18 Drug discovery and development

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18.1 HUMAN DISEASE AND DRUG THERAPY

18.1.1 Human disease

The wide range of diseases to which humans are exposed have in common the fact that each is the result of either some physiological dysfunction caused by a gene mutation or incorrect expression of the related protein, or of the exposure of the individual to an environmental factor, such as pesticides, diet, or bacterial, fungal or viral infection. The dysfunction gives rise to characteristic medical symptoms that enable the condition to be diagnosed, commonly by diagnostic tests of the type described in Chapter 16, and an evaluation made of the severity of the condition and the future prospects of the patient making a full recovery from it. Underlying many of the conditions at a molecular level is a change in the amount, function or activity of one or more proteins that in turn trigger changes in cellular, tissue or organ function. A large part of current worldwide medical research is aimed at the elucidation of the molecular mechanisms underlying diseases such as the various forms of cancer and neurological conditions such as Parkinson's disease, motor neurone disease and multiple sclerosis, in order to identify key proteins involved in the disease process with a view to selecting one of the proteins as a target for the development of a new drug and thereby to minimise or eliminate the symptoms.

18.1.2 The nature of drugs and their target proteins

At the present time there are just over 800 drugs in current use worldwide. The majority are organic molecules with a molecular weight of less than 500. However, other possibilities for the nature of the drug are receiving increasing attention. One such option is to develop a monoclonal antibody as the drug to target the protein. Thus an increasing number of monoclonal antibodies are being developed for the

treatment of specific forms of cancer. An example is transtuzumab (Herceptin®) used in the treatment of breast cancer. An alternative approach is to develop drugs to modify the expression of the gene producing the target protein rather than the protein itself. Our knowledge of gene replication and transcription has advanced to the stage where it has become possible to target the DNA or RNA responsible for the biosynthesis of a specific protein. Strategies based on the interference with the translation of mRNA, a process referred to as **RNA interference** (RNAi) have shown considerable potential in model studies (Section 6.8.5). Short interfering RNAs (siRNAs), for example, can be synthesised with a specific base sequence designed to complement and inactivate specific mRNAs or whole gene families. They work by activating a sequence-specific RNA-induced silencing complex (RISC) that cleaves the corresponding functional mRNA within the cell. Some siRNA 'libraries' are now available commercially. The main challenge with RNAi therapy is to devise an effective delivery system for the siRNAs as conventional oral administration would inevitably lead to their premature metabolism. A related potential therapy is the use of so-called DNAzymes. These are synthetic, single-stranded deoxyribonucleotides with the ability to bind and cleave RNA and thereby to suppress the expression of pathophysiologically active genes, for example in a number of cardiovascular states. They have the advantage over antisense molecules that they are less sensitive to nuclease activity.

Advances in recombinant DNA technology, particularly the discovery of restriction endonucleases, polynucleotide ligase and DNA polymerase (Section 5.5), created the technique of gene replacement therapy to correct inherited or acquired genetic defects affecting the availability of a specific protein underlying a disease state. DNA can be introduced into targeted cells, most commonly by incorporating it into a vector such as a modified virus, so there is strong reason to believe that this will become an increasingly important form of therapy in the future (see Table 6.9). Equally, cell-based therapies, particularly those based on the use of stem cells (Section 2.6), have proved to be successful in animal models and there is every reason to expect their future adaptation to the treatment of human disease.

The following discussion will concentrate on the discovery and development of small organic molecules as drugs but many of the principles and challenges discussed are equally applicable to these alternative forms of therapy. The vast majority of these small organic drugs target one of three specific types of proteins namely enzymes, membrane or nuclear receptors and transporters. It has been estimated that the current total number of different protein targets used by marketed drugs in humans is approximately 500. Nearly three-quarters of these targets are human proteins, the remainder are proteins in infecting organisms. Of those aimed at human targets nearly one-third are aimed at G-protein-coupled receptors (GPCRs) (Section 17.4.3) and one-third at enzymes.

18.1.3 Case studies

To illustrate how drug development is based on the targeting of a specific protein, three common human disorders – hypertension (high blood pressure), dyspepsia (heartburn) and bacterial infection – will be considered briefly. Two others, cardiovascular disease and HIV/AIDS are considered in greater detail in Section 18.2.2.

Case study 1 HYPERTENSION

Hypertension, also referred to as high blood pressure (bp), is defined as a systolic bp >140 mm Hg and a diastolic bp >90 mm Hg. Its cause may be primary or secondary to a range of conditions such as kidney disease. If unchecked, hypertension can lead to strokes and heart attacks. It can be reduced by a number of drugs acting by significantly different mechanisms:

- $\beta_1$adrenergic receptor antagonists such as propranolol and labetalol and the $\alpha_1$adrenergic receptor antagonist prazosin involve the blocking of the action of GPCRs.
- Inhibitors, such as captopril, of angiotensin converting enzyme (ACE), which converts angiotensin I to angiotensin II that in turn leads to an increase in blood pressure by its action on angiotensin II receptor, are the preferred first choice therapy to lower bp.
- Antagonists of the GPCR angiotensin II receptor, such as telmisartan, are a related drug therapy to that of ACE inhibitors.
- Antagonists of the dihydropyridine Ca^{2+} channel, such as nifedipine and verapamil, that block the movement of Ca^{2+} ions into smooth muscle cells lining coronary arteries and thereby lower bp, are also valuable therapeutic agents for the treatment of hypertension.
- Inhibitors of phosphodiesterases (PDE) found in vascular smooth muscle and involved in contractility, also reduce blood pressure. One such inhibitor is silendafil (Viagra®) but it specifically inhibits PDE5 which hydrolyses cGMP to 5′-GMP and thereby enhances the action of nitric oxide induced penile erection and is therefore widely prescribed for erection dysfunction and not for the treatment of hypertension!

Case study 2 DYSPEPSIA (INDIGESTION)

Dyspepsia presents as upper abdominal pain and is associated with excess production of acid in the stomach. If simple antacids are inadequate for its alleviation, drugs are available to reduce the acid secretion. In the 1970s it was shown that antagonists of the GPCR histamine H_2-receptor successfully inhibit stomach acid production. The first clinically used antagonist was cimetidine but it was soon replaced by ranitidine due to its better tolerability, longer action and greater activity. Cimetidine is also a significant inhibitor of several key cytochrome P450s involved in the metabolism of other drugs (see Table 18.1 below). However, the preferred choice of treatment for dyspepsia is now the use of a proton pump inhibitor (PPI) such as omeprazole and lansoprazole. These inhibit the action of the H^+/K^+ ATPase 'pump' that transports protons across membranes and which is the terminal stage in gastric acid secretion into the gastric lumen. Omeprazole (Losec® and Prilosec®) is one of the largest selling drugs ever produced. It is administered as a racemate of the R and S forms. The R form is inactive but is converted to the S form *in vivo* by the cytochrome enzyme CYP2C19 (Table 18.1).

It is evident from these case studies that a specific therapeutic outcome can be achieved by targeting one of a number of possible proteins. The challenge in the process of discovering and developing a new drug is firstly to identify the possible targets and then to take an informed decision on which one to select for the discovery process.

Case study 3 BACTERIAL INFECTION

Unlike the situation in the previous two cases, the drug target for the treatment of bacterial infection is not one of the patient's proteins, all of which are presumed to be functioning normally, but rather one of the proteins in the infecting organism. The aim of the therapy is either to prevent the replication of the infecting organism by administering a bacteriostatic drug, or to cause its death using a bacteriocidal drug. From knowledge of the mechanism of bacterial replication, five types of antibiotics have been developed:

- *Cell wall biosynthesis inhibitors*: Penicillin was the first such drug. It is one of a number of β-lactams that inhibit the enzyme DD-transpeptidase that is involved in the formation of peptidoglycan cross-links in bacterial cell walls. As a result of the absence of cross-links, the cell undergoes lysis and dies so the drug is bacteriocidal in its action. Penicillin G and penicillin V are commonly used to treat a wide range of streptococcal infections. The cephalosporins are also β-lactams and have a similar action to that of the penicillins.
- *Folic acid antagonists*: Trimethoprim, widely used in the treatment of urinary tract infections, acts by inhibiting the enzyme tetrahydrofolate reductase thereby inhibiting the synthesis of tetrahydrofolic acid, an essential precursor in the synthesis of the nucleotide thymidine. It is thus a bacteriostatic agent. The enzyme is an excellent target as this particular pathway is absent in humans for whom folic acid is an essential vitamin for the synthesis of thymidine. The sulphonamides act in a related way. They inhibit the enzyme dihydropteroate synthetase, an enzyme in the pathway to folic acid and hence thymidine.
- *Protein synthesis inhibitors*: The tetracyclines inhibit prokaryotic 30S ribosomes (not found in eukaryotes) by binding to aminoacyl-tRNA. Streptomycin acts in a related way. It binds to the bacterial ribosome 16S rRNA and thus inhibits the binding of formyl-methionyl-tRNA to the 30S subunit. It is very effective against tuberculosis. Erythromycin also inhibits aminoacyl translocation but in this case it binds to the 50S subunit of the 70S rRNA complex, thereby blocking protein synthesis. All these drugs are bacteriostatic.
- *RNA synthesis inhibitors*: Rifampicin acts against mRNA synthesis by inhibiting DNA-dependent RNA polymerase by blocking the β subunit, thus preventing transcription to mRNA and subsequent translation to proteins. Like the protein synthesis inhibitors, it is a bacteriostatic agent.
- *DNA synthesis inhibitors*: The quinolone bacteriocidal drugs inhibit the enzymes DNA gyrase and topoisomerase IV, neither of which are found in eukaryotic cells, and thereby inhibit bacterial DNA replication and transcription. Examples are ciprofloxacin and norfloxacin.

