51 Antibacterial drugs

OVERVIEW

In this chapter we continue to develop the ideas we introduced in the previous chapter. A detailed discussion of bacteriology is beyond the scope of this book but information about some clinically significant pathogens (see Table 51.1) is provided to give some context. The major classes of antibacterial[1] drugs are described (see Table 51.2), along with their mechanism of action, relevant pharmacokinetic properties and side effects. We conclude with an overview of new directions in research in this vital area.

INTRODUCTION

In 1928 Alexander Fleming, working at St Mary's Hospital in London, discovered that a culture plate on which staphylococci were being grown had become contaminated with a mould of the genus *Penicillium*. He made the crucial observation that bacterial growth in the vicinity of the mould had been inhibited. He subsequently isolated the mould in pure culture and demonstrated that it produced an antibacterial substance, which he called **penicillin**. This substance was subsequently prepared in bulk, extracted and its antibacterial effects analysed by Florey, Chain and their colleagues at Oxford in 1940. They showed that it was non-toxic to the host but killed the pathogens in infected mice, thus ushering in the 'antibiotic era'. Seventy years later, the number of different types of antibiotics had grown 10-fold and the practice of medicine would be unthinkable without them.

Gram staining and bacterial cell wall structure

Most bacteria can be classified as being either *Gram-positive* or *Gram-negative* depending on whether or not they stain with *Gram's stain*. This reflects fundamental differences in the structure of their cell walls, which has important implications for the action of antibiotics.

The cell wall of Gram-positive organisms is a relatively simple structure. It is some 15–50 nm thick and comprises about 50% peptidoglycan (see Ch. 50), 40–45% acidic polymer (which results in the cell surface being highly polar and negatively charged) together with 5–10% proteins and polysaccharides. The strongly polar polymer layer influences the penetration of ionised molecules and favours the penetration into the cell of positively charged compounds such as **streptomycin**.

The cell wall of Gram-negative organisms is much more complex. From the plasma membrane outwards, it consists of the following:

- A *periplasmic space* containing enzymes and other components.
- A *peptidoglycan layer* 2 nm in thickness, forming 5% of the cell wall mass, which is often linked to outwardly projecting lipoprotein molecules.
- An *outer membrane* consisting of a lipid bilayer, similar in some respects to the plasma membrane, that contains protein molecules and (on its inner aspect) lipoproteins linked to the peptidoglycan. Other proteins form transmembrane water-filled channels, termed *porins*, through which hydrophilic antibiotics can move freely.
- *Complex polysaccharides* forming important components of the outer surface. These differ between strains of bacteria and are the main determinants of their antigenicity. They are also the source of *endotoxin* which, when shed *in vivo*, triggers various aspects of the inflammatory reaction by activating complement and cytokines, causing fever, etc. (see Ch. 6).

Difficulty in penetrating this complex outer layer explains why some antibiotics are less active against Gram-negative than Gram-positive bacteria. It is also one reason for the extraordinary antibiotic resistance exhibited by *Pseudomonas aeruginosa*, a pathogen that can cause life-threatening infections in neutropenic patients and those with burns and wounds. The cell wall lipopolysaccharide is also a major barrier to penetration of some antibiotics, including **benzylpenicillin**, **meticillin**, the macrolides, **rifampicin**, **fusidic acid** and **vancomycin**.

In discussing the pharmacology of antibacterial drugs, it is convenient to divide them into different groups based upon their mechanism of action.

ANTIBACTERIAL AGENTS THAT INTERFERE WITH FOLATE SYNTHESIS OR ACTION

SULFONAMIDES

In a landmark discovery in the 1930s, before the advent of penicillin, Domagk demonstrated that it was possible for a drug to suppress a bacterial infection. The agent was prontosil,[2] a dye that proved to be an inactive prodrug that was metabolised *in vivo* to an active product, **sulfanilamide** (Fig. 51.1). Many sulfonamides have been developed since, but their importance has declined in the face of increasing resistance. The only sulfonamide drugs still commonly used as systemic antibacterials are **sulfamethoxazole** (usually in combination with **trimethoprim** as **co-trimoxazole**), **sulfasalazine** (poorly absorbed in the

[1]Strictly speaking, the term 'antibiotic' only applies to antibacterials that are produced by one organism to kill others (e.g. penicillin) in contrast to synthetic compounds such as the sulfonamides. In practice, however, this distinction is often ignored as many antibacterial drugs are 'semi-synthetic' (e.g. flucloxacillin).

[2]Domagk believed, wrongly, that the staining property of azo dyes, such as prontosil, was responsible for their antibacterial action. He used prontosil – a red dye – to treat his young daughter for a life-threatening streptococcal infection. She survived, but was left with permanently red-stained skin.

Table 51.1 Some clinically significant pathogenic bacteria

Genus	Morphology	Species	Disease
Gram-negative			
Bordetella	Cocci	*B. pertussis*	Whooping cough
Brucella	Curved rods	*B. abortus*	Brucellosis (cattle and humans)
Campylobacter	Spiral rods	*C. jejuni*	Food poisoning
Escherichia	Rods	*E. coli*	Septicaemia, wound infections, UTIs
Haemophilus	Rods	*H. influenzae*	Acute respiratory tract infection, meningitis
Helicobacter	Motile rods	*H. pylori*	Peptic ulcers, gastric cancer
Klebsiella	Capsulated rods	*K. pneumoniae*	Pneumonia, septicaemia
Legionella	Flagellated rods	*L. pneumophila*	Legionnaires' disease
Neisseria	Cocci, paired	*N. gonorrhea*	Gonorrhoea
Pseudomonas	Flagellated rods	*P. aeruginosa*	Septicaemia, respiratory infections, UTIs
Rickettsiae	Cocci or threads	Several spp.	Tick- and insect-borne infections
Salmonella	Motile rods	*S. typhimurium*	Food poisoning
Shigella	Rods	*S. dysenteriae*	Bacillary dysentry
Yersinia	Rods	*Y. pestis*	Bubonic plague
Vibrio	Flagellated rods	*V. cholerae*	Cholera
Gram-positive			
Bacillus	Rods, chains	*B. anthrax*	Anthrax
Clostridium	Rods	*C. tetani*	Tetanus
Corynebacterium	Rod	*C. diphtheriae*	Diphtheria
Mycobacterium	Rods	*M. tuberculosis*	Tuberculosis
		M. leprae	Leprosy
Staphylococcus	Cocci, clusters	*S. aureus*	Wound infections, boils, septicaemia
Streptococcus	Cocci, pairs	*S. pneumoniae*	Pneumonia, meningitis
	Cocci, chains	*S. pyogenes*	Scarlet fever, rheumatic fever, cellulitis
Other			
Chlamydia	Gram 'uncertain'	*C. trachomatis*	Eye disease, infertility
Treponema	Flagellated spiral rods	*T. pallidum*	Syphillis

UTI, urinary tract infection.

gastrointestinal tract, used to treat ulcerative colitis and Crohn's disease; see Chs 26 and 30). Silver **sulfadiazine** is used topically, for example to treat infected burns. Some drugs with quite different clinical uses (e.g. the antiplatelet drug **prasugrel**, Ch. 24, and the carbonic anhydrase inhibitor **acetazolamide**, Ch. 29), are sulfonamides and share some of the off-target adverse effects of this class.

Mechanism of action

Sulfanilamide is a structural analogue of *p*-aminobenzoic acid (PABA; see Fig. 51.1), which is an essential precursor in the synthesis of folic acid, required for the synthesis of DNA and RNA in bacteria (see Ch. 50). Sulfonamides

Clinical uses of sulfonamides

- Combined with **trimethoprim** (**co-trimoxazole**) for *Pneumocystis carinii* (now known as *P. jirovecii*), for toxoplamsosis and nocardiasis.
- Combined with **pyrimethamine** for drug-resistant malaria (Table 54.1) and for toxoplasmosis.
- In inflammatory bowel disease: **sulfasalazine** (sulfapyridine–aminosalicylate combination) is used (see Ch. 30).
- For infected burns (silver **sulfadiazine** given topically).

Table 51.2 A general overview of antibacterials and their mechanism of action

Family	Examples	Typical target organisms	Mechanism of action
Sulfonamides	Sulfadiazine, sulfamethoxazole, (trimethoprim)	*T. gondii*, *P. jirovecii*	Bacterial folate synthesis or action
β-lactams	PENICILLINS Benzylpenicillin, phenoxymethylpenicillin	Overall, mainly Gram-positive spp.; some Gram-negative spp.	Bacterial cell wall peptidoglycan synthesis
	Penicillinase-resistant penicillins Flucloxacillin, temocillin	Used for staphylococcal infections	
	Broad-spectrum penicillins Amoxicillin, ampicillin	A wide range of Gram-positive and Gram-negative spp.	
	Antipseudomonal penicillins Piperacillin, ticarcillin	Selected Gram-negative spp., especially *P. aeruginosa*	
	MECILLINAMS Pivmecillinam	Mainly Gram-negative spp.	
	CEPHALOSPORINS Cefalcor, cefadroxil, cefalexin, cefixime, cefotaxime, cefpodoxime, cefradine, ceftaroline, ceftazidime, ceftriaxone, cefuroxime	Broad spectrum of activity against Gram-negative and positive spp.	
	CARBAPENEMS Ertapenem, impenem, meropenem, doripenem.	Many Gram-negative and positive spp.	
	MONOBACTAMS Aztreonam	Gram-negative rods	
Glycopeptides	Vancomycin, teicoplanin, daptomycin	Gram-positive spp.	
Polymixins	Colistimethate, polymixin B	Gram-negative spp.	Bacterial outer cell membrane structure
Tetracyclines	Demeclocycline, doxycycline, lymecycline, minocycline, oxytetracycline, tetracycline tigecycline	Many Gram-negative and Gram-positive spp.	Bacterial protein synthesis (multiple mechanisms inhibited including initiation, transpeptidation and translocation; see text)
Aminogycosides	Amikacin, gentamicin, neomycin, tobramycin	Many Gram-negative, some Gram-positive spp.	
Macrolides	Azithromycin, clarithromycin, erythromycin, spiramycin, telithromycin	Similar to penicillin	
Oxazolidinones	Linezolid	Gram-positive spp.	
Lincosamides	Clindamycin	Gram-positive spp.	
Amphenicols	Chloramphenicol	Gram-negative and Gram-positive spp.	
Streptogramins	Quinupristin, dalfopristin	Gram-positive spp.	
Antimycobacterials	Capreomycin, clofazimine, cycloserine, dapsone, ethambutol, isoniazid, pyrazinamide, rifabutin, rifampicin	Most used for mycobacterial infections only	Various unrelated mechanisms (see text)
Quinolones	Ciprofloxacin, levofloxacin, moxifloxacin, nalidixic acid, norfloxacin, ofloxacin	Gram-negative and Gram-positive spp.	Bacterial DNA synthesis
Miscellaneous	Fusidic acid	Gram-positive spp.	Bacterial protein synthesis
	Nitrofurantoin	Gram-negative UTIs	Damages bacterial DNA
	Methenamine	Gram-negative UTIs	Formaldehyde pro-drug

Drug mixtures (e.g. co-fluampicil – flucloxacillin with ampicillin) are not shown.

