluoxetine was the most potent inhibitor, causing a three- to sixfold increase in plasma levels of desipramine; paroxetine caused a three- to fourfold increase, whilst citalpram showed a much less potent effect, which was around a 50 per cent increase. Sertraline and fluvoxamine showed impact on the levels of the TCA.

Fluoxetine (Prozac) is cleared to S-norfluoxetine, and this is among the most potent inhibitors of CYP2D6 in vitro and in vivo. Essentially, drugs such as TCAs, which have comparatively narrow therapeutic indexes, should be used with great care with fluoxetine (History 3). Inhibitor effects will be much worse in patients such as the elderly, who have compromised drug clearance already (Chapter 7). Fluoxetine is a substrate of CYP2D6, so it can even block its own metabolism, making dosage adjustments unusually difficult. Fluoxetine and S-norfluoxetine can also moderately inhibit CYP2C9 and CYP2C19, and clinically they can cause phentoin, a CYP2C9 substrate, to accumulate dangerously. Although fluoxetine is a weak inhibitor of CYP1A2 and CYP3A4, the clinical significance of such interactions is unclear. Interactions with SSRIs are best documented for 2D6 substrates, although more research is necessary to determine the clinical relevance of their inhibitory effects on other CYPs.

Other mechanism-based inhibitors

Herbal preparations contain some mechanism-based inhibitors of various CYP isoforms and these are capable of making a similar impact on the clearance of prescribed drugs as grapefruit juice. These preparations are often spontaneously adopted by patients on the recommendation of a friend or after reading some form of publicity. Although patients should tell their doctors and health care workers they are taking these substances, they often do not, as they do not feel that it is relevant or important.

Capsaicin

Found in various hot peppers and used as flavourings in spicy foodstuffs. Pepper extracts have been used medicinally to treat many conditions from diabetes to inflammatory diseases. However, capsaicin is oxidized by CYP2E1 to reactive metabolites such as epoxides and phenoxy radicals that irreversibly inhibit the CYP isoform.
Liquorice extract

Contains an isoflavon known as glabridin, which is a potent mechanism-based inhibitor of CYP3A4, although it can competitively inhibit CYP2C9 (History 4).

Extracts have been used in the South Pacific islands for many years for a wide variety of applications, although the kavalactones in the extract, methysticin and dihydromethysticin, are potent inhibitors of most human CYPs in vitro with the exception of 2E1. Kava-Kava has been associated with liver damage, from wild toxicity, all the way to liver failure requiring transplants.

5.4 Cell transport systems and CYP3A inhibitors

Efflux transporters (P-glycoprotein)

Although a great deal of effort is focused on the role of the liver in drug clearance, it is becoming more apparent that the gut wall has a particularly important role to play in the metabolism of drugs. In a recent study in human duodenum, jejunum and liver biopsies, it was found that CYP3A4 levels were three times higher in the gut wall than in the liver. Although inhibition of gut CYP3A by agents such as grapefruit juice should greatly increase bioavailability, this effect can be highly variable, partly due to the effects of such inhibitors on other cellular systems, such as mechanisms of drug cellular transport. P-glycoprotein is a molecular pump system, which is part of the group of ATP-binding cassette transporters (ABC transporters). P-glycoprotein, as well as CYP3A, is strongly expressed in intestinal cells where drugs are absorbed. Indeed, it is found in the apical areas of the human gut wall enterocytes at seven times the activity of liver tissue. As the drug enters the gut cells, the P-glycoprotein system actively pumps it out into the gut lumen again. The combination of the high apical P-glycoprotein and CYP3A4 levels in enterocytes appears to operate as a coordinated response to the threat posed by lipophilic xenobiotics. The effect of pumping the drug out is said to increase the possibility of the drug meeting the CYP on its return journey. The combination clearly retards drug absorption, but does not usually completely prevent it. P-glycoproteins are found in all species and have been the subject of intense interest, as bacteria, protozoa and human cancerous cells all use this system to protect themselves from potential toxic agents by simply pumping out the toxin, be it an antibiotic or an alkylating agent. If P-glycoprotein appears in sufficient quantity to clear the agent as fast as it enters, even potent cytotoxins will exert little or no effect. Attempts to use drugs such as verapamil, to inhibit P-glycoprotein to improve the
bioavailability of anticancer agents have not been as effective as predicted, partly due to a lack of specific P-glycoprotein inhibition. This is because many of these inhibitors also block CYP3A isoforms, which impedes the effectiveness of drugs such as cyclophosphamide that depend on CYP3A4 activation to kill tumour cells. Specific P-glycoprotein inhibitors are under development, but there is some scepticism as to whether they will be useful in clinical practice, as efflux pumps are found in most tissues and it may be difficult to target the effect of the inhibitors.