18.1.4 Basic characteristics of drugs

Pharmacological parameters

A number of parameters characterise the interaction of a drug with its target protein. Drugs acting as receptor agonists target the orthosteric sites, to which the physiological agonist binds, of the receptors that are present in very large numbers on each cell membrane. The three most important parameters of agonist action are **efficacy, potency** and **selectivity** (see Section 17.2 for full details). Efficacy is a measure of the ability of the drug to produce the maximum response from the receptors. **Potency** is a measure of the dose of the drug required to produce one-half (50%) of the maximum response from

the receptors and is expressed as an ED_{50}. Its value influences the clinical dose of a drug. **Selectivity** is a measure of the ability of the drug to discriminate between the target receptors and other receptors including isoforms of the target receptor. It therefore influences the side effects exerted by the drug. **Effectiveness** describes the ability of the drug to alleviate the symptoms in a large group of heterogeneous patients with apparently similar symptoms. Drugs acting as competitive receptor antagonists also bind to the orthosteric site of the receptors thereby blocking the action of the physiological agonist and reducing the receptor response. They are quantified by an IC_{50} value, which like ED_{50} measures the concentration required to reduce the receptor response by 50%. The majority of enzyme inhibitors compete with the natural substrate for the active site of the enzyme (Section 15.2.2). Alternatively, a drug may bind at an allosteric site on the enzyme producing a conformational change that either increases or decreases the activity of the substrate-binding site (Section 15.2.4).

Pharmacokinetic parameters

Pharmacokinetics relates to the way a drug is absorbed, distributed, metabolised and eliminated (hence the term **ADME** studies) from the body. For a drug to exert its desired effect it must be delivered to the site of action and normally this has to be achieved by its distribution about the body by the blood circulatory system. Most drugs are administered by the oral route, but the inhalation (via the lungs), sublingual (beneath the tongue), transdermal (across the skin) and subcutaneous injection (beneath the skin) routes are more appropriate for the administration of some drugs due to their particular physical properties. Drugs administered orally must be absorbed from the gastrointestinal tract in order to enter the circulatory system. For the majority of drugs passive diffusion is the mechanism by which this transfer occurs. The optimum requirement for this passive diffusion is that the drug is lipophilic, i.e. it possesses adequate lipid solubility and therefore is unionised. Many drugs are weak bases and as such exist in an ionised and hence hydrophilic state at the pH of 1 prevailing in the stomach. Thus, generally speaking, the extent of absorption of drugs from the stomach is low. In contrast, in the small intestine, with a pH of 7, most drugs are unionised and hence suitable for passive diffusion across the gut wall. In these cases, gastric emptying rate is normally the limiting factor for absorption and this is influenced by the food content of the stomach. On entering the circulatory system from the gut the drug is taken by the hepatic portal vein to the liver which is the major site of drug metabolism. Some drugs are readily metabolised by hepatic enzymes and therefore subject to significant inactivation by metabolism before they have entered the general circulatory system. Loss of active drug at this stage is referred to as the **first pass effect** – hence the importance of assessing the susceptibility of a candidate drug to hepatic metabolism in the early stages of drug discovery. The proportion of an oral dose of drug reaching the systemic circulation from its site of administration is referred to as its **bioavailability** (β). It is possible to avoid the first pass effect by administering the drug by routes such as sublingual and transdermal.

For an orally administered drug, its concentration in the blood increases as absorption from the gut continues. It eventually reaches a plateau, at which point the rate of absorption and the rate of loss of the drug are equal, and then declines as the drug is

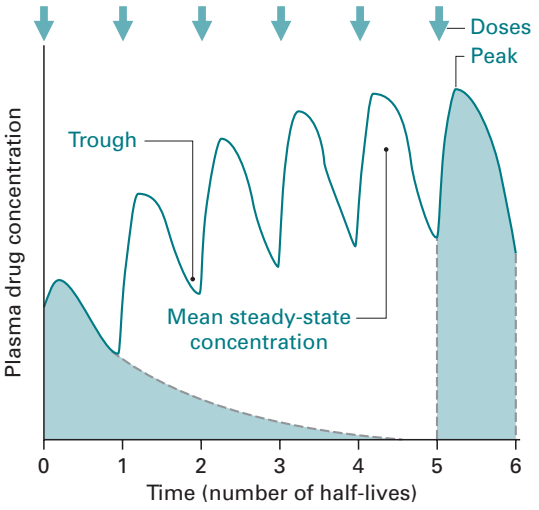


Fig. 18.1 Plasma drug concentration following repeated oral dosing. Following each dose the plasma concentration increases, reaches a peak and declines normally at an exponential rate. When the next dose is administered the plasma concentration profile is superimposed on the existing profile but if the dosing is given after one half-life the peak plasma concentration gradually reaches a maximum and thereafter remains at this level following further repeated dosing. This maximum is generally achieved after five doses. (Adapted from McLeod, H. L. (2008). Pharmacokinetics for the prescriber. *Medicine*, **36**, 350–354, by permission of Elsevier Science.)

eliminated from the body (Fig. 18.1). For a drug to exert its desired pharmacological effect its concentration in blood needs to exceed a threshold value referred to as the **minimum effective concentration**. At a higher concentration the drug may begin to display toxic side effects, referred to as the **toxicity threshold**. The ratio of these two thresholds is referred to as the **therapeutic index** or **therapeutic ratio**. The closer the index to 1, the more difficult the drug is in clinical use. Drugs such as the anti-epileptics phenytoin, carbamazepine and phenobarbitone, with an index in the region of 1, are often subject to **therapeutic drug monitoring** to ensure that the patient is not exposed to potential toxic effects. The aim of repeat dosing with any drug is to maintain the concentration of the drug in the **therapeutic range** or **window** in which toxic effects are not observed. If the dosing interval is adjusted correctly in relation to the plasma half-life (see below) of the drug, the drug plasma concentration will oscillate within the therapeutic range (Fig. 18.1). Once in the general circulation, the drug may bind to a plasma protein, especially albumin, and if the extent of binding is high (>90%) the ability of the drug to cross membranes to reach its site of action and exert a pharmacological effect may be impaired. Due to the high density of endothelial cells lining the brain, the so-called **blood–brain barrier**, drug entry into the brain from blood is slower than to other regions of the body and so drugs targeting the brain often exploit endogenous carrier-mediated transport systems rather than passive diffusion.

The pharmacokinetic parameters of a drug quantify the non-pharmacological behaviour of the drug from the time of its administration to the time of its removal from the body. There are three main parameters:

- *Intrinsic clearance*, Cl_{int}: This is defined as the volume of plasma apparently cleared of drug per unit time by all routes. It has units of $cm^3\ min^{-1}$. Drug is removed from the body by two main routes – metabolism, normally by hepatic enzymes, to one or more polar metabolites that generally lack pharmacological activity and which are readily excreted by the kidneys, and renal excretion of unchanged drug. Intrinsic clearance is therefore the sum of **hepatic clearance** Cl_{hep} (the volume of plasma apparently cleared of drug in unit time by hepatic metabolism) and **renal clearance** Cl_r (the volume of plasma apparently cleared of drug in unit time by renal excretion of unchanged drug). The value of Cl_{int} can be calculated by administering a dose (units: mg) of the drug by the oral route and dividing the dose by the area under the resulting plasma concentration/time curve (AUC) (units: mg.min cm^{-3}) making an allowance for the bioavailability of the drug:

$$CI_{int} = \frac{\text{dose} \times \beta}{\text{AUC}} \tag{18.1}$$

  Bioavailability is calculated from the ratio of the AUC values for an oral dose and for the same dose administered intravenously and which is not subject to first pass loss. It is expressed as a percentage and can vary from 0 to 100%.
- *Apparent volume of distribution*, V_d: This is defined as the volume of body fluid in which the drug appears to be distributed. It has units of dm^3. In an adult body the total volume of water is about 42 dm^3, and is made up of 3 dm^3 plasma water, 14 dm^3 extracellular water and 25 dm^3 intracellular water. The value of V_d is measured by administering a dose by bolus (fast) intravenous injection (to avoid the first pass loss) and using the equation:

$$V_d = \frac{\text{dose}}{\text{peak plasma concentration}} \tag{18.2}$$

  Many drugs have V_d values in the range of 42 dm^3 indicating that they are fully distributed in body water. Abnormally high values are the result of a low plasma concentration caused by the deposition of the drug in some particular tissue, most commonly fat tissue for highly lipophilic drugs. Generally speaking, this is an undesirable property of a drug. Equally, an abnormally low V_d due to a high plasma concentration is indicative of a poor ability of the drug to penetrate lipid barriers.
- *Plasma* or *elimination half-life*, $t_{1/2}$: This is defined as the time required for the plasma concentration to decline by 50% following its intravenous administration. It is a so-called hybrid constant as its value is linked to both Cl_{int} and V_d by the equation:

$$t_{1/2} = \frac{0.693 \times V_d}{Cl_{int}} \tag{18.3}$$

  Values of $t_{1/2}$ normally range from 1 to 24 hours. Clinically it is important as its value determines the frequency with which the drug needs to be administered to maintain the plasma concentration in the therapeutic range. Thus drugs with a short half-life need to be administered frequently whereas drugs with a long half-life can be given on a daily basis.