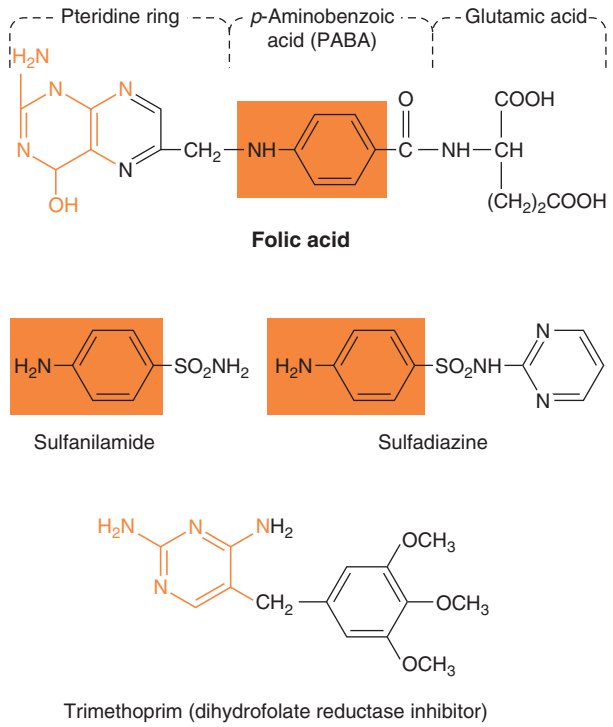

Fig. 51.1 Structures of two representative sulfonamides and trimethoprim. The structures illustrate the relationship between the sulfonamides and the *p*-aminobenzoic acid moiety in folic acid (orange box), as well as the possible relationship between the antifolate drugs and the pteridine moiety (orange). Co-trimoxazole is a mixture of sulfamethoxazole and trimethoprim.

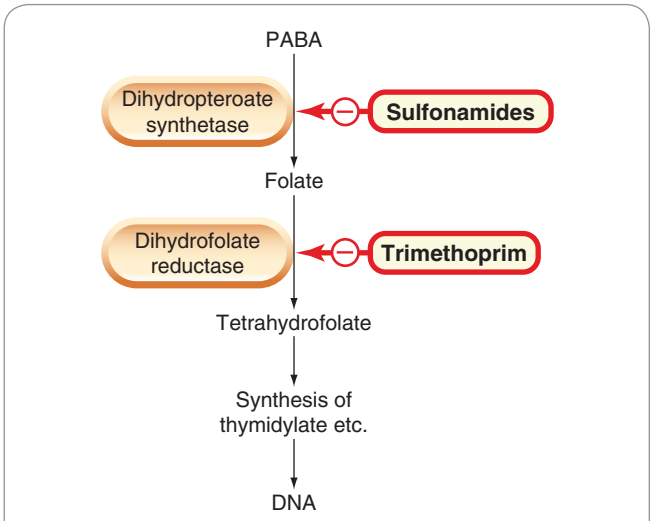

Fig. 51.2 The action of sulfonamides and trimethoprim on bacterial folate synthesis. See Chapter 25 for more detail of tetrahydrofolate synthesis, and Table 50.1 for comparisons of antifolate drugs. PABA, *p*-aminobenzoic acid.

compete with PABA for the enzyme *dihydropteroate synthetase*, and the effect of the sulfonamide may be overcome by adding excess PABA. This is why some local anaesthetics, which are PABA esters (such as **procaine**; see Ch. 43), can antagonise the antibacterial effect of these agents.

▼ While not necessarily clinically relevant, a general rule is that antibiotics that interfere with bacterial cell wall synthesis (e.g. penicillins: see Table 51.2) or inhibit crucial enzymes (such as the quinolones) generally kill bacteria (i.e. they are *bactericidal*). Those that inhibit protein synthesis such as the tetracyclines, tend to be *bacteriostatic*, that is they prevent growth and replication. Sulfonamides belong to the second group.

Sulfonamide action is vitiated in the presence of pus or products of tissue breakdown, because these contain thymidine and purines, which bacteria utilise directly, bypassing the requirement for folic acid. Resistance, which is common, is plasmid-mediated (see Ch. 50) and results from the synthesis of a bacterial enzyme insensitive to the drugs.

Pharmacokinetic aspects

Most sulfonamides are given orally and, apart from sulfasalazine, are well absorbed and widely distributed in the body. There is a risk of sensitisation or allergic reactions when these drugs are given topically.

The drugs pass into inflammatory exudates and cross both placental and blood–brain barriers. They are metabolised mainly in the liver, the major product being an acetylated derivative that lacks antibacterial action.

Unwanted effects

Serious adverse effects necessitating cessation of therapy include hepatitis, hypersensitivity reactions (rashes including Stevens–Johnson syndrome and toxic epidermal necrolysis, fever, anaphylactoid reactions – see Ch. 57), bone marrow depression and acute renal failure due to interstitial nephritis or crystalluria. This last effect results from the precipitation of acetylated metabolites in the urine (Ch. 29). Cyanosis caused by methaemoglobinaemia may occur but is a lot less alarming than it looks. Mild to moderate side effects include nausea and vomiting, headache and mental depression.

TRIMETHOPRIM

Mechanism of action

Trimethoprim is chemically related to the antimalarial drug **pyrimethamine** (Ch. 54), both being folate antagonists. Structurally (Fig. 51.1), it resembles the pteridine moiety of folate and the similarity is close enough to fool the bacterial dihydrofolate reductase, which is many times more sensitive to trimethoprim than is the equivalent enzyme in humans.

Trimethoprim, also bacteriostatic, is active against most common bacterial pathogens as well as protozoa, and is used to treat various urinary, pulmonary and other infections. It is sometimes given as a mixture with sulfamethoxazole as co-trimoxazole (Fig. 51.1). Because sulfonamides inhibit a different stage on the same bacterial metabolic pathway, they can potentiate the action of trimethoprim (see Fig. 51.2). In the UK, the use of co-trimoxazole is generally restricted to the treatment of *Pneumocystis carinii* (now known as *P. jirovecii*) pneumonia (a fungal infection), toxoplasmosis (a protozoan infection) or nocardiasis (a bacterial infection).

Pharmacokinetic aspects

Trimethoprim is well absorbed orally, and widely distributed throughout the tissues and body fluids. It reaches high concentrations in the lungs and kidneys, and fairly high concentrations in the cerebrospinal fluid (CSF). When given with sulfamethoxazole, about half the dose of each is excreted within 24 h. Because trimethoprim is a weak base, its elimination by the kidney increases with decreasing urinary pH.

Unwanted effects

Folate deficiency, with resultant *megaloblastic anaemia* (see Ch. 25) is a danger of long-term administration of trimethoprim. Other unwanted effects include nausea, vomiting, blood disorders and rashes.

Antimicrobial agents that interfere with the synthesis or action of folate

- Sulfonamides are bacteriostatic; they act by interfering with folate synthesis and thus with nucleotide synthesis. Unwanted effects include crystalluria and hypersensitivities.
- **Trimethoprim** is bacteriostatic. It acts by antagonising folate.
- **Co-trimoxazole** is a mixture of **trimethoprim** with **sulfamethoxazole**, which affects bacterial nucleotide synthesis at two points in the pathway.
- **Pyrimethamine** and **proguanil** are also antimalarial agents (see Ch. 54).

β-LACTAM ANTIBIOTICS

PENICILLINS

The remarkable antibacterial effects of systemic penicillin in humans were clearly demonstrated in 1941.[3] A small amount of penicillin, extracted laboriously from crude cultures in the laboratories of the Dunn School of Pathology in Oxford, was given to a desperately ill policeman who had septicaemia with multiple abscesses. Although sulfonamides were available, they would have had no effect in the presence of pus. Intravenous injections of penicillin were given every 3 h. All the patient's urine was collected, and each day the bulk of the excreted penicillin was extracted and reused. After 5 days, the patient's condition was vastly improved, and there was obvious resolution of the abscesses. Furthermore, there seemed to be no toxic effects of the drug. Unfortunately, when the supply of penicillin was finally exhausted his condition gradually deteriorated and he died a month later.

Penicillins, often combined with other antibiotics, remain crucially important in antibacterial chemotherapy, but they can be destroyed by bacterial *amidases* and *β-lactamases* (*penicillinases*; see Fig. 51.3). This forms the basis of one of the principal types of antibiotic resistance.

Mechanisms of action

All β-lactam antibiotics interfere with the synthesis of the bacterial cell wall peptidoglycan (see Ch. 50, Fig. 50.3). After attachment to *penicillin-binding proteins* on bacteria (there may be seven or more types in different organisms), they inhibit the transpeptidation enzyme that crosslinks the peptide chains attached to the backbone of the peptidoglycan.