The genes that code for P-glycoproteins have been termed MDR or multidrug resistant genes and their substrate specificity is so wide as to be almost non-specific and has been described as ‘fuzzy’. Some studies have suggested that substrates are likely to be lipophilic, with molecular weights greater than 400, to have pKa’s greater than 4, and the sum of their nitrogen and oxygen molecules to be greater than or equal to eight. Non-substrates are low in nitrogen and oxygen, less than 400 Mwt and a basic pKa of less than eight.

It is logical to expect that there would be considerable overlap between MDR proteins and CYPs as they are effectively carrying out a similar function using different means, that is, to protect the cell from small lipophilic molecules. If a drug is a potent inhibitor of gut CYP3A, it is often, but not always, capable of blocking P-glycoprotein.

In terms of what is known about P-glycoprotein, an inhibitor such as verapamil or grapefruit juice will increase the bioavailability of other drugs that are substrates for the pump system, such as cyclosporine. The anti-rejection agent tacrolimus has a narrow therapeutic index, so small changes in its bioavailability may have serious clinical effects. Tacrolimus blood levels increase in the presence of pomelo, a citrus fruit related to grapefruit, as this agent is a CYP3A4 and P-glycoprotein substrate.

However, digoxin is a known substrate of P-glycoprotein and it also has a narrow therapeutic index. Inhibitors of P-glycoprotein can certainly elevate digoxin to fatal levels in animal studies, but grapefruit juice does not appear to affect digoxin bioavailability in man, possibly because P-glycoprotein transport does not normally have as much effect on total digoxin availability as it does with other drugs. However, other P-glycoprotein substrates such as atorvastatin are believed to increase the bioavailability of digoxin, possibly by competition for P-glycoprotein transport. Interestingly, atorvastatin is not thought to change the clearances of other 3A4 substrates, i.e. ritonavir, nelfinavir or terfenadine.

To further complicate the picture, there are a series of organic anion-transporting polypeptides, known as OATPs. These transport systems can also be inhibited by grapefruit juice and can reduce the absorption of fexofenadine that is a known OATP substrate.

Overall, the contribution of the multi-factorial complexity of pre-systemic metabolism is still being researched and it is often difficult to establish what contribution cellular transport systems make to bioavailability. Indeed, it is
emerging that one of the reasons for the very wide variety of drug bioavailability in modern medicine could be the sheer number of possible inhibitors and substrates that exist for P-glycoprotein in the diet. These range from natural products to food contaminants. Since no two people’s diets are identical, the impact of P-glycoprotein modulation on drug absorption may never be fully realized. Efflux transporter systems are discussed again under the heading of Phase III of metabolism, where MDR-type transporters remove conjugated metabolites from the cell using efflux pumps of similar structure and function to P-glycoprotein (Chapter 6).

5.5 Clinical consequences of drug inhibition

Introduction

Although CYP inhibition can be competitive, non-competitive, uncompetitive or mechanism dependent, in clinical practice the main concern is how rapidly the inhibitor causes drug levels to climb towards toxicity and whether the toxic effects can be treated before serious injury or death results. As has been mentioned already, there are a number of major clinical conditions caused by inhibition of drug clearance that can overtake even healthy individuals in a matter of hours. The speed at which these problems can be manifested cannot be overemphasized. Clearly, the best option is prevention:

• Firstly, by ensuring that health care professionals do not make mistakes; if these do occur, someone should immediately ‘pick up the ball’ and ensure that the mistake is not translated to a potentially fatal prescription that could be handed to a patient.