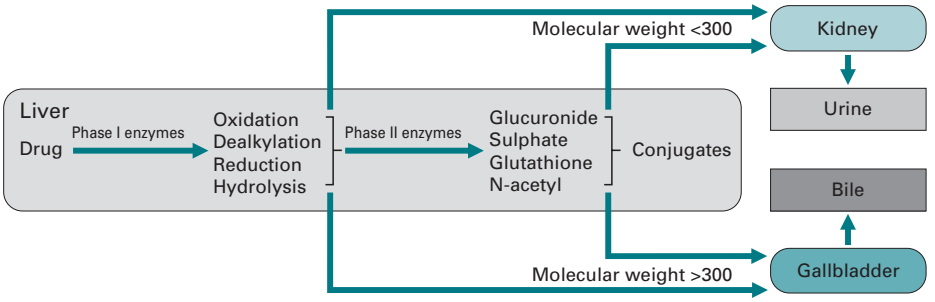


Fig. 18.2 Drug metabolism. Phase I enzymes catalyse the modification of existing functional groups in drug molecules (oxidation reactions). Conjugating enzymes (Phase II) facilitate the addition of endogenous molecules such as sulphate, glucuronic acid and glutathione to the original drug or its Phase I metabolites. (Adapted from McLeod, H. L. (2008). Pharmacokinetics for the prescriber. *Medicine*, **36**, 350–354, by permission of Elsevier Science.)

Knowledge of all of these pharmacokinetic properties of a drug is fundamental to its clinical use as they dictate the dose size and frequency. Hence pharmacokinetic studies form a vital part of the drug discovery and development processes.

Drug metabolism

Most drugs are sufficiently lipophilic to be poorly excreted by the kidneys and hence would be retained by the body for very long periods of time were it not for the intervention of metabolism, mainly in the liver. Metabolism occurs in two phases (Fig. 18.2). Phase I mainly involves oxidation reactions and Phase II conjugation of either the drug or its Phase 1 metabolites with glucuronic acid, sulphate or glutathione to increase the polarity of the drug or its metabolite(s) and hence ease of renal excretion. The oxidation reactions are carried out by a group of haem-containing enzymes collectively known as cytochrome P450 monooxygenases (CYP), so-called because of their absorption maximum at 450 nm when combined with CO. They are membrane-bound and associated with the endoplasmic reticulum. They operate in conjunction with a single NADPH-cytochrome P450 reductase and are capable of oxidising drugs at C, N and S atoms. More than 50 CYP human genes have been sequenced and divided into four families (CYP1–4) of which CYP2 is the largest. Five CYPs appear to be responsible for the metabolism of the majority of drugs in humans: CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 (Table 18.1). The genes for these cytochromes have been cloned and expressed in cell lines suitable for drug metabolism studies. Several of these cytochromes are expressed polymorphically (i.e. they exist in several forms due to their expression by related genes) in humans resulting in considerable interindividual variation in the rate of metabolism of some drugs. There are also ethnic variations in the expression of some of these isoforms. This can be critically important in the use of drugs that have a narrow therapeutic index. In principle, it is possible to genotype individual patients for their CYP activity and hence to 'personalise' drug dosage but in practice this has yet to be put into widespread clinical practice. One other problem associated with the clinical use of

Table 18.1 **Action of some drugs on the major human cytochrome P450 isoforms**

P450 isoform	Substrate	Inhibitor	Inducer
CYP1A2	Imipramine, oestradiol, paracetamol, verapramil, propranolol	Fluvoxamine,[a] cimetidine, ciprofloxacin	Omeprazole, cigarette smoke
CYP2C9	Fluvastatin, ibuprofen, phenytoin, amitriptyline, tamoxifen	Fluconazole,[a] fluvastatin, lovastatin, sulphaphenazole, phenylbutazone	Rifampicin, secobarbital
CYP2C19	Diazepam, propranolol, amitriptyline, omeprazole, lansoprazole	Lansoprazole, omeprazole, cimetidine	Carbamazepine, rifampacin, prednisone
CYP2D6,	Amitriptyline, imipramine, propranolol	Quinidine,[a] bupropion,[a] cimetidine, ranitidine	Rifampicin, dexamethazone
CYP2E1	Paracetamol, theophylline, ethanol	Cimetidine, disulfiram	Ethanol
CYP3A4	Indinavir, diazepam, lansoprazole, saquinavir, lovastatin	Ketoconazole, indinavir,[a] nelfinavir,[a] ritonavir[a]	Carbamazepine, nevirapine, phenytoin

Note: [a]Strong inhibitors that cause at least 80% decrease in clearance.

some drugs is that they either inhibit or induce one or more of the cytochrome P450s (Table 18.1). Inhibition means that the intrinsic clearance of the drug and that of other concomitantly administered drugs is impaired and this may have toxicological consequences whilst enzyme induction results either in increased clearance of the drug that may render its therapy ineffective and/or in the production of toxic metabolites.

18.1.5 Desirable properties of a new drug

From the foregoing discussion it is evident that the drug discovery and development processes must lead to a drug that meets a number of criteria:

- *Chemical structure*: It must possess structural features that allow it to specifically interact with and bind to the target protein. To this end it must possess flexibility and hydrogen binding potential and be of an appropriate molecular size.
- *Physical properties*: It must possess some aqueous solubility and adequate lipophilicity to allow it to cross membranes to access the target site. Linked to these properties, its pK_a must be such that it exists *in vivo* predominantly in the unionised state.
- *Pharmacological properties*: It must have acceptable potency, efficacy, selectivity and effectiveness for receptor agonists or binding properties for enzyme inhibitors or receptor antagonists. It must also fill an unmet clinical niche. The chemical structure and pharmacological properties must be novel to allow patent protection.

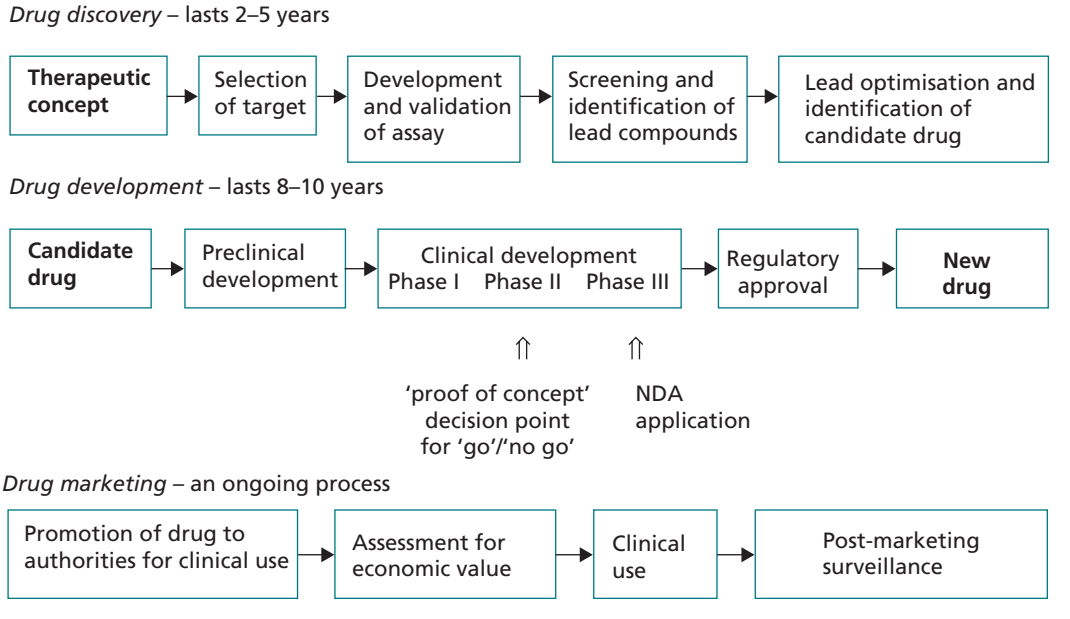
- *Pharmacokinetic properties*: It must have an acceptable rate of absorption, bioavailability, clearance, volume of distribution and plasma half-life to ensure that its dosing size and frequency and onset and duration of action meet patient needs.
- *Toxicological properties*: Ideally it must possess a large therapeutic index, but in some forms of therapy, notably with cytotoxic drugs, this is not possible.

Most candidate drugs in clinical development fail to reach the clinical market for one of four reasons – inappropriate pharmacokinetics, lack of efficacy, unacceptable toxicology and adverse effects in humans.

18.2 DRUG DISCOVERY

18.2.1 Drug discovery and development processes

The launching of a new drug onto the clinical market is the culmination of three distinct processes – drug discovery, drug development and drug marketing. Schematically the processes can be represented as follows:



Although presented as three linear stages, in practice it is essentially a parallel activity supporting a 'learn and confirm' approach with the 'proof of concept' as an important decision point. The high cost of drug discovery and development, variously estimated at $500 m to $3 bn, and the long-term nature of drug discovery and development coupled with the competitive need to get the drug licensed and in clinical use as quickly as possible, drive the management strategy underlying the discovery and development processes. To minimise potential development losses there is a

need to ensure that if the candidate drug is to fail to meet its pharmacokinetic, pharmacological and toxicological targets it should do so early in its development phase ('fail early fail cheaply'). A working deadline for the final 'go ahead' with drug development is typically the end of Phase IIa clinical studies in which the drug is administered to patients for the first time (Section 18.3.2). The strategic decision taken at this time is referred to as **proof of concept**. In the recent past there have been several examples of drugs being withdrawn from the market after they have been awarded a marketing licence by which time maximum development costs have been incurred with no prospect for future sales. For the above reasons, multidisciplinary management teams are created to define the **critical path** through the development process and to expedite the process in a time and cost effective manner.