[3]Although *topical* penicillin had actually been used with success in five patients with eye infections 10 years previously by Paine, a graduate of St Mary's who had obtained some penicillin mould from Fleming.

Clinical uses of the penicillins

- Penicillins are given by mouth or, in more severe infections, intravenously, and often in combination with other antibiotics.
- Uses are for sensitive organisms and may (but may not: individual sensitivity testing is often appropriate depending on local conditions) include:
  - *bacterial meningitis* (e.g. caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*): **benzylpenicillin**, high doses intravenously
  - *bone* and *joint infections* (e.g. with *Staphylococcus aureus*): **flucloxacillin**
  - *skin* and *soft tissue infections* (e.g. with *Streptococcus pyogenes* or *S. aureus*): **benzylpenicillin**, **flucloxacillin**; animal bites: **co-amoxiclav**
  - *pharyngitis* (from *S. pyogenes*): **phenoxylmethylpenicillin**
  - *otitis media* (organisms commonly include *S. pyogenes*, *Haemophilus influenzae*): **amoxicillin**
  - *bronchitis* (mixed infections common): **amoxicillin**
  - *pneumonia*: **amoxicillin**
  - *urinary tract infections* (e.g. with *Escherichia coli*): **amoxicillin**
  - *gonorrhea*: **amoxicillin** (plus **probenecid**)
  - *syphilis*: **procaine benzylpenicillin**
  - *endocarditis* (e.g. with *Streptococcus viridans* or *Enterococcus faecalis*): high-dose intravenous **benzylpenicillin** sometimes with an aminoglycoside
  - serious infections with *Psuedomonas aeruginosa*: **ticarcillin**, **piperacillin**.

This list is not exhaustive. Treatment with penicillins is sometimes started empirically, if the likely causative organism is one thought to be susceptible to penicillin, while awaiting the results of laboratory tests to identify the organism and determine its antibiotic susceptibility.

The final bactericidal event is the inactivation of an inhibitor of autolytic enzymes in the cell wall, leading to lysis of the bacterium. Some organisms, referred to as 'tolerant', have defective autolytic enzymes in which case lysis does not occur in response to the drug. Resistance to penicillin may result from a number of different causes and is discussed in detail in Chapter 50.

Types of penicillin and their antimicrobial activity

The first penicillins were the naturally occurring **benzylpenicillin** (**penicillin G**) and its congeners, including **phenoxymethylpenicillin** (**penicillin V**). Benzylpenicillin is active against a wide range of organisms and is the drug of first choice for many infections (see clinical box, above). Its main drawbacks are poor absorption in the gastrointestinal tract (which means it must be given by injection) and its susceptibility to bacterial β-lactamases.

Semisynthetic penicillins, incorporating different side-chains attached to the penicillin nucleus (at R_1 in Fig. 51.3),

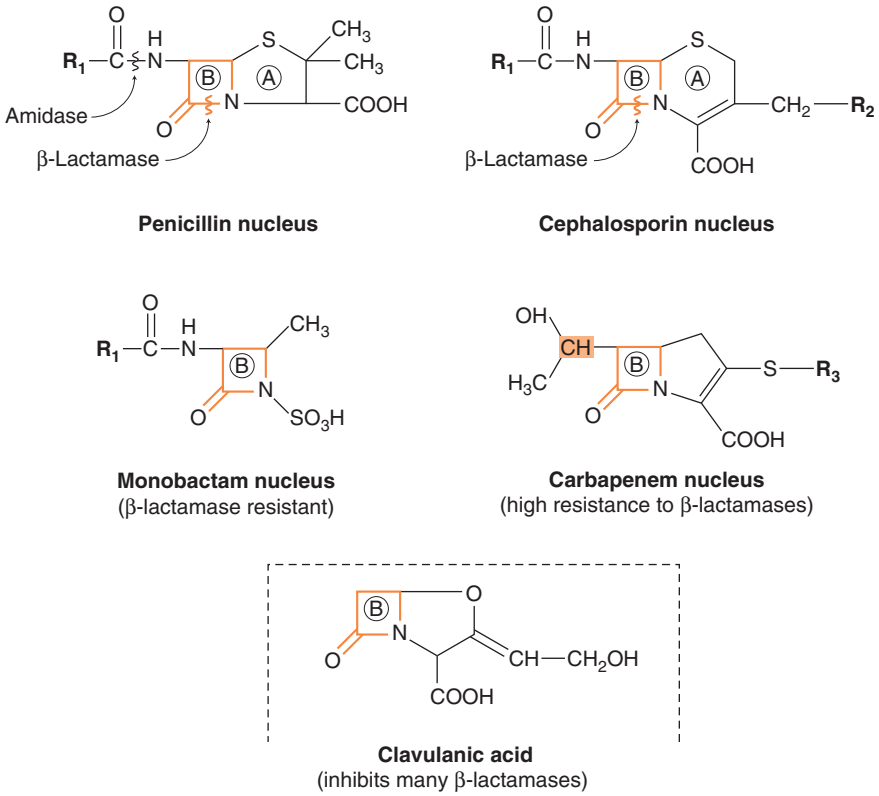

Fig. 51.3 Basic structures of four groups of β-lactam antibiotics and clavulanic acid. The structures illustrate the β-lactam ring (marked B) and the sites of action of bacterial enzymes that inactivate these antibiotics (A, thiazolidine ring). Various substituents are added at R_1, R_2 and R_3 to produce agents with different properties. In carbapenems, the stereochemical configuration of the part of the β-lactam ring shown shaded in orange here is different from the corresponding part of the penicillin and cephalosporin molecules; this is probably the basis of the β-lactamase resistance of the carbapenems. The β-lactam ring of clavulanic acid is thought to bind strongly to β-lactamase, meanwhile protecting other β-lactams from the enzyme.

include *β-lactamase-resistant* penicillins (e.g. **meticillin**,[4] **flucloxacillin**, **temocillin**) and *broad-spectrum* penicillins (e.g. **ampicillin**, **amoxicillin**). *Extended-spectrum* penicillins (e.g. **ticarcillin**, **piperacillin**) with antipseudomonal activity have gone some way to overcoming the problem of serious infections caused by *P. aeruginosa*. Amoxicillin and ticarcillin are sometimes given in combination with the β-lactamase inhibitor **clavulanic acid** (e.g. **co-amoxiclav**). **Pivmecillinam** is a prodrug of mecillinam, which also has a wide spectrum of action.

Pharmacokinetic aspects

Oral absorption of penicillins varies, depending on their stability in acid and their adsorption to foodstuffs in the gut. Penicillins can also be given by intravenous injection. Preparations for intramuscular injection are also available, including slow-release preparations such as **benzathine benzylpenicillin** is useful for treating syphilis since *Treponema pallidum* is a very slowly dividing organism. Intrathecal administration of benzylpenicillin (used historically to treat meningitis) is no longer used, as it can cause convulsions.[5]

The penicillins are widely distributed in body fluids, passing into joints; into pleural and pericardial cavities; into bile, saliva and milk; and across the placenta. Being lipid-insoluble, they do not enter mammalian cells, and cross the blood–brain barrier only if the meninges are inflamed, in which case they may reach therapeutically effective concentrations in the cerebrospinal fluid.

Elimination of most penicillins occurs rapidly and is mainly renal, 90% being through tubular secretion. The relatively short plasma half-life is a potential problem in the clinical use of benzylpenicillin, although because penicillin works by preventing cell wall synthesis in dividing organisms, intermittent rather than continuous exposure to the drug can be an advantage.

Unwanted effects

Penicillins are relatively free from direct toxic effects (other than their proconvulsant effect when given intrathecally). The main unwanted effects are hypersensitivity reactions caused by the degradation products of penicillin, which combine with host protein and become antigenic. Skin rashes and fever are common; a delayed type of serum sickness occurs infrequently. Much more serious is acute anaphylactic shock which, although rare, may be fatal. When given orally, penicillins, particularly the broad-spectrum type, alter the bacterial flora in the gut. This can be associated with gastrointestinal disturbances and in some cases with suprainfection by other, penicillin-insensitive, microorganisms leading to problems such as pseudomembranous colitis (caused by *Clostridium difficile*, see below).

CEPHALOSPORINS AND CEPHAMYCINS

Cephalosporins and cephamycins are β-lactam antibiotics, first isolated from fungi. They all have the same mechanism of action as penicillins.

[4]Meticillin (previous name: methicillin) was the first β-lactamase-resistant penicillin. It is not now used clinically because it was associated with interstitial nephritis, but is remembered in the acronym 'MRSA' – meticillin-resistant *Staphylococcus aureus*.

[5]Indeed, penicillins applied topically to the cortex are used to induce convulsions in an animal model of epilepsy (see Ch. 45).

Semisynthetic broad-spectrum cephalosporins have been produced by addition, to the cephalosporin C nucleus, of different side-chains at R_1 and/or R_2 (see Fig. 51.3). These agents are water-soluble and relatively acid stable. They vary in susceptibility to β-lactamases. Many cephalosporins and cephamycins are now available for clinical use (see list in Table 51.2). Resistance to this group of drugs has increased because of plasmid-encoded or chromosomal β-lactamase. The latter is present in nearly all Gram-negative bacteria and it is more active in hydrolysing cephalosporins than penicillins. In several organisms a single mutation can result in high-level constitutive production of this enzyme. Resistance also occurs when there is decreased penetration of the drug as a result of alterations to outer membrane proteins, or mutations of the binding-site proteins.

Clinical uses of the cephalosporins

Cephalosporins are used to treat infections caused by sensitive organisms. As with other antibiotics, patterns of sensitivity vary geographically, and treatment is often started empirically. Many different kinds of infection may be treated, including:

- *septicaemia* (e.g. **cefuroxime**, **cefotaxime**)
- *pneumonia* caused by susceptible organisms
- *meningitis* (e.g. **ceftriaxone**, **cefotaxime**)
- *biliary tract infection*
- *urinary tract infection* (especially in pregnancy or in patients unresponsive to other drugs)
- *sinusitis* (e.g. **cefadroxil**).