• Secondly, the patient must be informed about the dangers of some drugs in combination with inhibitors. This should prevent patient intake of both dietary inhibitors and over-the-counter/herbal preparations that could block the metabolism of prescribed drugs.

Torsades des Pointes

This is an effect where a compound can prolong the cardiac ventricular QT interval, which is controlled by potassium ion channels. The expression QT is described in an ECG wave analysis of the period elapsed between ventricular depolarization and repolarization – this effectively means the time taken for heart muscle to contract and then recover. The recommended treatment for Torsades des Pointes is to withdraw the suspected causative agent and
then administer intravenous magnesium sulphate. If the QT interval increases beyond 0.45 of a second, this leads to ventricular tachycardia, arrhythmia and eventually fibrillation, with total cardiac disorganization and no detectable QRS complex. The patient collapses and the only treatment is rapid defibrillation (History 1). It is most often caused by myocardial infarction, but a significant list of drugs can also trigger it. These include:

Amiodarone, sotalol, procainamide, disopyramide, pimozide, (cisapride was withdrawn in 2000 for this reason) and non-sedative antihistamines such as terfenadine and astemizole.

Obviously any inhibitor that prevents clearance of these agents could precipitate QT interval prolongation. This has been found to be the case with a number of 3A4 substrates, including amiodarone, pimozide, cisapride, terfenadine and astemizole.

Clearly it is important to avoid any possibility of triggering QT interval problems; for example, terfenadine can be replaced with its active metabolite, fexofenadine (carboxyterfenadine). It has also been suggested that less potent azoles such as fluconazole would be much less of a risk in triggering QT interval change with a CYP3A4 substrate as it is a much less potent inhibitor compared with the other azoles, although it is known to increase cyclosporine plasma levels and is a risk with low therapeutic index 3A4 substrates in general.

**Sedative effects**

The risk of sedation is obviously less of a problem in the home, rather than perhaps operating heavy machinery with razor-sharp rotating blades. The co-administration of inhibitors with drugs such as the benzodiazepines and others such as buspirone can potentiate their sedative effects markedly. Midazolam is used as a sedative in intensive care units and particularly in children, there is already a large variation in individual clearances, so any inhibition of the metabolism of this drug may cause excessive sedation. The azole inhibitors, in the expected order of severity, can seriously retard midazolam clearance: ketoconazole > itraconazole > fluconazole. The selective serotonin reuptake inhibitor (SSRI) fluoxetine and its principal metabolite, norfluoxetine, are also potent inhibitors of midazolam clearance, and the norfluoxetine derivative is a particularly potent inhibitor of any 3A4 substrate. Fluvoxetine is also a strong 3A4 inhibitor. However, these can be replaced by sertraline or paroxetine that have much less significant inhibitory effects to prevent excessive sedation with benzodiazepines. Co-administration of SSRIs and other 3A4 inhibitors cause accumulation of other 3A4-cleared drugs such as carbamazepine (History 4). In addition to sedation, the effects
of high plasma levels of carbamazepine can lead to mental confusion, ataxia (staggering gait) and even unconsciousness.