18.2.2 Selection of the drug target

Four main approaches are employed in drug discovery to identify potential protein targets for a drug discovery programme:

- *Pathophysiological approach*: This is based on the elucidation of the biochemical mechanism underlying the selected disease state. The approach has been successful for such conditions as atherosclerosis and HIV/AIDS but is inherently slow in complex conditions such as cancer, Parkinson's and Alzheimer's diseases.
- *Gene expression profiling*: This approach screens the gene profile of patients with the selected disease in an attempt to identify genes that are either up- or down-regulated relative to control subjects (see Sections 6.8, 6.9 and 6.10).
- *Gene knock-out screening*: This commonly uses transgenic strains of mice in which specific genes have been deleted and the metabolic consequences studied (see Section 6.8.4). An increasingly common alternative to mice is the zebrafish (*Danio*). However, approaches using both species are slow relative to the use of the yeast *Saccharomyces cerevisiae* that has a strong genome similarity to that of the human genome and which is much easier to manipulate genetically.
- *Genetic approach*: This approach uses either whole animals or isolated cells and involves the use of antisense oligonucleotides or RNA interference (RNAi). The former bind to mRNA and prevent its translation whilst the latter destroy by cleavage the functional mRNA (see Sections 6.8.5, 6.8.6 and 6.9.2). The technique of looking for single nucleotide polymorphisms (SNPs) in patients with a common disease is growing in importance as a way of gaining quick insight into the genetic background for a disease.

The application of such approaches coupled with knowledge gained from the current scientific literature, commonly identify a number of potential protein targets and strategic decisions have to be made to select one. Two contrasting case studies illustrate this point:

Case study Target selection: Atherosclerosis

This multifactorial condition is associated with the thickening of the arterial walls due to the ingress of atherogenic lipoproteins into the intima where they are oxidised

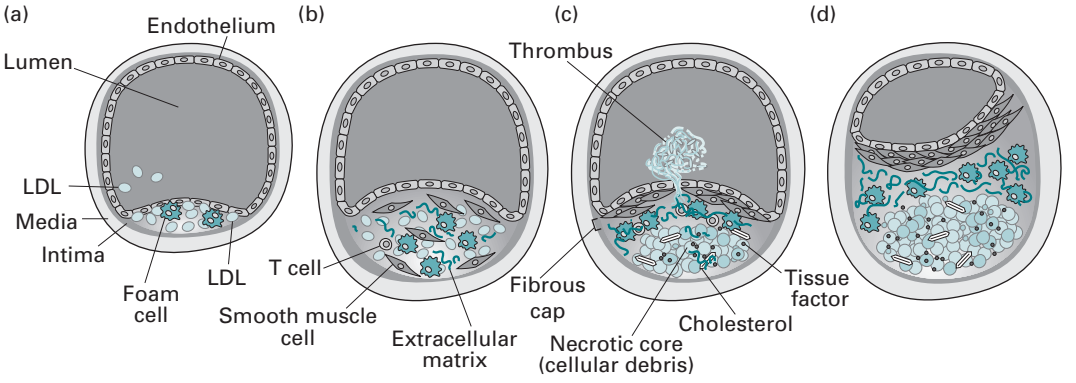


Fig. 18.3 Initiation and progression of atherosclerosis. (a) Low density lipoprotein (LDL) particles enter the intima where they are oxidised and aggregate within the extracellular intimal space. They are then phagocytosed by macrophages eventually leading to the formation of lipid-laden foam cells and fatty streaks, the initial lesion leading to the development of atherosclerotic lesions. (b) Smooth muscle cells that secrete matrix components such as collagen facilitate the formation of these lesions by increasing the retention of LDL. T cells are recruited to the lesion and perpetuate a state of chronic inflammation. The diameter of the lumen gradually increases. (c) Foam cells eventually die releasing cellular debris and crystalline cholesterol. The smooth muscle cells form a fibrous cap that walls off the plaque from the blood. This further promotes the recruitment of inflammatory cells. The plaque may rupture resulting in the formation of a thrombus in the lumen. If large enough, the thrombus may block the artery and cause a heart attack. (d) If the plaque does not rupture, it continues to grow and eventually blocks the lumen. (Adapted from Rader, D. J. and Daugherty, A. (2008). Translating molecular discoveries into new therapies for atherosclerosis. *Nature*, **451**, 904–912, by permission of the Nature Publishing Group.)

and subsequently phagocytosed by macrophages to form foam cells loaded with lipid, mainly cholesterol, and eventually fatty streaks and an atherosclerotic plaque (Fig. 18.3). The progressive growth of the plaque leads either to a restriction of blood flow and eventually a total blockage or if the plaque eventually ruptures it may cause a blood clot again causing a blockage and as a result a heart attack or stroke (Section 16.3.2).

Body cholesterol originates from dietary cholesterol and *de novo* synthesis in cells, most importantly in the liver. Cholesterol is distributed about the body in the form of five types of lipoprotein particles: chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) that differ in size and composition and which are linked metabolically via the enzyme lipoprotein lipase. The two most abundant types of particle are LDL and HDL. LDL is the main carrier of cholesterol from the liver to peripheral cells including those within the developing atherosclerotic plaque and has been referred to as 'bad' cholesterol. HDL is the carrier of cholesterol from peripheral cells to the liver, a process referred to as **reverse cholesterol transport** (RCT), hence HDL has been termed 'good' cholesterol (Fig. 18.4).

Patients with homozygous familial hypercholesterolaemia suffer from premature atherosclerosis due to a mutation in the gene coding for the LDL receptor located

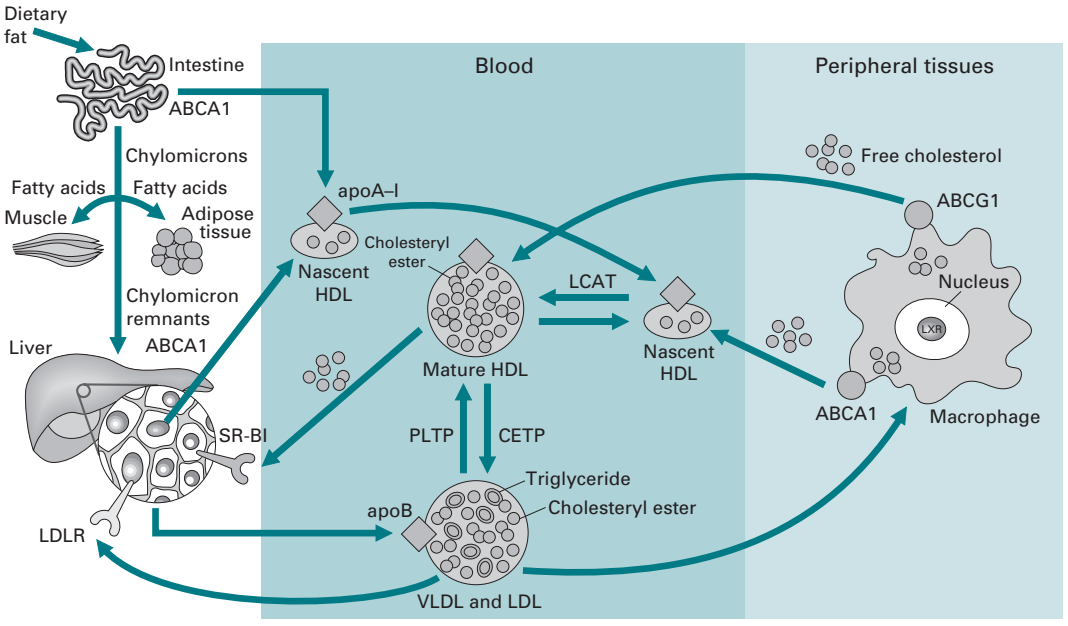
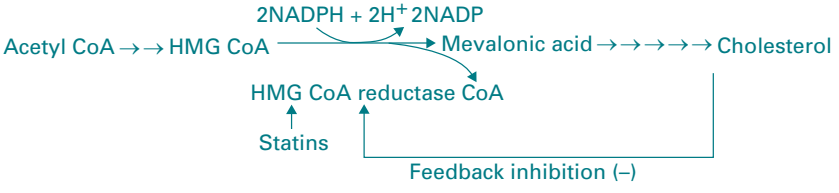


Fig. 18.4 Lipoprotein metabolism. The intestine absorbs dietary fat and packages it into chylomicrons that are transported to peripheral tissues through the blood. In muscle and adipose tissues the enzyme lipoprotein lipase breaks down the chylomicrons, and fatty acids enter these tissues. The liver takes up the chylomicron remnants, loads lipids onto apoB and secretes VLDL that undergoes lipolysis by lipoprotein lipase to form LDL. LDLs are taken up by LDL receptors (LDLR) on liver cells. The intestine and liver generate HDL particles through the secretion of lipid-free apoA1. This recruits cholesterol through the action of the transporter ABCA1 forming nascent HDLs. The free cholesterol in nascent HDLs is esterified by the enzyme lecithin cholesterol acyltransferase (LCAT) creating mature HDLs. The cholesterol in mature HDLs is returned to the liver directly through the receptor SR-B1 and indirectly by transfer to LDLs and VLDLs through the enzyme cholesteryl ester transfer protein (CETP). The lipid content of HDLs is also altered by the enzymes hepatic lipase and endothelial lipase and the phospholipids transfer protein (PLTP). (Adapted from Rader, D. J. and Daugherty, A. (2008). Translating molecular discoveries into new therapies for atherosclerosis. *Nature*, **451**, 904–912, by permission of the Nature Publishing Group.)

on the hepatocyte surface and which is the main mechanism for the removal of LDL from plasma. This observation stimulated the research for drugs that would increase LDL receptor expression and a reduction in plasma LDL. However, to date the main attention for the therapeutic reduction of plasma cholesterol has focussed on the metabolic pathway, starting with acetyl CoA, which leads to its biosynthesis. The controlling enzyme in this pathway is hydroxymethylglutaryl CoA reductase (HMG CoA reductase):



Twelve drugs, collectively called *statins*, have been developed as competitive inhibitors of this enzyme and approved for therapeutic use. They have proved to be very effective in producing a substantial reduction in plasma cholesterol so that statins are currently the most widely used drugs in the developed world. However, they are associated with a number of side effects such as muscle cramps and disturbance of liver enzymes and less commonly acute renal failure. Some statins are known to damage mitochondria and this may underlie some of these side effects.