Pharmacokinetic aspects

Some cephalosporins may be given orally, but most are given parenterally, intramuscularly (which may be painful) or intravenously. After absorption, they are widely distributed in the body and some, such as **cefotaxime**, **cefuroxime** and **ceftriaxone**, cross the blood–brain barrier. Excretion is mostly via the kidney, largely by tubular secretion, but 40% of ceftriaxone is eliminated in the bile.

Unwanted effects

Hypersensitivity reactions, very similar to those seen with penicillin, may occur, and there may be some cross-sensitivity; about 10% of penicillin-sensitive individuals will have allergic reactions to cephalosporins. Nephrotoxicity has been reported (especially with **cefradine**), as has drug-induced alcohol intolerance. Diarrhoea is common and can be due to *C. difficile*.

OTHER β-LACTAM ANTIBIOTICS

Carbapenems and monobactams (see Fig. 51.3) were developed to deal with β-lactamase-producing Gram-negative organisms resistant to penicillins.

CARBAPENEMS

Imipenem, an example of a carbapenem, acts in the same way as the other β-lactams (see Fig. 51.3). It has a very broad spectrum of antimicrobial activity, being active against many aerobic and anaerobic Gram-positive and Gram-negative organisms. However, many of the 'meticillin-resistant' staphylococci are less susceptible, and resistant strains of *P. aeruginosa* have emerged during therapy. Resistance to imipenem was low, but is increasing as some organisms now have chromosomal genes that code for imipenem-hydrolysing β-lactamases. It is sometimes given together with **cilastatin**, which inhibits its inactivation by renal enzymes. **Meropenem** is similar but is not metabolised by the kidney. **Ertapenem** has a broad spectrum of antibacterial actions but is licensed only for a limited range of indications. Most carbapenems are not orally active, and are used only in special situations.

Unwanted effects are generally similar to those seen with other β-lactams, nausea and vomiting being the most frequently seen. Neurotoxicity can occur with high plasma concentrations.

MONOBACTAMS

The main monobactam is **aztreonam** (see Fig. 51.3), which is resistant to most β-lactamases. It is given by injection and has a plasma half-life of 2 h. Aztreonam has an unusual spectrum of activity and is effective only against Gram-negative aerobic bacilli such as pseudomonas species, *Neisseria meningitidis* and *Haemophilus influenzae*. It has no action against Gram-positive organisms or anaerobes.

Unwanted effects are, in general, similar to those of other β-lactam antibiotics, but this agent does not necessarily cross-react immunologically with penicillin and its products, and so does not usually cause allergic reactions in penicillin-sensitive individuals.

GLYCOPEPTIDES

Vancomycin is a glycopeptide antibiotic, and **teicoplanin** is similar but longer lasting. Vancomycin inhibits cell wall synthesis (Ch. 50, Fig. 50.3). It is effective mainly against Gram-positive bacteria. Vancomycin is not absorbed from the gut and is only given by the oral route for treatment of gastrointestinal infection with *C. difficile*. For systemic use, it is given intravenously and has a plasma half-life of about 8 h.

The main clinical use of vancomycin is the treatment of MRSA (it is often the drug of last resort for this condition) and some other serious infections. It is also valuable in severe staphylococcal infections in patients allergic to both penicillins and cephalosporins.

Unwanted effects include fever, rashes and local phlebitis at the site of injection. Ototoxicity and nephrotoxicity can occur, and hypersensitivity reactions are occasionally seen.

Daptomycin is a new lipopeptide antibacterial with a similar spectrum of actions to vancomycin. It is usually used, in combination with other drugs, for the treatment of MRSA.

ANTIMICROBIAL AGENTS AFFECTING BACTERIAL PROTEIN SYNTHESIS

TETRACYCLINES

The tetracyclines are broad-spectrum antibiotics. The group includes **tetracycline**, **oxytetracycline**, **demeclocycline**, **lymecycline**, **doxycycline**, **minocycline** and **tigecycline**.

β-Lactam antibiotics

Bactericidal because they inhibit peptidoglycan synthesis.

Penicillins

- The first choice for many infections.
- **Benzylpenicillin**:
  - given by injection, short half-life and is destroyed by β-lactamases
  - spectrum: Gram-positive and Gram-negative cocci and some Gram-negative bacteria
  - many staphylococci are now resistant.
- β-Lactamase-resistant penicillins (e.g. **flucloxacillin**):
  - given orally
  - spectrum: as for benzylpenicillin
  - many staphylococci are now resistant.
- Broad-spectrum penicillins (e.g. **amoxicillin**):
  - given orally; they are destroyed by β-lactamases
  - spectrum: as for **benzylpenicillin** (although less potent); they are also active against Gram-negative bacteria.
- Extended-spectrum penicillins (e.g. **ticarcillin**):
  - given orally; they are susceptible to β-lactamases
  - spectrum: as for broad-spectrum penicillins; they are also active against pseudomonads.
- Unwanted effects of penicillins: mainly hypersensitivities.
- A combination of **clavulanic acid** plus **amoxicillin** or **ticarcillin** is effective against many β-lactamase-producing organisms.

Cephalosporins and cephamycins

- Second choice for many infections.
- Oral drugs (e.g. **cefaclor**) are used in urinary infections.
- Parenteral drugs (e.g. **cefuroxime**, which is active against *S. aureus*, *H. influenzae*, Enterobacteriaceae).
- Unwanted effects: mainly hypersensitivities.

Carbapenems

- **Imipenem** is a broad-spectrum antibiotic.
- Imipenem is used with **cilastin**, which prevents its breakdown in the kidney.

Monobactams

- **Aztreonam**: Active only against Gram-negative aerobic bacteria and resistant to most β-lactamases.

Miscellaneous antibacterial agents that prevent cell wall or membrane synthesis

- *Glycopeptide antibiotics* (e.g. **vancomycin**). **Vancomycin** is bactericidal, acting by inhibiting cell wall synthesis. It is used intravenously for multiresistant staphylococcal infections and orally for pseudomembranous colitis. Unwanted effects include ototoxicity and nephrotoxicity.
- *Polymixins* (e.g. **colistimethate**). They are bactericidal, acting by disrupting bacterial cell membranes. They are highly neurotoxic and nephrotoxic, and are only used topically.

Mechanism of action

Following uptake into susceptible organisms by active transport, tetracyclines act by inhibiting protein synthesis (see Ch. 50, Fig. 50.4). They are regarded as bacteriostatic, not bactericidal.

Antibacterial spectrum

The spectrum of antimicrobial activity of the tetracyclines is very wide and includes Gram-positive and Gram-negative bacteria, *Mycoplasma*, *Rickettsia*, *Chlamydia* spp., spirochaetes and some protozoa (e.g. amoebae). Minocycline is also effective against *N. meningitidis* and has been used to eradicate this organism from the nasopharynx of carriers. However, widespread resistance to these agents has decreased their usefulness. Resistance is transmitted mainly by plasmids and, because the genes controlling resistance to tetracyclines are closely associated with genes for resistance to other antibiotics, organisms may develop resistance to many drugs simultaneously.

Clinical uses of tetracyclines

- The use of tetracyclines declined because of widespread drug resistance, but has staged a comeback, e.g. for respiratory infections, as resistance has receded with reduced use. Most members of the group are microbiologically similar; **doxycycline** is given once daily and may be used in patients with renal impairment. Uses (sometimes in combination with other antibiotics) include:
  - rickettsial and chlamydial infections, brucellosis, anthrax and Lyme disease
  - as useful second choice, for example in patients with allergies, for several infections (see Table 51.1), including mycoplasma and leptospira
  - respiratory tract infections (e.g. exacerbations of chronic bronchitis, community-acquired pneumonia)
  - acne
  - inappropriate secretion of antidiuretic hormone (e.g. by some malignant lung tumours), causing hyponatraemia: **demeclocycline** inhibits the action of this hormone by an entirely distinct action from its antibacterial effect (Ch. 33).

Pharmacokinetic aspects

The tetracyclines are generally given orally but can also be administered parenterally. Minocycline and doxycycline are well absorbed orally. The absorption of most other tetracyclines is irregular and incomplete but is improved in the absence of food. Because tetracyclines chelate metal ions (calcium, magnesium, iron, aluminium), forming non-absorbable complexes, absorption is decreased in the presence of milk, certain antacids and iron preparations.

Unwanted effects

The commonest unwanted effects are gastrointestinal disturbances caused initially by direct irritation and later by modification of the gut flora. Vitamin B complex deficiency can occur, as can suprainfection. Because they chelate Ca^{2+}, tetracyclines are deposited in growing bones and teeth, causing staining and sometimes dental hypoplasia and bone deformities. They should therefore not be given to children, pregnant women or nursing mothers. Another hazard to pregnant women is hepatotoxicity. Phototoxicity (sensitisation to sunlight) has also been seen, particularly with demeclocycline. Minocycline can produce vestibular disturbances (dizziness and nausea). High doses of tetracyclines can decrease protein synthesis in host cells, an antianabolic effect that may result in renal damage. Long-term therapy can cause disturbances of the bone marrow.

CHLORAMPHENICOL

Chloramphenicol was originally isolated from cultures of *Streptomyces*. It inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit (see Ch. 50, Fig. 50.4).

Antibacterial spectrum

Chloramphenicol has a wide spectrum of antimicrobial activity, including Gram-negative and Gram-positive organisms and rickettsiae. It is bacteriostatic for most organisms but kills *H. influenzae*. Resistance, caused by the production of *chloramphenicol acetyltransferase*, is plasmid-mediated.

Clinical uses of chloramphenicol

- Systemic use should be reserved for serious infections in which the benefit of the drug outweighs its uncommon but serious haematological toxicity. Such uses may include:
  - infections caused by *Haemophilus influenzae* resistant to other drugs
  - *meningitis* in patients in whom penicillin cannot be used
  - *typhoid fever*, but **ciprofloxacin** or **amoxicillin** and **co-trimoxazole** are similarly effective and less toxic.
- Topical use safe and effective in bacterial conjunctivitis.