**Muscle damage (rhabdomyolysis)**

This is when striated muscle disintegrates and the released myoglobin enters the blood and then the urine, eventually leading to renal failure. Blunt force trauma, some infections, burns, ischaemia, or severe exercise usually cause it. However, it can also occur in response to exposure of some drugs and chemicals. Heroin and solvent abusers can develop it, but it can occur in response to statin treatment. The use of cerivastatin has been curtailed as rhabdomyolysis has occurred when the drug was used with gemfibrozil. It seems that if statin plasma levels rise to high levels, creatine kinase levels are elevated in plasma and this can lead to rhabdomyolysis. The statins have become increasingly important as their cholesterol-lowering effects are seen as a valuable component of the general effort to reduce ischaemic cardiovascular disease. They are seen as quite safe drugs and some statins are now available OTC at relatively low doses. However, simvastatin, lovastatin, atorvastatin and cerivastatin are 3A4 substrates and are vulnerable to elevation of plasma levels in the presence of potent 3A4 inhibitors. This is a particular concern with inhibitors such as grapefruit juice, the azoles and erythromycin. If statin therapy must be continued in the presence of a 3A4 inhibitor, it would be wise to use those which are cleared by other CYPs, such as fluvastatin (CYP2C9) or pravastatin. Patients taking immunosuppressants and those with renal problems are more prone to develop rhabdomyolysis than others and are at particular risk. It is safest to avoid the interaction by substituting non-inhibiting drugs or statins cleared by other CYPs.

**Excessive hypotension**

As you will, of course, no doubt remember from your pharmacology studies, there are several different drug options in the management of hypertension. This is fortunate, as this condition is very common, sometimes does not respond to therapy and deteriorates with age. This means that antihypertensives of various types are prescribed in vast amounts to older patients, usually for many years. These include CYP3A4 substrates, such as the dihydropyridines (nifedipine, felodipine, nicardipine, and nimodipine). These calcium channel blockers very useful and well tolerated. Nicardipine is more selective for heart vessels, while nimodipine is more effective in cerebral vessels. However, the most common problem with them is excessive vasodilatation that can lead to postural hypotension, dizziness, and headache. They can work too well, to the point where blood pressure is insufficient to force blood
through diseased coronary arteries and they can cause reflex tachycardia; these effects can make some forms of angina worse.

Obviously any marked changes in the clearance of these potent drugs could lead to potentially major deleterious changes in cardiovascular function. It is easy to see how the azoles and the macrolide inhibitors could cause severe cardiovascular problems due to non-clearance of dihydropyridines. Their high pre-systemic metabolism means that grapefruit juice would have a particularly potent and possibly life-threatening effect. Interestingly, the lack of gut metabolism of amlodipine makes this agent less susceptible to grapefruit juice interactions. Any antihypertensive agent that is a 3A4 substrate will be liable to cause excessive reductions in blood pressure if they accumulate in the presence of an inhibitor. This is a particularly problematic effect in the case of suicide inhibitors like grapefruit juice and norfluoxetine.

**Ergotism**

Until the advent of the highly effective group of triptan 5HT agonists, severe migraine sufferers were faced with the prospect of using ergotamine tartrate or suffering the extremely unpleasant pain that this syndrome can inflict. As a migraine sufferer myself, my one experience with ergotamine in 1980 led to a painful effect as if there were wires tightening inside my calf muscles. This took two days to wear off and I still suffered the headache. Ergotamine can also cause severe neural derangement known as ‘St Anthony’s fire’, which sometimes affected people where mouldy flour was used to make bread that contained a considerable dose of ergot alkaloids. Ergotamine is cleared by CYP3A4, so the effects of any inhibitor of this isoform on ergot clearance would lead to an extremely grim series of peripheral and CNS symptoms. Fortunately, the triptans (sumatriptan, naratriptan, zolmatriptan, etc.) are the mainstay of acute migraine treatment and the ergotism problem with CYP3A4 inhibitors should now be very rare.