One obvious target for a new candidate drug is HDL, the aim being to increase its plasma levels and hence facilitate increased reverse cholesterol transport. Some Japanese individuals have raised HDL due to a genetic deficiency of the enzyme cholesteryl ester transfer protein (CETP) (Table 6.4), and this led to the development of CETP inhibitors. One such candidate drug, torcetapib, was developed but had to be withdrawn from Phase III clinical trials (Section 18.3.2) because although it increased serum HDL it resulted in an increased death rate. Whether or not this is a general property of all CETP inhibitors or indeed of all agents that raise HDL, or one specific to torcetapib, remains to be investigated. Two transporter proteins, ABCG1 and ABCA1, located on cell membranes have been shown to promote the efflux of cholesterol from macrophages to form nascent HDL and hence to stimulate reverse cholesterol transport. The genes encoding these two transporters are stimulated by liver X receptors (LXR) that are nuclear transcription factor receptors, and agonists of this receptor have been shown to increase HDL cholesterol by 48% and to improve atherosclerosis in animals. Some of these agonists are now in human clinical trials. Other research has indicated that raising the amount of apoA-1 in HDL could be beneficial provided they do not modify its structure and some novel candidate drugs that may increase apoA-1 are currently in development. Recent research has also indicated that the protein proprotein convertase subtilisin/kexin type 9 (PCSK9) plays an important role in the regulation of the LDL receptor. Claims have been made that the use of iRNA to silence the gene for PCSK9 in mice reduces plasma cholesterol levels by half. Whether or not this approach can be applied to humans remains to be seen.

Case study Target selection: HIV/AIDS therapy

These conditions are induced by the human immunodeficiency viruses HIV-1 and HIV-2 that were discovered in 1983 and 1985 respectively. HIV-1 is the most virulent and most prevalent in the worldwide pandemic: 33 million people worldwide are believed to be living with HIV. They are retroviruses and have a RNA genome. They attack the immune system, especially T-helper cells, resulting in an increased rate of cell apoptosis and death. The virus attacks these cells via attachment of the viral envelope glycoprotein gp120 to a CD4 receptor on the host cell. Membrane penetration by the virus requires the additional involvement of either one of two cytokine co-receptors CCR5 and CXCR4 on the surface of the patient's cells that are linked to GPCRs. Once attached to the CD4 and the co-receptor, the envelope protein undergoes a conformational change that allows penetration into the host cell. The virus then injects various enzymes including a reverse transcriptase, protease, integrase and RNase into the cell. The reverse transcriptase promotes the synthesis of a DNA copy

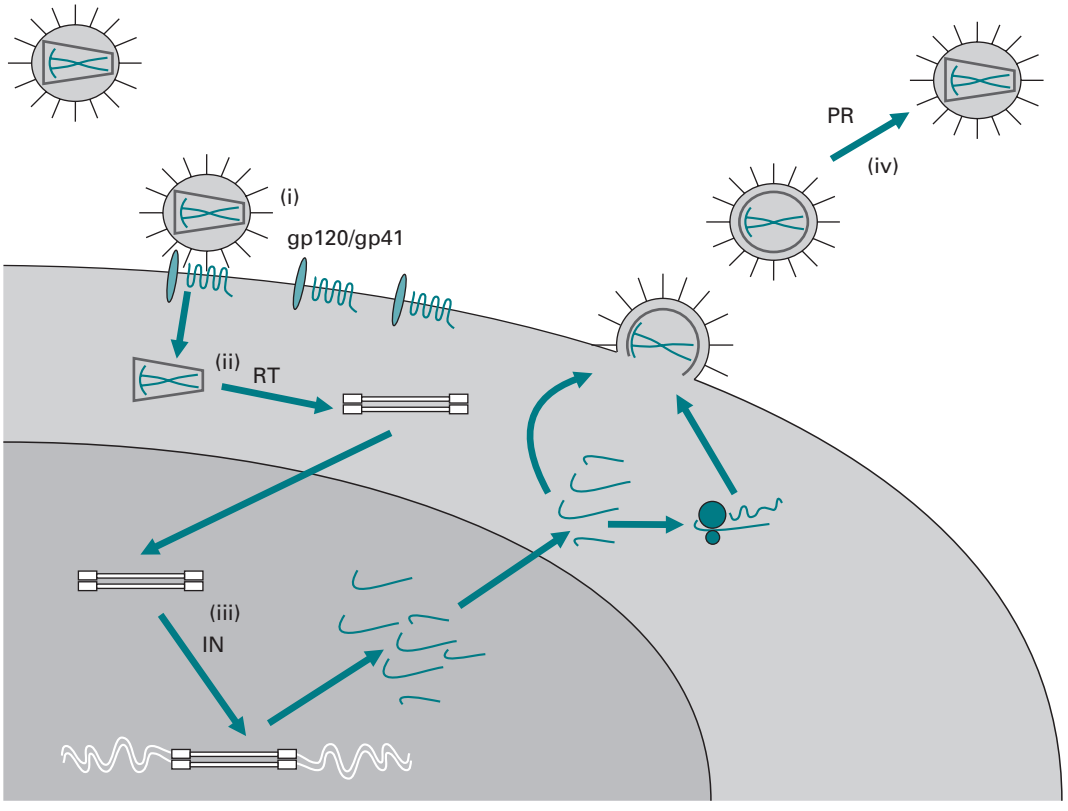


Fig. 18.5 Targets for the therapeutic intervention in the HIV cycle. Currently approved antiretroviral drugs block HIV infection at different steps of the viral life cycle: (i) virus entry through their interaction with gp120 or gp41; (ii) reverse transcription (i.e. reverse transcriptase inhibitors); (iii) integration (i.e. integrase (N) inhibitors); and (iv) maturation (i.e. inhibitors (PR) that block the conversion of immature virus into mature infectious virons). (Reproduced from Menendez-Arias, L. and Tozser, J. (2008). HIV-1 protease inhibitors: effects on HIV-2 replication and resistance. *Trends in Pharmacological Sciences*, **29**, 42–49, by permission of Elsevier Science.)

of the viral RNA genome and integrase inserts it into the chromosome of the host cell; the protease is crucial to the replication process. The viral genome carries nine genes, three of which code for structural proteins and six for regulatory proteins that control the ability of the virus to infect cells. One of the features of the action of reverse transcriptase is that it frequently mismatches bases and this is exploited in the action of the nucleotide reverse transcriptase inhibitors (see below) which mimic natural bases and are incorporated into the DNA strand thereby terminating the chain. The enzyme makes an average of one mistake every time it copies the RNA so that thousands of variants, some viable some not, are produced daily.

From a detailed knowledge of the molecular mechanisms involved in virus infection and replication, numerous potential targets for drug discovery are evident (Fig. 18.5) but to date development has concentrated on the three viral-specific enzymes – reverse transcriptase, protease and integrase. Inhibitors of each of these enzymes have been developed and approved for therapeutic use against HIV-1 and

their use has led to the slowing of the development of AIDS. The first anti-HIV drug to be licensed was the HIV nucleotide reverse transcriptase inhibitor (NRTI) azidothymidine (AZT) in 1987. Its discovery and development paralleled that of acyclovir for the inhibition of DNA replication of herpes simplex virus (HSV) in the late 1970s. In the 1990s the discovery that the protease was crucial for the replication of the HIV lead to the development of HIV protease inhibitors (PI). Whilst individually these two classes of drugs are effective in many patients, by the combination of a PI with two NRTIs (so-called **highly active antiretroviral therapy**, HAART) better reductions in viral burden have been achieved. A third class of drugs, the non-nucleotide reverse transcriptase inhibitors (NNRTI), has been added to these combinations, and modern treatment is based on combination therapy with drugs such as Atrivir®, Trizivir® and Combivir®. A one dose per day formulation, Atripla®, which contains one NNRTI and two NRTIs, is now a popular first choice drug. However, many patients on long-term antiretroviral therapy experience adverse effects and develop resistance to these drugs and new drugs aimed at different targets are being developed. Drug resistance is a consequence of the fact that reverse transcriptase does not carry out proofreading allowing the emergence of mutants that are not sensitive to the therapy. Two main types of new therapy have so far been developed in an attempt to circumvent this resistance problem. **Maturation inhibitors** interfere with the assembly and maturing of new virons prior to their release by the host cell. One such agent, Bevirimat®, is currently undergoing clinical trials. **Fusion inhibitors** block the binding of the virus to a receptor on the host cell. Selzentry® is a co-receptor antagonist for the receptor CCR5 involved in the attachment of the virus to the host cell. It was licensed for human use in 2007. Recent research has shown that the infection process is more complex than originally believed and that over 250 human proteins, many of which are as yet unidentified, are needed by the HIV to enable it to spread throughout the body, so potentially a large number of other drug targets are available for exploitation. Of particular interest is the observation that human cells possess antiviral activity that inhibits the release of retrovirus particles including HIV-1 and that this activity is antagonised by the HIV-1 accessory protein Vpu. The antiviral activity is due to protein-based 'tethers' that can be induced by interferon-α. The protein CD317 has been identified as one such tether. It is obvious that Vpu and CD317 are potential future therapeutic targets. It is hoped that the use of new classes of drugs, in combination with existing therapies, will lead to a reduction in the number of cases of drug resistance. Whether or not this will have a beneficial effect on patients on long-term therapy remains to be seen as will the consequence of changing a drug protein target from the virus to the human host.