Pharmacokinetic aspects

Given orally, chloramphenicol is rapidly and completely absorbed and reaches its maximum concentration in the plasma within 2 h. It can also be given parenterally. The drug is widely distributed throughout the tissues and body fluids including the CSF. Its half-life is approximately 2 h. About 10% is excreted unchanged in the urine, and the remainder is inactivated in the liver.

Unwanted effects

The most important unwanted effect of chloramphenicol is severe, idiosyncratic depression of the bone marrow, resulting in *pancytopenia* (a decrease in all blood cell elements) – an effect that, although rare, can occur even with low doses in some individuals. Chloramphenicol must be used with great care in newborns, with monitoring of plasma concentrations, because inadequate inactivation and excretion of the drug can result in the 'grey baby syndrome' – vomiting, diarrhoea, flaccidity, low temperature and an ashen-grey colour – which carries 40% mortality. Hypersensitivity reactions can occur, as can gastrointestinal disturbances secondary to alteration of the intestinal microbial flora.

AMINOGLYCOSIDES

The aminoglycosides are a group of antibiotics of complex chemical structure, resembling each other in antimicrobial activity, pharmacokinetic characteristics and toxicity. The main agents are **gentamicin**, **streptomycin**, **amikacin**, **tobramycin** and **neomycin**.

Mechanism of action

Aminoglycosides inhibit bacterial protein synthesis (see Ch. 50). There are several possible sites of action. Their penetration through the cell membrane of the bacterium depends partly on oxygen-dependent active transport by a polyamine carrier system (which, incidentally, is blocked by chloramphenicol) and they have minimal action against anaerobic organisms. The effect of the aminoglycosides is bactericidal and is enhanced by agents that interfere with cell wall synthesis (e.g. penicillins).

Resistance

Resistance to aminoglycosides is becoming a problem. It occurs through several different mechanisms, the most important being inactivation by microbial enzymes, of which nine or more are known. Amikacin was designed as a poor substrate for these enzymes, but some organisms can inactivate this agent as well. Resistance as a result of failure of penetration can be largely overcome by the concomitant use of penicillin and/or vancomycin, at the cost of an increased risk of severe adverse effects.

Antibacterial spectrum

The aminoglycosides are effective against many aerobic Gram-negative and some Gram-positive organisms. They are most widely used against Gram-negative enteric organisms and in sepsis. They may be given together with a penicillin in streptococcal infections and those caused by *Listeria* spp. and *P. aeruginosa* (see Table 51.1). Gentamicin is the aminoglycoside most commonly used, although tobramycin is the preferred member of this group for *P. aeruginosa* infections. Amikacin has the widest antimicrobial spectrum and can be effective in infections with organisms resistant to gentamicin and tobramycin.

Pharmacokinetic aspects

The aminoglycosides are polycations and therefore highly polar. They are not absorbed from the gastrointestinal tract and are usually given intramuscularly or intravenously. They cross the placenta but do not cross the blood–brain barrier, although high concentrations can be attained in joint and pleural fluids. The plasma half-life is 2–3 h. Elimination is virtually entirely by glomerular filtration in the kidney, 50–60% of a dose being excreted unchanged within 24 h. If renal function is impaired, accumulation occurs rapidly, with a resultant increase in those toxic effects (such as ototoxicity and nephrotoxicity) that are dose related.

Unwanted effects

Serious, dose-related toxic effects, which may increase as treatment proceeds, can occur with the aminoglycosides, the main hazards being ototoxicity and nephrotoxicity.

The ototoxicity involves progressive damage to, and eventually destruction of, the sensory cells in the cochlea and vestibular organ of the ear. The result, usually irreversible, may manifest as vertigo, ataxia and loss of balance in the case of vestibular damage, and auditory disturbances or deafness in the case of cochlear damage. Any aminoglycoside may produce both types of effect, but streptomycin and gentamicin are more likely to interfere with vestibular function, whereas neomycin and amikacin mostly affect hearing. Ototoxicity is potentiated by the concomitant use of other ototoxic drugs (e.g. loop diuretics; Ch. 29) and susceptibility is genetically determined via mitochondrial DNA (see Ch. 11).

The nephrotoxicity consists of damage to the kidney tubules and may necessitate dialysis, although function usually recovers when administration ceases. Nephrotoxicity is more likely to occur in patients with pre-existing renal disease or in conditions in which urine volume is reduced, and concomitant use of other nephrotoxic agents (e.g. first-generation cephalosporins, vancomycin) increases the risk. As the elimination of these drugs is almost entirely renal, this nephrotoxic action can impair their own excretion and a vicious cycle may develop. Plasma concentrations should be monitored regularly and the dose adjusted accordingly.

A rare but serious toxic reaction is paralysis caused by neuromuscular blockade. This is usually seen only if the agents are given concurrently with neuromuscular-blocking agents. It results from inhibition of the Ca^{2+} uptake necessary for the exocytotic release of acetylcholine (see Ch. 13).

MACROLIDES

The term *macrolide* relates to the structure – a many-membered lactone ring to which one or more deoxy sugars are attached. The main macrolide and related antibiotics are **erythromycin**, **clarithromycin** and **azithromycin**. **Spiramycin** and **telithromycin** are of minor utility.

Mechanism of action

The macrolides inhibit bacterial protein synthesis by an effect on ribosomal translocation (Ch. 50, Fig. 50.4). The drugs bind to the same 50S subunit of the bacterial ribosome as chloramphenicol and **clindamycin**, and any of these drugs may compete if given concurrently.

Antimicrobial spectrum

The antimicrobial spectrum of erythromycin is very similar to that of penicillin, and it is a safe and effective alternative for penicillin-sensitive patients. Erythromycin is effective against Gram-positive bacteria and spirochaetes but not against most Gram-negative organisms, exceptions being *N. gonorrhoeae* and, to a lesser extent, *H. influenzae*. *Mycoplasma pneumoniae*, *Legionella* spp. and some chlamydial organisms are also susceptible (see Table 51.1). Resistance can occur and results from a plasmid-controlled alteration of the binding site for erythromycin on the bacterial ribosome (Ch. 50, Fig. 50.4).

Azithromycin is less active than erythromycin against Gram-positive bacteria but is considerably more effective against *H. influenzae* and may be more active against *Legionella*. It can be used to treat *Toxoplasma gondii*, as it kills the cysts. Clarithromycin is as active, and its metabolite is twice as active, against *H. influenzae* as erythromycin. It is also effective against *Mycobacterium avium-intracellulare* (which can infect immunologically compromised individuals and elderly patients with chronic lung disease), and it may also be useful in leprosy and against *Helicobacter pylori* (see Ch. 30). Both these macrolides are also effective in *Lyme disease*.

Pharmacokinetic aspects

The macrolides are administered orally or parenterally, although intravenous injections can be followed by local thrombophlebitis. They diffuse readily into most tissues but do not cross the blood–brain barrier, and there is poor penetration into synovial fluid. The plasma half-life of erythromycin is about 90 min; that of clarithromycin is three times longer, and that of azithromycin 8–16 times longer. Macrolides enter and indeed are concentrated within phagocytes – azithromycin concentrations in phagocyte lysosomes can be 40 times higher than in the blood – and they can enhance intracellular killing of bacteria by phagocytes.

Erythromycin is partly inactivated in the liver; azithromycin is more resistant to inactivation, and clarithromycin is converted to an active metabolite. Inhibition of the P450 cytochrome system by these agents can affect the bioavailability of other drugs leading to clinically important interactions, for example with theophylline. The major route of elimination is in the bile.

Unwanted effects

Gastrointestinal disturbances are common and unpleasant but not serious. With erythromycin, the following have also been reported: hypersensitivity reactions such as rashes and fever, transient hearing disturbances and rarely, following treatment for longer than 2 weeks, cholestatic jaundice. Opportunistic infections of the gastrointestinal tract or vagina can occur.

ANTIMICROBIAL AGENTS AFFECTING TOPOISOMERASE

QUINOLONES

The quinolones include the broad-spectrum agents **ciprofloxacin**, **levofloxacin**, **ofloxacin**, **norfloxacin** and **moxifloxacin** as well as **nalidixic acid,** a narrow-spectrum drug used in urinary tract infections. Most are fluorinated (fluoroquinolones). These agents inhibit topoisomerase II (a bacterial DNA gyrase), the enzyme that produces a negative supercoil in DNA and thus permits transcription or replication (see Fig. 51.4).

Antibacterial spectrum and clinical use

Ciprofloxacin is the most commonly used and typical of the group. It is a broad-spectrum antibiotic effective against both Gram-positive and Gram-negative organisms, including the *Enterobacteriaceae* (enteric Gram-negative bacilli), many organisms resistant to penicillins, cephalosporins and aminoglycosides, and against *H. influenzae*, penicillinase-producing *N. gonorrhoeae*, *Campylobacter* spp. and pseudomonads. Of the Gram-positive organisms, streptococci and pneumococci are only weakly inhibited, and there is a high incidence of staphylococcal resistance. Ciprofloxacin should be avoided in MRSA infections. Clinically, the fluoroquinolones are best reserved for infections with facultative and aerobic

Antimicrobial agents affecting bacterial protein synthesis

- *Tetracyclines* (e.g. **minocycline**). These are orally active, bacteriostatic, broad-spectrum antibiotics. Resistance is increasing. Gastrointestinal disorders are common. They also chelate calcium and are deposited in growing bone. They are contraindicated in children and pregnant women.
- ***Chloramphenicol***. This is an orally active, bacteriostatic, broad-spectrum antibiotic. Serious toxic effects are possible, including bone marrow depression and 'grey baby syndrome'. It should be reserved for life-threatening infections.
- *Aminoglycosides* (e.g. **gentamicin**). These are given by injection. They are bactericidal, broad-spectrum antibiotics (but with low activity against anaerobes, streptococci and pneumococci). Resistance is increasing. The main unwanted effects are dose-related nephrotoxicity and ototoxicity. Serum levels should be monitored. (**Streptomycin** is an anti-tuberculosis aminoglycoside.)
- *Macrolides* (e.g. **erythromycin**). Can be given orally and parenterally. They are bactericidal/bacteriostatic. The antibacterial spectrum is the same as for penicillin. **Erythromycin** can cause jaundice. Newer agents are **clarithromycin** and **azithromycin**.
- *Lincosamides* (e.g. **clindamycin**). Can be given orally and parenterally. It can cause pseudomembranous colitis.
- *Streptogramins* (e.g. **quinupristin/dalfopristin**). Given by intravenous infusion as a combination. Considerably less active when administered separately. Active against several strains of drug-resistant bacteria.
- ***Fusidic acid***. This is a narrow-spectrum antibiotic that acts by inhibiting protein synthesis. It penetrates bone. Unwanted effects include gastrointestinal disorders.
- ***Linezolid***. Given orally or by intravenous injection. Active against several strains of drug-resistant bacteria.