**Excessive anticoagulation**

Although a number of new anticoagulants are under development, warfarin remains the most commonly used agent for the treatment of a number of conditions where thrombosis is at high risk, such as those with replacement heart valves, atrial fibrillation and deep venous thrombosis. It is a reflection of the vast patient mortality and morbidity due to cardiovascular disease in developed countries that warfarin is the fourth most commonly prescribed agent in the US alone. Warfarin therapy is closely monitored through its pharmacological effect (prothrombin time and INR; international normalized ratio, usually set at 1.5–3), rather than its plasma levels. The drug is given as a racemic mixture (R and S-isomers) and the S-isomer is more potent than the
R-isomer. The clearance of the drug to hydroxylated metabolites occurs stereo-selectively, with 1A2 and 2C19 metabolizing the R-isomer, whilst 2C9 is responsible for the clearance of the more potent S-isomer. Inhibition of 2C9-mediated S-isomer clearance has more impact on warfarin’s pharmacological effect than effects on the other CYPs, although they do show some impact. Cimetidine, an inhibitor of 1A2 and 2C19 (Case History 5), is not recommended for concurrent therapy with warfarin, although it is available OTC and thus there is potential for a moderate increase in prothrombin time. Potent CYP2C9 inhibitors, such as some azoles and SSRIs, can cause a major increase in warfarin’s half-life and thus dangerously magnify its anticoagulating effects. A number of other drugs can also partially inhibit warfarin metabolism and lead to increases in prothrombin time; these include amiodarone, trimethoprim, isoniazid, sulphamethoxazole (in the bactrim combination with trimethoprim), sulfipyrazone, propafenone, metronidazole (inhibits S-isomer), some statins and disulfiram.

Warfarin acts by antagonizing the effects of vitamin K, which is necessary for the formation of several clotting factors. A therapeutic dose basically knock out around half the usual formation of these factors. It is worth noting that changes to warfarin clearance can take some time to be reflected in changes in prothrombin time. This is because the effect of the drug depends on the rate of removal of blood clotting factors that had already been formed before the drug took effect. From an initial dose, it can take up to a day and a half before any change in INR occurs. The drug already has a long half-life (1–3 days) so when a drug increases warfarin’s clearance, it will take perhaps 1–2 days before an effect is seen in terms of prothrombin time and this effect will not disappear for a few days. The most serious effects of excessive coagulation are GI tract bleeding and intracranial haemorrhage, both of which can be fatal.

5.6 Use of inhibitors for positive clinical intervention

Introduction

The effects of inhibitors on the concentrations of CYP substrates can be so dramatic that it has occurred to a number of scientists and clinicians to explore various strategies to exploit this effect to provide some form of benefit to the patient. This can take the form of preventing the formation of a toxic metabolite, to modulate hormone levels in cancer chemotherapy, or even to reduce the cost of prescribing an expensive drug. The key factor in this approach is whether the increased burden and risk to the patient of taking another drug in what is effectively an unlicensed application is really beneficial to the patient. As you can imagine, these applications have met with varying levels of success and acceptance and are discussed below.
The use of inhibitors to arrest hormone-dependent tumours

This is by far the most successful clinical application of inhibitors of CYP-mediated metabolism, although the development of these drugs has taken nearly 30 years. Perhaps a quarter of breast cancers are hormone dependent for their progression, so two treatment strategies can be pursued, one to block the receptors (tamoxifen) and the other to prevent the oestrogens being formed from androgenic precursors, such as 4-hydroxyandrostenedione. In some cases both approaches are used at once. The main distinguishing characteristic of an oestrogenic molecule to an oestrogen receptor is the aromatized ‘A’ ring and the androgenic precursor is subject to a series of CYP19 ‘aromatase’-mediated reactions, rather like a spot-welding robot in car advertisements, which result in the aromatic ‘A’ ring; the oestrogen is completed by CYP17 that also alters the substituents on the D (far right-hand) ring of the steroid. The first aromatase inhibitor to be useful clinically was aminoglutethimide, which was effective in blocking oestrogen formation in peripheral tissues. However, this drug was quite toxic and resulted in haematological problems (methaemoglobin formation, of which more later) and agranulocytosis (loss of all neutrophils), which can be fatal.

It was shown in the 1970s that ketoconazole could retard the progression of some breast cancers, although it was not pursued due to its hepatotoxicity. A great deal of research into aromatase led to the development of anastrozole (Arimidex®), exemestane (Aromasin®), and letrozole (Femara®). These agents are vastly more potent than aminoglutethimide, although the latter agent is still used in Cushing’s syndrome and to control adrenal hormone formation in post-menopausal women. Anastrozole is highly effective in abolishing oestrogen formation, although it does show the side effects expected from loss of oestrogen, such as loss of body strength, nausea and hot flushes. As mentioned above, it can be used in conjunction with clomiphene (another oestrogen receptor antagonist) and tamoxifen. Interestingly, a new generation of agents has emerged which provide a third strategy to fight hormone-dependent tumours. These oestrogen receptor downregulators are designed to degrade and destroy oestrogen receptors. The most effective of these agents at the time of writing is faslodex. This may supersede the use of aromatase inhibitors in certain contexts in the future.