In both of the above cases studies the state of knowledge of the underlying biochemistry is considerable and there are numerous existing drugs for therapeutic use. Any decision to select either a new target or a new combination of existing drugs for new drug development in either of these two therapeutic areas is far from straightforward and requires the combined expertise, experience and intuition of the strategic multidisciplinary development team to make an informed judgement. Marketing potential increasingly drives such strategic decisions. A new drug may be the 'first in class' in which case it will have no competitors and may become a

'blockbuster' drug with annual sales in excess of $1bn. However, the risk with such a drug programme is that the concept underlying its action will not previously have been confirmed. Alternatively, the drug may be in an existing class in which case it will have competitors and the aim will be to make it 'best in class'.

18.2.3 Selection of a screening target and assay procedure

In silico pharmacology

It has been estimated that in the process of discovering and developing the 800 drugs that are currently licensed for clinical use, pharmaceutical companies have synthesised and evaluated against their particular narrow target field over 10 million compounds. Individual pharmaceutical companies currently hold chemical 'libraries' of up to 2 or 3 million compounds. Such a large resource is available for screening against new therapeutic targets. Moreover, in the last decade huge advances have been made in compiling computer databases of both the structural, physical and biological properties of existing drugs and structural classification of all known protein targets. These databases are highly sophisticated. For example, the ligand database will contain such information as molecular weight, molecular structure, H-bond donor numbers, polarity, surface area, lipophilicity (expressed as a log P value, where P is a measure of the partition coefficient between water and an organic solvent usually 1-octanol), three-dimensional conformation, spectral properties and known biological targets (receptors, enzymes), quantitative structure–activity relationships (QSARs) and structure–absorption, distribution, metabolism and excretion relationships. Similarly, the protein database will contain all known information about primary, secondary, tertiary and quaternary structure, binding site details of the protein and structural details of known ligands. Knowledge of the amino acid sequence of a target receptor enables a computer model of the structure of the agonist-binding site to be made.

The availability of such data sets has given rise to the interdisciplinary field of **chemogenomics** which aims to predict gene/protein/ligand relationships and hence enable predictions to be made of potential ligands for new targets long before any screening has taken place. Such predictions will be based on the assumptions that chemically similar compounds should share common targets and targets sharing similar ligands should share similarities in their binding sites. This use of computer-based data to aid drug discovery is known under the names of ***in silico* pharmacology**, **computational pharmacology** and **computational therapeutics**. Such computer-based predictions are used to identify potential drug targets and to test the models. They are run in parallel with high-throughput screening techniques in drug discovery programmes.

High-throughput screening

The screening of large chemical libraries for a specific type of biological activity is generally based either on binding activity of the test compound against a selected target preparation or the ability of the compound to activate or inhibit some particular

signalling pathway in a test preparation (Section 17.4.1). Assays need to be adaptable to automation to enable very large numbers of compounds to be screened. Such **high-throughput screening** is generally performed robotically and involves detection systems such as fluorescence-based techniques that do not require the separation of reaction product from substrate. In **high-content screening** automated microscopes are used to take multiple images of the fluorescent cells either in microtitre plates or in a flow cytometer. The images are analysed using image analysis algorithms that enable both biochemical and morphological parameters in the assay system to be evaluated simultaneously (see Sections 4.4 and 4.7).

In recent years high-throughput screening has been adapted to include **fragment-based** approaches to lead discovery. This involves screening the target against a library of small molecules rather than drug-sized molecules. The philosophy behind this approach is that it tests potential binding interactions in fewer assays than does the use of drug-sized molecules. The idea is then to combine the structural elements involved in the binding of these small molecules into a larger lead compound. The difficulties with the technique are firstly that the smaller molecules bind more weakly than do larger molecules so the assay system has to display the sensitivity to detect this weaker interaction with the target, and secondly that the approach will only work if the two or more fragments that are combined to give the larger molecule bind to the same or compatible conformational microstates of the target protein.

Assay types

The assay chosen for a particular target needs to be as specific and relevant to the desired final physiological response as possible. High-throughput screening can be carried out using **cell-free assays** or **cell-based assays**. In the former case, the target is isolated and may be a receptor preparation (Section 17.3.2) or enzyme preparation. They are commonly used for GPCR targets (Fig. 17.6). These *in vitro* systems are simple, unambiguous, cheap and easy to interpret. On the other hand, they give relatively little specific information and are insensitive to issues such as membrane permeability but are effective in the early phase of screening programmes. Cell-based assays, in contrast, give a better appreciation of a likely biological response, allow several responses, such as protein–protein interactions and signalling pathways, to be monitored simultaneously and can distinguish between receptor agonists and antagonists. On the negative side, they require more of the test compound and are generally more expensive. This is an important consideration in the early phase of screening when up to a million candidates may need to be screened.

Screening and developing 'hits'

In the early stages of screening, mixtures of compounds rather than individual compounds are interacted with the target. After the assay is complete, bound and unbound compounds are separated, commonly by size exclusion chromatography (Section 11.7), the bound compounds then released and their identity obtained by LC-MS. This gives rise to a number of 'hits'. To check the authenticity of these hits, the compounds in question will be re-screened individually in duplicate or triplicate. The compounds will then be re-screened using different concentrations and varying

experimental conditions. It is also common for them to be tested in fully functional assays and at the same time checks will be made on their potential patentability. The molecular structure of the confirmed hits will then be compared to identify common structural features that can then be built into a newly synthesised series of candidate drugs. The technique of **combinatorial chemistry** is widely used by medicinal chemists to synthesise a large number of structurally related compounds on a small scale. These compounds are then screened for activity to identify further important structural features and the process repeated on an iterative basis. At the same time, attention will be paid to the physical properties of the emerging 'lead' compounds. For a compound to be an effective therapeutic agent it needs to possess a number of important physical and biological properties. These include a balanced water/lipid solubility expressed by its log *P* value, the extent to which it binds to human albumin, its interaction with the human cytochrome P450 system and its lack of toxicity. Simple *in vitro* screening methods are available for these properties without resorting to animal studies. The potential selectivity of the compounds will also be evaluated against other related physiological targets. The application of these various tests enables compounds with inappropriate properties to be rejected at an early stage.

Pharmacological and toxicological profiling

The purpose of these studies is to evaluate the full pharmacodynamic profile of the compounds in order to establish that they possess the required molecular and cellular properties in tissues and animals, that they possess adequate potency, efficacy and selectivity, appropriate pharmacokinetic properties and that repeated dosing with the compound or its withdrawal after repeated dosing do not lead to toxic effects. Such profiling will be undertaken using both *in vitro* and *in vivo* methods. Studies are likely to include evaluation in animal cell lines expressing cloned human genes and animal models to study both acute and chronic physiological, pharmacological and toxicological properties. The outcome of the drug discovery phase is the identification of a drug candidate, with a few 'back-up' candidates, for development as a licensed drug.

18.3 DRUG DEVELOPMENT

At the end of the drug discovery process the management team will know that the candidate drug has displayed encouraging properties in the screening programme and has appropriate physical and pharmacokinetic properties for clinical use. The team will also have reason to believe that the intellectual property represented by the drug can be protected by international patents. At this stage, however, the group has no evidence that it will display acceptable efficacy, potency and selectivity in human subjects. Thus the priority is to obtain 'proof of concept' that the drug will demonstrate the anticipated clinical properties. To achieve this, data from human subjects are needed and this is the initial priority of the development phase. Unlike the discovery process, the development phase is highly prescribed in that it

has to meet national and international regulations and standards and is subject to review by regulatory and licensing bodies. It is a long and expensive process that accounts for up to two-thirds of the total cost of launching a new drug. It has two main phases:

- *Preclinical phase*: This involves the establishment of small-scale production of the test drug and the creation of a formulation for its clinical use; the establishment of the pharmacokinetic profile of the drug and the evaluation of its acute *in vitro* and *in vivo* toxicity in the rat and normally one other species; the evaluation of its genetic and reproductive toxicology, and the development of analytical methods for the drug and its metabolites.
- *Clinical phase*: This involves the first studies in healthy volunteers (Phase Ia and Ib) paralleled by chronic toxicology evaluation in animals and followed by first studies in patients (Phase IIa and IIb), further toxicology studies and large-scale studies in patients (Phase III and Phase IV).

The aim is to establish that the drug acts in the expected way, that it has therapeutic value and can be produced in a cost-effective way. 'Proof of concept' is therefore sought by the end of the Phase IIa clinical studies before large-scale human studies are commenced.

18.3.1 Preclinical phase

Pharmaceutical development

At the beginning of the development process the candidate drug is likely to only have been prepared in gram quantities. An initial task of the medicinal chemists is to develop a synthetic route capable of delivering the drug in kilogram quantities. The emphasis will be on purity and cost. A parallel task is to produce a formulation for pharmacological and safety evaluation for the chosen administrative route in human studies. In the case of drugs to be administered orally, the formulation will include excipients (pharmacologically inert materials) that will produce an appropriate bulk (bearing in mind that the active component in a tablet is likely to be a few milligrams at most) and stability that will ensure that when it has been administered it will release the active component at the required rate and physiological location (e.g. stomach or small intestine). Stability and dissolution tests will also need to be carried out on the formulation. As the development of the drug progresses there will be a need for manufacture scale-up coupled with a refined specification and finally there will be the need for mass production.