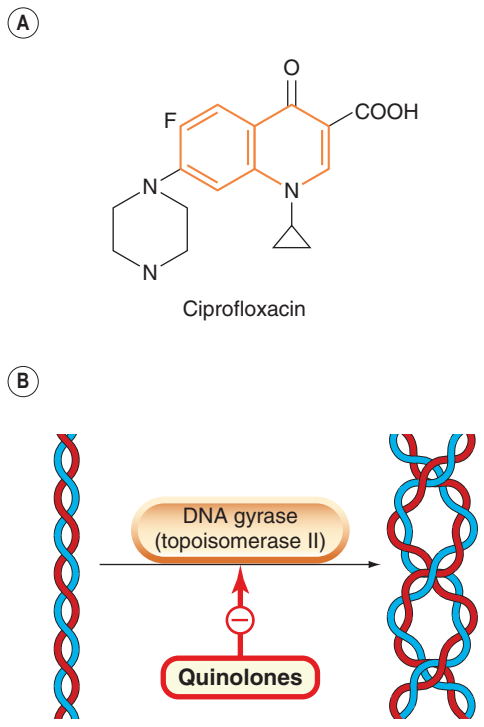

Fig. 51.4 A simplified diagram of the mechanism of action of the fluoroquinolones. [**A**] An example of a quinolone (the quinolone moiety is shown in orange). [**B**] Schematic diagram of (left) the double helix and (right) the double helix in supercoiled form (see also Fig. 50.6). In essence, the DNA gyrase unwinds the RNA-induced positive supercoil (not shown) and introduces a negative supercoil.

Gram-negative bacilli and cocci.[6] Resistant strains of *Staphylococcus aureus* and *P. aeruginosa* have emerged.

Clinical uses of the fluoroquinolones

- Complicated *urinary tract infections* (**norfloxacin**, **ofloxacin**).
- *Pseudomonas aeruginosa* respiratory infections in patients with cystic fibrosis.
- Invasive external otitis ('malignant otitis') caused by *P. aeruginosa*.
- Chronic Gram-negative bacillary osteomyelitis.
- Eradication of *Salmonella typhi* in carriers.
- *Gonorrhoea* (**norfloxacin**, **ofloxacin**).
- Bacterial *prostatitis* (**norfloxacin**).
- *Cervicitis* (**ofloxacin**).
- *Anthrax*.

Pharmacokinetic aspects

Fluoroquinolones are well absorbed orally. The drugs accumulate in several tissues, particularly in the kidney, prostate and lung. All quinolones are concentrated in phagocytes. Most fail to cross the blood–brain barrier, but ofloxacin does so. Aluminium and magnesium antacids interfere with the absorption of the quinolones. Elimination of ciprofloxacin and norfloxacin is partly by hepatic metabolism by P450 enzymes (which they can

[6]When ciprofloxacin was introduced, clinical pharmacologists and microbiologists sensibly suggested that it should be reserved for organisms already resistant to other drugs so as to prevent emergence of resistance. However, by 1989 it was already estimated that it was prescribed for 1 in 44 of Americans, so it would seem that the horse had not only left the stable but had bolted into the blue!

inhibit, giving rise to interactions with other drugs) and partly by renal excretion. Ofloxacin is excreted in the urine.

Unwanted effects

In hospitals, infection with *C. difficile* may prove hazardous but otherwise unwanted effects are infrequent, usually mild and reversible. The most frequent manifestations are gastrointestinal disorders and skin rashes. Arthropathy has been reported in young individuals. Central nervous system symptoms – headache and dizziness – have occurred, as have, less frequently, convulsions associated with central nervous system pathology or concurrent use of **theophylline** or an non-steroidal anti-inflammatory drug (NSAID) (Ch. 26).

There is a clinically important interaction between ciprofloxacin and theophylline (through inhibition of P450 enzymes), which can lead to theophylline toxicity in asthmatics treated with the fluoroquinolones. The topic is discussed further in Chapter 28. Moxifloxacin prolongs the electrocardiographic QT interval and is used extensively, following Food and Drug Administration guidance, as a positive control in studies in healthy volunteers examining possible effects of new drugs on cardiac repolarisation.

Antimicrobial agents affecting DNA topoisomerase II

- The quinolones interfere with the supercoiling of DNA.
- **Ciprofloxacin** has a wide antibacterial spectrum, being especially active against Gram-negative enteric coliform organisms, including many organisms resistant to penicillins, cephalosporins and aminoglycosides; it is also effective against *H. influenzae*, penicillinase-producing *N. gonorrhoeae*, *Campylobacter* spp. and pseudomonads. There is a high incidence of staphylococcal resistance.
- Unwanted effects include gastrointestinal tract upsets, hypersensitivity reactions and, rarely, central nervous system disturbances.

MISCELLANEOUS AND LESS COMMON ANTIBACTERIAL AGENTS

METRONIDAZOLE

▼ **Metronidazole** was introduced as an antiprotozoal agent (see Ch. 54), but it is also active against anaerobic bacteria such as *Bacteroides*, *Clostridia* spp. and some streptococci. It is effective in the therapy of *pseudomembranous colitis*, and is important in the treatment of serious anaerobic infections (e.g. sepsis secondary to bowel disease). It has a disulfiram-like action (see Ch. 49), so patients must avoid alcohol while taking metronidazole.

STREPTOGRAMINS

▼ **Quinupristin** and **dalfopristin** are cyclic peptides, which inhibit bacterial protein synthesis by binding to the 50S subunit of the bacterial ribosome. Dalfopristin changes the structure of the ribosome so as to promote the binding of quinupristin. Individually, they exhibit only very modest bacteriostatic activity, but combined together as an intravenous injection they are active against many Gram-positive bacteria. The combination is used to treat serious infections, usually where no other antibacterial is suitable. For example, the combination is effective against MRSA and vancomycin-resistant *Enterococcus faecium*. They are not currently used in the UK.

Both drugs undergo extensive first-pass hepatic metabolism and must therefore be given as an intravenous infusion. The half-life of each compound is 1–2 h.

Unwanted effects include inflammation and pain at the infusion site, arthralgia, myalgia and nausea, vomiting and diarrhoea. To date, resistance to quinupristin and dalfopristin does not seem to be a major problem.

CLINDAMYCIN

▼ The lincosamide clindamycin is active against Gram-positive cocci, including many penicillin-resistant staphylococci and many anaerobic bacteria such as *Bacteroides* spp. It acts in the same way as macrolides and chloramphenicol (Ch. 50, Fig. 50.4). In addition to its use in infections caused by *Bacteroides* organisms, it is used to treat staphylococcal infections of bones and joints. It is also given topically, as eye drops, for staphylococcal conjunctivitis and as an anti-protozoal drug (see Ch. 54).

Unwanted effects consist mainly of gastrointestinal disturbances, ranging from uncomfortable diarrhoea to potentially lethal pseudomembranous colitis, caused by a toxin-forming *C. difficile*.[7]

OXAZOLIDINONES

▼ Originally hailed as the 'first truly new class of antibacterial agents to reach the marketplace in several decades' (Zurenko et al., 2001), the oxazolidinones inhibit bacterial protein synthesis by a novel mechanism: inhibition of *N*-formylmethionyl-tRNA binding to the 70S ribosome. **Linezolid** is the first member of this new antibiotic family to be introduced. It is active against a wide variety of Gram-positive bacteria and is particularly useful for the treatment of drug-resistant bacteria such as MRSA, penicillin-resistant *Streptococcus pneumoniae* and vancomycin-resistant enterococci. The drug is also effective against some anaerobes, such as *C. difficile*. Most common Gram-negative organisms are not susceptible to the drug. Linezolid can be used to treat pneumonia, septicaemia, and skin and soft tissue infections. Its use is restricted to serious bacterial infections where other antibiotics have failed, and there have so far been few reports of resistance.

Unwanted effects include thrombocytopenia, diarrhoea, nausea and, rarely, rash and dizziness. Linezolid is a non-selective inhibitor of monoamine oxidase, and appropriate precautions need to be observed (see Ch. 47).

FUSIDIC ACID

▼ Fusidic acid is a narrow-spectrum steroid antibiotic active mainly against Gram-positive bacteria. It acts by inhibiting bacterial protein synthesis (Ch. 50, Fig. 50.4). As the sodium salt, the drug is well absorbed from the gut and is distributed widely in the tissues. Some is excreted in the bile and some metabolised. It is used in combination with other antistaphylococcal agents in staphylococcal sepsis, and is very widely used topically for staphylococcal infections (e.g. as eye drops or cream).

Unwanted effects such as gastrointestinal disturbances are fairly common. Skin eruptions and jaundice can occur. Resistance occurs if it is used systemically as a single agent so it is always combined with other antibacterial drugs when used systemically.