The use of inhibitors to reduce toxic metabolite formation

There are a number of P450-mediated metabolic reactions which result in short-lived, highly unstable and exceedingly toxic products which are capable of severe toxicity. Some of these agents will be described in detail in Chapter
8. The capacity of CYPs to form potentially toxic metabolites is usually rooted in the oxidation of a metabolized molecule, which, according to its structure, may become highly unstable. Some metabolites are so reactive they destroy the enzyme, which has been seen in ‘suicide’ inhibitors mentioned earlier. Slightly less reactive metabolites might enter the rest of the hepatocyte and react with protein structures resulting in change in function and eventual necrosis or apoptosis, depending on the rapidity of formation and the reactivity.

**Paracetamol-mediated hepatic necrosis**

The best example of this is the metabolism of paracetamol in overdose to reactive quinine-imine derivatives. The resulting damage leads to necrosis of the liver. This process is covered in more detail in Chapter 8. It was shown in the 1980s that various inhibitors could slow or prevent the metabolism of paracetamol to its reactive metabolites and several animal studies were carried out to show that this could work clinically. However, this approach never became a clinical reality. Acutely, patients presenting before liver damage was sufficient to cause necrosis could be saved with glutathione precursor supplements, such as N-acetylcysteine. After liver damage was too severe, then it would either be transplant time or death. Considering a preventative approach, including an inhibitor in paracetamol tablets could potentially prevent the formation of the toxic metabolite without affecting clearance that much, as 95 per cent of paracetamol clearance is accomplished by sulphation and glucuronidation. However, the main inhibitors of CYP2E1 are mostly sulphur-containing agents and inhibit other enzymes such as alcohol dehydrogenase and aldehyde dehydrogenase. It would not be practical to include a 2E1 inhibitor in paracetamol tablets because as soon as the patient drank any alcohol, they would be violently ill. So the use of a CYP inhibitor to prevent paracetamol-mediated hepatic necrosis is a dead end.

However, another route of inhibition may in the future have some therapeutic value. A small proportion of paracetamol is cleared to a reactive cytotoxic metabolite (NAPQI: Chapter 8) and this can be detoxified by GSH, either directly, or through catalysis by the cytosolic Phase II enzymes, glutathione-S-transferases (GSTs). Studies in knockout mice have shown that in animals where GST pi has been deleted they sustain less hepatotoxicity than those with intact enzyme. This is bizarre, as it might be expected that the enzyme would be necessary to catalyse NAPQI clearance to a benign mercapturate. In both knockout and control animals, GSH were depleted, so thiol consumption during detoxification occurs. So it is possible that GST pi depletes GSH in a process that is not relevant to NAPQI clearance. Whatever the role of GST in this experiment, it raises the possibility that future GST inhibitors might prevent hepatotoxicity in the later stages of liver damage in paracetamol overdose and in combination with N-acetyl cysteine, the standard antidote, may rescue those previously doomed to liver failure.
Dapsone-mediated methaemoglobin formation