Pharmacokinetic studies

The preclinical phase of drug development includes both *in vitro* and whole-animal kinetic and metabolism studies. The ability of the drug to cross membranes by passive diffusion can be assessed by use of Caco-2 cells in monolayer culture on permeable supports. The drug is placed on one side of the layer and the rate at which it crosses to the other side measured. Comparisons are then made with standard reference compounds. Values are generally in good agreement with *in vivo* data. The possible

role of carrier-mediated transport in drug distribution can be studied using an assay system based on the use of cells containing a cloned transporter.

As discussed in Section 18.1.4, the key pharmacokinetic parameters that dictate the clinical use of a drug are V_d, Cl_{int} and $t_{1/2}$. In addition, the nature and enzymology of the drug's metabolism are crucially important to the safe use of the drug. Isolated hepatocytes have proved to be a good model for the *in vitro* study of drug pharmacokinetics and metabolism. Using these cells, generally obtained from rat and /or dog and occasionally man, it is possible to study the metabolism of candidate drugs and to determine the constants K_m and V_{max} by established graphical means (Section 15.2.1). Cl_{int} is equal to V_{max}/K_m and from knowledge of it, Cl_{hep} can be estimated. From knowledge of the weight of the liver from which the cells were obtained, values can then be scaled to predict *in vivo* hepatic clearance. The values of these *in vitro* derived constants are generally in good agreement with the *in vivo* values obtained in whole animal and human studies. Automated multi-well plate-based assays coupled to fluorescence or LC-MS detection are widely available for the evaluation of the interaction of candidate drugs with the key five cytochrome P450s important to the drug discovery process. The technique of UPLC (Section 11.3.3) is now widely used in support of metabolic studies in drug discovery and development because of its increased speed and sensitivity relative to conventional HPLC.

The possibility of carrying out human pharmacokinetic studies as early as the drug discovery phase has arisen as a result of developments in accelerator mass spectrometry (AMS) (Section 9.3.6). Unlike other forms of mass spectrometry, the ions are accelerated to high kinetic energies before they are mass analysed. As a result, the technique is exceptionally sensitive and accurate in its analysis so that it can be applied to human dosing studies using the principle of **microdosing**, also known as **Phase Zero** studies. The European Medicines Evaluation Agency (EMEA) and the US Food and Drug Administration (FDA) have jointly defined a microdose as one-hundredth of the pharmacological dose and never greater than 100 μg. This is sufficient to enable AMS to study the absorption, distribution, metabolism and excretion of an investigational dose. Moreover, such studies have been defined as research rather than clinical studies and as a result are not subject to the regulatory requirements of normal clinical studies. Following microdosing by the chosen route in a volunteer, plasma or other biological fluid samples are taken at periodic intervals and analysed by HPLC or UPLC coupled to AMS. Quantities of drug and metabolites in the attomole range can be studied. A full pharmacokinetic profile can thus be obtained. Such data are invaluable in the selection of the best candidate drug. It is therefore easy in the early stages of the drug discovery process to eliminate candidate drugs that have unacceptable pharmacokinetic characteristics including either being rapidly metabolised by the cytochrome P450s or acting as activators or inhibitors of them.

Assessment of drug safety

Drug safety considerations are paramount throughout the whole drug discovery and development processes. *In silico* methods can give an advanced warning of potential safety problems associated with a specific chemical structure but many forms of safety problems are unpredictable and have to be identified by both *in vitro* and *in vivo*

methods from a very early stage in a drug's evolution. A battery of exploratory *in vitro* screens has been developed to assess mutagenicity, cytotoxicity, immunotoxicity, hepatotoxicity, embryotoxicity and genotoxicity. Such assays are commenced early in the discovery phase and are used in the selection of the candidate drug in order to give confidence that the selected candidate drug will meet regulatory standards in the later stages of drug development. Once the strategic decision is taken to proceed to the preclinical phase of drug development, regulatory toxicology requirements come into play. These commence with 28-day repeat dose studies in two species, one of which is non-rodent. Rat and marmoset are most commonly selected. As the clinical phase is reached, the toxicology requirements progress to 3–12 month chronic studies in two species and reproductive toxicology studies, generally in rabbit, begin. These latter studies cover fertility and implantation, foetal development and pre- and postnatal effects. Two-year carcinogenicity studies in two species begin after the 'go' decision has been taken for the continued development of the drug. There are regulatory recommendations for the number of animals and species to be studied in all of these safety studies.

Safety pharmacology

In addition to a range of pharmacological studies being undertaken to fully establish the efficacy and selectivity of the candidate drug, studies are required by regulatory bodies to detect any undesirable effects of the drug. These include studies on the central nervous system, cardiovascular system, respiratory system and the autonomic nervous system.

18.3.2 Clinical phase

In order to obtain 'proof of concept' for the drug, clinical studies in human volunteers is essential. Human studies take place in four highly regulated stages:

- *Phase I*: This involves the evaluation of the drug in healthy volunteers, generally male in the age range 18–45. The emphasis of the initial studies (Phase Ia) is on its tolerability and basic pharmacokinetics rather than on the efficacy. The studies start with single sub-therapeutic doses (as predicted by animal studies) that are gradually increased as safety is demonstrated. A satisfactory outcome to this stage leads to Phase Ib in which volunteers receive repeat doses in a randomised, placebo-controlled and 'double-blind' trial (referred to as a RCT); the investigator is unaware of which subjects are receiving the drug and which placebo. Each stage involves about 50 volunteers and typically each takes 6 months to complete. Only if the drug is a cytotoxic agent, intended for treatment of specific types of cancer, are patients instead of healthy volunteers involved in this phase.
- *Phase II*: At this stage the volunteers are patients with the medical condition to be treated and the emphasis is on the assessment of the drug's efficacy but pharmacokinetic data will also be collected. Studies are again normally placebo and double-blind controlled, and would also involve comparative studies with an established drug (if one exists) to evaluate its therapeutic and economic

advantages. The dosage regimen is based on the outcome of the Phase I studies. In Phase IIa up to 200 patients are studied lasting up to 2 years. The results of this phase are then used to assess the efficacy and tolerability of the treatment and thus to either establish or reject the 'proof of concept' and hence to make a 'go'/'no-go' decision. A 'go' decision leads to Phase IIb studies in which a further 200–500 patients are studied for a further 2–3 years. The main aim of these studies is to confirm the results of Phase IIa and then establish the optimum dose and dosing regimen. The total number of subjects involved in Phase II trials must be such that the eventual statistical evaluation of the results has sufficient 'power calculation' to make the outcome, relative to the controls, unambiguous.

- *Phase III*: These studies are designed to extend the efficacy, tolerability and pharmacokinetic studies begun in Phase II and are carried out to a similar design but this time the emphasis is on safety and economic advantages relative to other 'in class' drugs. The selection of the volunteer patients for this stage will not be so restrictive and exclusive as in Phase II studies. The studies will therefore expose the drug to a wider patient population in terms of age and ethnicity. Up to 5000 patients may be involved with the studies being carried out in multiple centres often in different countries and taking 2–5 years to complete.
- *Phase IV*: By this stage the drug will have received a marketing license and its use will be at the discretion of the medical profession. The aims of Phase IV trials include the wish of the manufacturer to extend the therapeutic uses of the drug and to undertake a large-scale study of the mortality associated with the drug if it is a 'first in class' drug. Closely associated with Phase IV trials is the concept of **pharmacovigilance** – the monitoring of unexpected side effects that may only occur in a small minority of patients and hence to improve the safe use of the drug. It relies on general practitioners and pharmacists reporting the adverse drug reactions experienced by their patients but although there are recognised procedures for this reporting, it is estimated that only a small percentage, possibly as low as 10%, are reported. Licence holders are required to provide the licensing authority with regular updates on safety information relating to the use of the drug. This may lead to restrictions on the use of the drug. In 2004 the anti-inflammatory drug rofecoxib (Vioxx®) was withdrawn from the market after over 80 million patients had received it. Intended for the treatment of acute pain, concern was expressed over its association with increased risk of heart attack and stroke in patients on long-term therapy. Since 2004 a number of other high-profile new drugs have also been withdrawn from the market for similar concerns.

It is inevitable that with thousands of individuals involved in the clinical trials of a new drug that there will be reports of side effects. Symptoms such as headache, dizziness, feeling tired, constipation, muscle pain and confusion are quite commonly reported and in many cases it is difficult to establish that they are actually caused by the drug under evaluation but will be listed as 'possible side effects' for the marketed drug. Of greater concern are the occasional 'adverse reactions' of the heart attack and liver damage type. These cannot be ignored and, as in the case of Vioxx®, can lead to the withdrawal of a drug even though it affects an extremely small number of the total

number of patients receiving it. In an attempt to address this problem, a group of seven major pharmaceutical companies has formed the International Severe Adverse Events Consortium (SAEC) to identify genetic DNA variants that may predispose an individual to drug adverse reactions. A parallel strategy that is also being considered is to establish a patient register to record all users of a particular drug so that should fear of an adverse reaction arise it may be possible to trace patients potentially at risk. Such an approach might prevent a drug such as Vioxx® from being withdrawn from the market to the disadvantage of the majority of its users.