NITROFURANTOIN

▼ **Nitrofurantoin** is a synthetic compound active against a range of Gram-positive and Gram-negative organisms. The development of resistance in susceptible organisms is rare, and there is no cross-resistance. Its mechanism of action is probably related to its ability to damage bacterial DNA. It is given orally and is rapidly and totally

[7]This may also occur with broad-spectrum penicillins and cephalosporins.

absorbed from the gastrointestinal tract and just as rapidly excreted by the kidney. Its use is confined to the treatment of urinary tract infections.

Unwanted effects such as gastrointestinal disturbances are relatively common, and hypersensitivity reactions involving the skin and the bone marrow (e.g. leukopenia) can occur. Hepatotoxicity and peripheral neuropathy have also been reported.

Methanamine has a similar clinical utility to nitrofurantoin and shares several of its unwanted effects. It exerts its effects following slow conversion (in acidic urine) to formaldehyde.

POLYMIXINS

▼ The polymixin antibiotics in use are **polymixin B** and **colistimethate**. They have cationic detergent properties and disrupt the bacterial outer cell membrane (Ch. 50). They have a selective, rapidly bactericidal action on Gram-negative bacilli, especially pseudomonads and coliform organisms. They are not absorbed from the gastrointestinal tract. Clinical use of these drugs is limited by their toxicity and is confined largely to gut sterilisation and topical treatment of ear, eye or skin infections caused by susceptible organisms.

Unwanted effects may be serious and include neurotoxicity and nephrotoxicity.

ANTIMYCOBACTERIAL AGENTS

The main mycobacterial infections in humans are tuberculosis and leprosy, chronic infections caused by *Mycobacterium tuberculosis* and *M. leprae*, respectively. Another mycobacterial infection of less significance here is *M. avium-intracellulare* (actually two organisms), which can infect some AIDS patients. A particular problem with mycobacteria is that they can survive inside macrophages after phagocytosis, unless these cells are 'activated' by cytokines produced by T-helper (Th)1 lymphocytes (see Chs 6 and 18).

DRUGS USED TO TREAT TUBERCULOSIS

For centuries, tuberculosis was a major killer disease, but the introduction of **streptomycin** in the late 1940s followed by **isoniazid** and, in the 1960s, of **rifampicin** and **ethambutol** revolutionised therapy. Tuberculosis came to be regarded as an easily treatable condition but, regrettably, this is so no longer true and strains with increased virulence or exhibiting multidrug resistance are now common (Bloom & Small, 1998). It now causes more deaths than any other single agent even though infection rates are slowly falling. In 2012, the World Health Organization (WHO) estimated that 8.6 million people contracted the disease and some 1.3 million died as a result of the infection. One-third of the world's population (2 billion people) harbour the bacillus, 10% of whom will develop the disease at some point in their lifetime. Poverty-stricken countries in Africa and Asia bear the brunt of the disease, partly because of an ominous synergy between mycobacteria (e.g. *M. tuberculosis*, *M. avium-intercellulare*) and HIV. About a quarter of HIV-associated deaths are caused by tuberculosis.

Treatment is led by the first-line drugs isoniazid, rifampicin, **rifabutin**, ethambutol and **pyrazinamide**. Second-line drugs include **capreomycin**, **cycloserine**, streptomycin (rarely used now in the UK), **clarithromycin** and ciprofloxacin. These are used to treat infections likely to be resistant to first-line drugs, or when the first-line agents have to be abandoned because of unwanted reactions.

To decrease the probability of the emergence of resistant organisms, combination drug therapy is a frequent strategy. This commonly involves:

- an initial phase of treatment (about 2 months) with a combination of isoniazid, rifampicin and pyrazinamide (plus ethambutol if the organism is suspected to be resistant)
- a second, continuation phase (about 4 months) of therapy, with isoniazid and rifampicin; longer-term treatment is needed for patients with meningitis, bone/joint involvement or drug-resistant infection.

ISONIAZID

The antibacterial activity of isoniazid is limited to mycobacteria. It halts the growth of resting organisms (i.e. is bacteriostatic) but can kill dividing bacteria. It passes freely into mammalian cells and is thus effective against intracellular organisms. Isoniazid is a prodrug that must be activated by bacterial enzymes before it can exert its inhibitory activity on the synthesis of *mycolic acids*, important constituents of the cell wall peculiar to mycobacteria. Resistance to the drug, secondary to reduced penetration into the bacterium, may be present, but cross-resistance with other tuberculostatic drugs does not occur.

Isoniazid is readily absorbed from the gastrointestinal tract and is widely distributed throughout the tissues and body fluids, including the CSF. An important point is that it penetrates well into 'caseous' tuberculous lesions (i.e. necrotic lesions with a cheese-like consistency). Metabolism, which involves acetylation, depends on genetic factors that determine whether a person is a slow or rapid acetylator of the drug (see Ch. 11), with slow inactivators enjoying a better therapeutic response. The half-life in slow inactivators is 3 h and in rapid inactivators, 1 h. Isoniazid is excreted in the urine partly as unchanged drug and partly in the acetylated or otherwise inactivated form.

Unwanted effects depend on the dosage and occur in about 5% of individuals, the commonest being allergic skin eruptions. A variety of other adverse reactions have been reported, including fever, hepatotoxicity, haematological changes, arthritic symptoms and vasculitis. Adverse effects involving the central or peripheral nervous systems are largely consequences of pyridoxine deficiency and are common in malnourished patients unless prevented by administration of this substance. Isoniazid may cause haemolytic anaemia in individuals with glucose 6-phosphate dehydrogenase deficiency, and it decreases the metabolism of the antiepileptic agents **phenytoin**, **ethosuximide** and **carbamazepine**, resulting in an increase in the plasma concentration and toxicity of these drugs.

RIFAMPICIN

Rifampicin (also called **rifampin**) acts by binding to, and inhibiting, DNA-dependent RNA polymerase in prokaryotic but not in eukaryotic cells (Ch. 50). It is one of the most active antituberculosis agents known, and is also effective against leprosy and most Gram-positive bacteria as well as many Gram-negative species. It enters phagocytic cells and can therefore kill intracellular microorganisms including the tubercle bacillus. Resistance can develop rapidly in a one-step process in which a chromosomal mutation changes its target site on microbial DNA-dependent RNA polymerase (see Ch. 50).

Rifampicin is given orally and is widely distributed in the tissues and body fluids (including CSF), giving an orange tinge to saliva, sputum, tears and sweat. It is excreted partly in the urine and partly in the bile, some of it undergoing enterohepatic cycling. The metabolite retains antibacterial activity but is less well absorbed from the gastrointestinal tract. The half-life is 1–5 h, becoming shorter during treatment because of induction of hepatic microsomal enzymes.

Unwanted effects are relatively infrequent. The commonest are skin eruptions, fever and gastrointestinal disturbances. Liver damage with jaundice has been reported and has proved fatal in a very small proportion of patients, and liver function should be assessed before treatment is started. Rifampicin causes induction of hepatic metabolising enzymes (Ch. 10), resulting in an increase in the degradation of warfarin, glucocorticoids, narcotic analgesics, oral antidiabetic drugs, **dapsone** and oestrogens, the last effect leading to failure of oral contraception.

ETHAMBUTOL

Ethambutol has no effect on organisms other than mycobacteria. It is taken up by the bacteria and exerts a bacteriostatic effect after a period of 24 h, probably by inhibiting mycobacterial cell wall synthesis. Resistance emerges rapidly if the drug is used alone. Ethambutol is given orally and is well absorbed. It can reach therapeutic concentrations in the CSF in tuberculous meningitis. In the blood, it is taken up by erythrocytes and slowly released. Ethambutol is partly metabolised and is excreted in the urine.

Unwanted effects are uncommon, the most significant being optic neuritis, which is dose-related and is more likely to occur if renal function is decreased. This results in visual disturbances manifesting initially as red–green colour blindness progressing to a decreased visual acuity. Colour vision should be monitored before and during prolonged treatment.

PYRAZINAMIDE

Pyrazinamide is inactive at neutral pH but tuberculostatic at acid pH. It is effective against the intracellular organisms in macrophages because, after phagocytosis, the organisms are contained in phagolysosomes where the pH is low. The drug probably inhibits bacterial fatty acid synthesis. Resistance develops rather readily, but cross-resistance with isoniazid does not occur. The drug is well absorbed after oral administration and is widely distributed, penetrating the meninges. It is excreted through the kidney, mainly by glomerular filtration.

Unwanted effects include gout, which is associated with high concentrations of plasma urates. Gastrointestinal upsets, malaise and fever have also been reported. Serious hepatic damage due to high doses was once a problem but is less likely with lower dose/shorter course regimens now used; nevertheless, liver function should be assessed before treatment.

CAPREOMYCIN

▼ Capreomycin is a peptide antibiotic given by intramuscular injection. Unwanted effects include kidney damage and injury to the auditory nerve, with consequent deafness and ataxia. The drug should not be given at the same time as streptomycin or other drugs that may cause deafness.

CYCLOSERINE

▼ Cycloserine is a broad-spectrum antibiotic that inhibits the growth of many bacteria, including coliforms and mycobacteria. It is water-soluble and destroyed at acid pH. It acts by competitively inhibiting bacterial cell wall synthesis. It does this by preventing the formation of D-alanine and the D-Ala-D-Ala dipeptide that is added to the initial tripeptide side-chain on *N*-acetylmuramic acid, i.e. it prevents completion of the major building block of peptidoglycan (Ch. 50, Fig. 50.3). It is absorbed orally and distributed throughout the tissues and body fluids, including CSF. Most of the drug is eliminated in active form in the urine, but approximately 35% is metabolised.

Cycloserine has unwanted effects, mainly on the central nervous system. A wide variety of disturbances may occur, ranging from headache and irritability to depression, convulsions and psychotic states. Its use is limited to tuberculosis that is resistant to other drugs.

Antituberculosis drugs

To avoid the emergence of resistant organisms, compound therapy is used (e.g. three drugs initially, followed by a two-drug regimen later).