The sulphone drug dapsone is used in leprosy therapy in the Third World, but is also a useful anti-inflammatory agent in conditions which feature the infiltration of activated neutrophils, such as the skin condition dermatitis herpetiformis (DH). The drug is very effective and will suppress DH symptoms, such as intense pruritus and skin eruptions within hours of dosage. This brings rapid relief from a condition that can make patient’s lives intolerable. However, the drug causes methaemoglobin formation, which is due to the CYP2C9-mediated oxidation of the drug to a hydroxylamine (Chapter 8). This particular hydroxylamine is a relatively poor substrate for Phase II glucuronyl transferase and the greater the drug dose, the more hydroxylamine escapes Phase II, enters the circulation and oxidizes haemoglobin to methaemoglobin, which cannot carry oxygen. The more methaemoglobin formed as a percentage of total haemoglobin, the more tissue anoxia occurs; symptoms range from a headache/hangover-like effects at sub 10 per cent levels to hospitalization (nausea, tiredness and breathing problems) at 20 per cent. The standard daily dosage in leprosy of around 100mg of dapsone usually leads to around the 5–8 per cent level of methaemoglobin and it is just about tolerable to most patients, in the light of the alternative of the progression of the disease. However, with DH, the dosage varies wildly from patient to patient. Some can be fully controlled on 25mg per week, whilst others must take 400 plus mg of dapsone daily and the condition is only partially suppressed. At this dosage, the patient’s quality of life is much diminished by the drug and the only reason to persist with treatment might be a lack of effect of the only other drug alternative (sulphapyridine). Even moderately effective drug therapy with high side effects is better than the recurrence of the disease symptoms. In rat studies a number of potential inhibitors were tested for their ability to retard or arrest dapsone-dependent methaemoglobin formation. Piperonyl butoxide, an insecticide and broad CYP inhibitor, was effective, as was cimetidine, although ketoconazole and methimizole were not. Although it was known then that animal and human CYPs were not the same, the main families of CYPs were still being unravelled. These animal studies were reinforced by in vitro work with human liver microsomes, which again showed that cimetidine could be effective. This work led to volunteer studies that showed that cimetidine on a single dose would reduce hydroxylamine formation. Multiple dose studies in animals were also promising and a clinical study in DH patients finally showed that the hydroxylamine formation could be reduced, but not abolished, that methaemoglobin formation fell by nearly 30 per cent and the drug retained its clinical effects and improved patient tolerance. Subsequent studies underscored the possibilities of using cimetidine in patients who could only respond to high dapsone doses and would normally have had to endure considerable methaemoglobin formation. Interestingly, it was clear that the rat was a poor
model for man, in that cimetidine was far more effective as an inhibitor in the rat. It would probably have been undesirable, though, to use too potent an inhibitor long term, as endogenous CYP functions would have been severely affected. As a coda to this work, in 2003 the antioxidant dihydrolipoic acid (formed from lipoic acid in human erythrocytes \textit{in vivo}) was found to partially block the reaction between the hydroxylamine and oxyhaemoglobin \textit{in vitro}. Hence, a study could now be designed to use lipoic acid and cimetidine in combination to make an even larger reduction in methaemoglobin formation in patients on high-dosage dapsone, without completely blocking the CYPs.

\textbf{Use of inhibition in alcoholism}

The effects of alcoholism are covered in more detail in Chapter 8. Among the treatments for alcoholism is the use of the potent inhibitor of aldehyde dehydrogenase and CYP2E1, known as disulfiram (antabuse). This compound is taken by the alcoholic to help the abstinence process. In the alcoholic, ethanol is cleared by CYP2E1 and alcohol dehydrogenase to acetaldehyde, which is cleared by aldehyde dehydrogenase to acetic acid and water. If alcohol is imbibed during antabuse treatment, the clearance of ethanol to acetaldehyde occurs, but the process stops there and acetaldehyde accumulates causing a severe effect that includes flushing, nausea, vomiting and sweating. Even small amounts of alcohol will show this effect, even if the patient is genuinely abstinent. There are many medicinal and hygiene-based products, ranging from cough mixtures to mouthwashes, that can contain up to 30 per cent ethanol, so patient awareness is valuable in this context.

\textbf{Summary}

Inhibition of drug clearance has the greatest clinical impact on a patient’s well-being, in terms of the rapidity of the effect and its severity. This is particularly important when the patient or health care professional is for a time unaware that a potent inhibitor has been consumed. Currently, in the light of the numbers of potent dietary and OTC inhibitors available to the patient, it is as important to educate the patient in the dangers of inhibition of narrow therapeutic index drugs as it is to educate the health care professional.