Regulatory and ethical approval for clinical trials

Although the decision to enter a candidate drug into clinical trials is taken by the company, responsibility for authorising the studies lies with the national regulatory authority. In European Union countries, the European Medicines Evaluation Agency (EMEA) coordinates the control of clinical trials. The European Union Drug Regulating Authorities Clinical Trials (EudraCT) is the database for all clinical trials commencing in the Community. Each member state has a 'competent authority' with the power to award a Clinical Trial Authorisation (CTA). In the UK, that power rests with the Medicines and Health Regulatory Authority (MHRA). In the US the Food and Drug Administration (FDA) has the responsibility for issuing an Investigational New Drug Application (IND). Applications for a CTA/IND have to be supported by details of the proposed clinical trial's protocol which must define the formulation to be used, its route of administration, maximum dose and whether or not the drug is of biological origin and if it is whether or not it is of recombinant technology or a gene transfer product. In addition, details of the location of the clinical trial, including its emergency facilities, and the identity and expertise of the principal investigators have to be provided together with details of the insurance cover in the event of adverse effects on the trial subjects. Paralleling the award of a CTA/IND, a clinical trial has to be approved by an independent Research Ethics Committee (REC) or Institutional Review Board (IRB). These are nationally approved bodies that are subject to both national and international guidelines and regulations. In Europe there is the European Clinical Trials Directive (2001) and the EC Good Clinical Practice Directive (2005) both of which define the responsibilities, duties and functions of all those involved in clinical trials, all of which are enforced by law. All human studies are subject to the Declaration of Helsinki first signed in 1964 but subsequently modified on several occasions. The declaration identified the basic importance of 'risk assessment' and 'informed consent' in the review and approval of protocols for clinical trials. Essentially, benefits must outweigh risk and the interests of the study subjects must take precedence over potential advances in medicine. Subjects must be informed in lay language about the design, objectives and potential risks of the study and must give their written informed consent to participate. Moreover, they must be free to withdraw from a study at any point without giving a reason and all their personal details collected for the study must remain confidential and not put into the public domain. RECs and IRBs consist of 'expert' (medical and scientific) and 'lay'members and their task is to assess the study protocol, to evaluate its design and risks and to ensure that the interests of the recruited subjects are protected. Since Phase I studies mainly involve healthy

subjects who by definition cannot personally gain from the study, the risk/benefit balance is difficult to judge. Healthy subjects are allowed to receive financial payment that should be proportionate to their time and inconvenience incurred, but such payments are sometimes judged to be an incentive to recruitment. Phase I studies are carried out either in a designated commercial or non-commercial unit or in a hospital. All Phase II to IV studies are carried out in a hospital. Once a CTA/IND and ethical approval have been granted investigators are not permitted to deviate from the approved design and must immediately report any adverse events to the approving bodies. Both the regulatory authority and the ethics committee have the power to stop a clinical trial if adverse events justify such action. Equally, the regulatory authority can agree to the premature termination of a trial if the therapeutic advantages of the trial drug over the control drug are statistically clear-cut.

The CTA system for approval of clinical trials means that the number of such trials worldwide can be monitored (www.ClinicalTrials.gov). At the end of 2007 more than 5000 trials were in progress at over 127 000 sites. The six most common therapeutic areas were oncology, central nervous system disorders, cardiology, infectious diseases, endocrinology and respiratory diseases. These six areas represented 68% of all protocols and 74% of all sites.

18.3.3 Patent protection

The large sums of money involved in drug discovery and development can only be recouped by the protection of the intellectual property inherent in the process thereby giving the company a monopoly over the sales of the drug subject to the approval of the appropriate licensing authority. For a drug to be patentable, it must be novel (i.e. new and not covered by previous patents), its discovery must involve an 'inventive step' represented by the pharmacological, biochemical and chemical research underlying the development, and the drug must have a 'utility' represented by its therapeutic use for a particular medical condition. One of the dilemmas facing the developing company is the timing of the filing of the patent application. Too early and the range of chemical structures possessing the particular pharmacological property may not have been fully defined thus allowing competitors the potential opportunity for developing a 'me-too' drug without infringing the patent. In addition, the earlier the application is filed the earlier it will subsequently expire. Too late and there is a risk that a competitor may 'prior date' with an identical application. In practice, most new drug applications are made towards the end of the discovery phase when the candidate drug is being identified. Most countries in the world now recognise the Patent Cooperation Treaty (PCT) under which filing a single application in one signatory country gives protection in the remainder. Once the application has been filed, officers of the World Intellectual Property Office in Geneva are charged with the task of producing a search report assessing the case for granting a patent. Once granted, a patent lasts for 20 years from the priority date. During this period the company must pay an annual fee that increases with the life of the patent to keep the patent in force. In the case of patents for new drugs this 20-year term may subsequently be extended by up to 5 years if the company can demonstrate that due

to the long development time it has not had a reasonable opportunity to exploit the patent. By the time the patent protection of most drugs expires, more therapeutically effective drugs will have been licensed so lack of protection is not necessarily a major concern.

18.3.4 Marketing authorisation

When the Phase III clinical trials are complete, the sponsor must submit to the regulatory authority, such as the MHRA or FDA, a New Drug Application (NDA) for permission to market the drug. In the EU there is a mutual recognition approval procedure coordinated by the EMEA through its Committee on Human Medicinal Products (CHMP) whereby one member country assesses the application and seeks to gain recognition in others. Applications for marketing authorisation must be accompanied by large amounts of documentation supporting all the clinical trials data that underpin the application. Marketing authorisation is granted on a risk–benefit analysis basis. The authorities recognise that no drug is ever risk-free and their task is to assess whether the balance of the evidence submitted by the company is in favour of the benefits. Approval may take up to 2 years but the authorities do attempt to accelerate the process for drugs that represent totally new therapies. In the UK, the National Committee for Health and Clinical Excellence (NICE) has the additional power to decide whether or not the National Health Service (NHS) will agree to make a newly authorised drug available to UK patients. Decisions by NICE depend not only on the drug's novelty but also on the economic benefits ensuing from its use. This latter criterion is particularly important in approvals for expensive new therapies such as monoclonal antibodies and is an increasingly important factor in the clinical acceptability for all new medicines in many countries.

18.4 SUGGESTIONS FOR FURTHER READING

General texts

Anonymous (2007). *Guidelines on the Practice of Ethics Committees in Medical Research with Human Participants*, 4th edn. London: Royal College of Physicians.

Crooke, S. T. (ed.) (2008). *Antisense Drug Technology: Principles, Strategies and Applications*, 2nd edn. New York: CRC Press. (Gives a review of the mechanisms of action, pharmacokinetics and therapeutic potential of antisense drugs in the context of a wide range of clinical conditions.)

Kshirsagar, T. (ed.) (2008). *High-Throughput Lead Optimization in Drug Discovery*. New York: CRC Press. (Uses real examples to illustrate the application of the technique in modern drug development.)

Rang, H. P. (ed.) (2006). *Drug Discovery and Development: Technology in Transition*. London: Elsevier. (An in-depth coverage written in an authoritative style by experts from the pharmaceutical industry.)

Review articles

Cross, D. M. and Bayliss, M. K. (2000). A commentary on the use of hepatocytes in drug metabolism studies during drug discovery. *Drug Metabolism Reviews*, **32**, 219–240.

Eichler, H.-G., Pinatti, F., Flamion, B., Leufkens, H. and Breckenridge, A. (2008). Balancing early market access to new drugs with the need for benefit/risk data: a mounting dilemma. *Nature Reviews Drug Discovery*, **7**, 818–826.

Ekins, S., Mestres, J. and Tasta, B. (2007). In silico pharmacology for drug discovery: methods for virtual ligand screening and profiling. *British Journal of Pharmacology*, **152**, 9–20.
Gomez-Hens, A. and Aguillar-Caballos, M. P. (2007). Modern analytical approaches to high-throughput drug discovery. *Trends in Analytical Chemistry*, **26**, 171–180.
Imming, P., Sinning, C. and Meyer, A. (2006). Drugs, their targets and the nature and number of drug targets. *Nature Reviews Drug Discovery*, **5**, 821–832.
Joy, T. and Hegele, R. A. (2008). Is raising HDL a futile strategy for atheroprotection? *Nature Reviews Drug Discovery*, **7**, 143–154.
Karlberg, J. P. E. (2008). Trends in disease focus of drug development. *Nature Reviews Drug Discovery*, **7**, 639–640.
Lagerstrom, M. C. and Schioth, H. B. (2008). Structural diversity of G-protein coupled receptors and significance for drug discovery. *Nature Reviews Drug Discovery*, **7**, 339–357.
Lindsay, A. C. and Choudhury, R. P. (2008). Form to function: current and future roles for atherosclerosis imaging in drug discovery. *Nature Reviews Drug Discovery*, **7**, 517–530.
Neil, S. J., Zang, T. and Bieniasz, P. D. (2008). Tetherin inhibits retrovirus release and is antagonised by HIV-1 Vpu. *Nature*, **451**, 425–431.
Rader, D. J. and Daugherty, A. (2008). Translating molecular discoveries into new therapies for atherosclerosis. *Nature*, **451**, 904–912.
Rognan, D. (2007). Chemogenomic approaches to rational drug design. *British Journal of Pharmacology*, **152**, 38–52.
Rose, A. D. (2008). Pharmacogenetics in drug discovery and development: a translational perspective. *Nature Reviews Drug Discovery*, **7**, 807–817.
Starkuviene, V. and Pepperkok, R. (2007). The potential of high-content high-throughput microscopy in drug discovery. *British Journal of Pharmacology*, **152**, 62–71.
Wang, C.-Y., Liu, P. Y. and Liao, J. K. (2008). Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. *Trends in Molecular Medicine*, **14**, 32–44.