First-line drugs

- **Isoniazid** kills actively growing mycobacteria within host cells. Given orally, it penetrates necrotic lesions, also the cerebrospinal fluid (CSF). 'Slow acetylators' (genetically determined) respond well. It has low toxicity. Pyridoxine deficiency increases risk of neurotoxicity. No cross-resistance with other agents.
- **Rifampicin** is a potent, orally active drug that inhibits mycobacterial RNA polymerase. It penetrates CSF. Unwanted effects are infrequent (but serious liver damage has occurred). It induces hepatic drug-metabolising enzymes. Resistance can develop rapidly.
- **Ethambutol** inhibits growth of mycobacteria. It is given orally and can penetrate CSF. Unwanted effects are uncommon, but optic neuritis can occur. Resistance can emerge rapidly.
- **Pyrazinamide** is tuberculostatic against intracellular mycobacteria. Given orally, it penetrates CSF. Resistance can develop rapidly. Unwanted effects include increased plasma urate and liver toxicity with high doses.

Second-line drugs

- **Capreomycin** is given intramuscularly. Unwanted effects include damage to the kidney and to the auditory nerve.
- **Cycloserine** is a broad-spectrum agent. It inhibits an early stage of peptidoglycan synthesis. Given orally, it penetrates the CSF. Unwanted effects affect mostly the central nervous system.
- **Streptomycin**, an aminoglycoside antibiotic, acts by inhibiting bacterial protein synthesis. It is given intramuscularly. Unwanted effects are ototoxicity (mainly vestibular) and nephrotoxicity.

DRUGS USED TO TREAT LEPROSY

Leprosy is one of the most ancient diseases known to mankind and has been mentioned in texts dating back to 600 BC. The causative organism is *M. leprae*. It is a chronic

disfiguring illness with a long latency, and historically sufferers have been ostracised and forced to live apart from their communities even though the disease is not actually particularly contagious. Once viewed as incurable, the introduction in the 1940s of dapsone, and subsequently rifampicin and **clofazimine** in the 1960s, completely changed our perspective on leprosy. It is now generally curable, and the global figures show that the prevalence rates for the disease have dropped by 90% over the last 20 years as a result of public health measures and **M**ulti**d**rug **T**reatment (MDT) regimens (to avoid drug resistance) implemented by WHO and supported by some pharmaceutical companies. The disease has been eliminated from 119 out of 122 countries where it was considered to be a major health problem. In 2012, some 180 000 new cases were reported mainly in Asia and Africa.

Paucibacillary leprosy, leprosy characterised by one to five numb patches, is mainly *tuberculoid*[8] in type and is treated for 6 months with dapsone and rifampicin. *Multibacillary leprosy*, characterised by more than five numb skin patches, is mainly *lepromatous* in type and is treated for at least 2 years with rifampicin, dapsone and clofazimine.

DAPSONE

Dapsone is chemically related to the sulfonamides and, because its action is antagonised by PABA, probably acts through inhibition of bacterial folate synthesis. Resistance to the drug has steadily increased since its introduction and treatment in combination with other drugs is now recommended.

Dapsone is given orally; it is well absorbed and widely distributed through the body water and in all tissues. The plasma half-life is 24–48 h, but some drug persists in liver, kidney (and, to some extent, skin and muscle) for much longer periods. There is enterohepatic recycling of the drug, but some is acetylated and excreted in the urine. Dapsone is also used to treat *dermatitis herpetiformis*, a chronic blistering skin condition associated with coeliac disease.

Unwanted effects occur fairly frequently and include haemolysis of red cells (usually not severe enough to lead to frank anaemia), methaemoglobinaemia, anorexia, nausea and vomiting, fever, allergic dermatitis and neuropathy. *Lepra reactions* (an exacerbation of lepromatous lesions) can occur, and a potentially fatal syndrome resembling infectious mononucleosis has occasionally been seen.

CLOFAZIMINE

Clofazimine is a dye of complex structure. Its mechanism of action against leprosy bacilli may involve an action on DNA. It also has anti-inflammatory activity and is useful in patients in whom dapsone causes inflammatory side effects.

Clofazimine is given orally and accumulates in the body, being sequestered in the mononuclear phagocyte system. The plasma half-life may be as long as 8 weeks. The anti-leprotic effect is delayed and is usually not evident for 6–7 weeks.

Unwanted effects may be related to the fact that clofazimine is a dye. The skin and urine can develop a reddish colour and the lesions a blue–black discoloration. Dose-related nausea, giddiness, headache and gastrointestinal disturbances can also occur.

Antileprosy drugs

- For *tuberculoid leprosy*: **dapsone** and **rifampicin** (**rifampin**).
  - **Dapsone** is sulfonamide-like and may inhibit folate synthesis. It is given orally. Unwanted effects are fairly frequent; a few are serious. Resistance is increasing.
  - **Rifampicin** (see *Antituberculosis drugs* box).
- For *lepromatous leprosy*: dapsone, **rifampicin** and **clofazimine**.
  - **Clofazimine** is a dye that is given orally and can accumulate by sequestering in macrophages. Action is delayed for 6–7 weeks, and its half-life is 8 weeks. Unwanted effects include red skin and urine, sometimes gastrointesinal disturbances.

POSSIBLE NEW ANTIBACTERIAL DRUGS

In contrast to the rapid discoveries and developments that characterised the 'heroic' years of antibiotic research spanning approximately 1950–1980, and which produced virtually all our existing drugs, the flow has since dried up, with only two totally novel antibiotics introduced during this period (Jagusztyn-Krynicka & Wysznska, 2008). At the same time, resistance has been increasing, with about half the infection-related deaths in Europe now attributable to drug resistance (Watson, 2008).[9]

Resistance normally appears within 2 years or so of the introduction of a new agent (Bax et al., 2000). In a disquieting review and meta-analysis, Costelloe et al. (2010) concluded that most patients prescribed antibiotics for a respiratory or urinary tract infection develop individual resistance to the drug within a few weeks and that this may persist for up to a year after treatment. Since about half the antibiotic use is for veterinary purposes, it is not just human medicine that is implicated in this phenomenon.

The reason for the failure to develop new drugs is complex and has been analysed in detail by Coates et al. (2011), who also evaluate many new leads arising from academic and industrial research. Their overall message is rather depressing, however: they point out that another 20 new classes of antibiotics would need to be discovered in the next 50 years to keep up with the challenges posed by the increasing prevalence of drug resistance.

On a more optimistic note, novel antibiotic candidates continue to be discovered in plants (Limsuwan et al., 2009) and bacteria (Sit & Vederas, 2008) as well as through

[8]The difference between *tuberculoid* and *lepromatous* disease appears to be that the T cells from patients with the former vigorously produce interferon-γ, which enables macrophages to kill intracellular microbes, whereas in the latter case the immune response is dominated by interleukin-4, which blocks the action of interferon-γ (see Ch. 18).

[9]The worst offenders are sometimes collectively referred to, rather fittingly, as 'ESKAPE pathogens'. The acronym is formed of the initial letters of *E. faecium*, *S. aureus*, *K. pneumonia*, *A. baumanii*, *P. aeruginosa* and *Enterobacter* spp.

traditional medicinal chemistry approaches. In addition, researchers in the front line of this important field are recruiting all the latest conceptual technologies into the fray: bioinformatics, utilising information derived from pathogen genome sequencing, is one such approach (Bansal, 2008). The hunt for, and targeting of, bacterial *virulence factors* is showing some promise (Escaich, 2008). New types of screening procedures have been devised (Falconer & Brown, 2009) which would reveal novel targets, and sophisticated pharmacodynamic profiling is being brought to bear on the problem (Lister, 2006).

The world awaits developments with bated breath.

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<www.who.int>. (*Once again, the World Health Organization website is a mine of information about the demographics and treatment of infectious diseases. The sections on leprosy and tuberculosis are especially worthwhile studying. The site includes photographs, maps and much statistical information, as well as information on drug resistance. Highly recommended*)

52 Antiviral drugs

OVERVIEW

This chapter deals with drugs used to treat infections caused by viruses. We provide some basic information about viruses: a simple outline of their structure, a list of the main pathogenic species and a brief summary of the life history of an infectious virus. We then continue with a consideration of the host–virus interaction: the defences deployed by the human host against viruses and the strategies employed by viruses to evade these measures. We then discuss the various types of antiviral drugs and their mechanisms of action, with particular reference to the treatment of acquired immunodeficiency syndrome (AIDS), an infection caused by the human immunodeficiency virus (HIV).

BACKGROUND INFORMATION ABOUT VIRUSES

AN OUTLINE OF VIRUS STRUCTURE

Viruses are small (usually in the range 20–30 nm) infective agents that are incapable of reproduction outside their host cells. The free-living (e.g. outside its host) virus particle is termed a *virion*, and consists of segments of nucleic acid (either RNA or DNA) enclosed in a protein coat comprised of symmetrical repeating structural units and called a *capsid* (Fig. 52.1). The viral coat, together with the nucleic acid core, is termed the *nucleocapsid*. Some viruses have a further external lipoprotein envelope, which may be decorated with antigenic viral glycoproteins or phospholipids acquired from its host when the nucleocapsid buds through the membranes of the infected cell. Certain viruses also contain enzymes that initiate their replication in the host cell.

Viruses are generally characterised either as *DNA* or *RNA viruses* depending on the nature of their nucleic acid content. These two broad categories are conventionally subdivided into some six subgroups, which classify viruses according to whether they contain single- or double-stranded nucleic acids and how this functions during replication.

EXAMPLES OF PATHOGENIC VIRUSES

Viruses can infect virtually all living organisms, and they are common causes of disease in humans. Some important examples are as follows:

- ▼ *DNA viruses*: poxviruses (smallpox), herpesviruses (chickenpox, shingles, cold sores, glandular fever), adenoviruses (sore throat, conjunctivitis) and papillomaviruses (warts).
- *RNA viruses*: orthomyxoviruses (influenza), paramyxoviruses (measles, mumps, respiratory tract infections), rubella virus (German measles), rhabdoviruses (rabies), picornaviruses (colds, meningitis, poliomyelitis), retroviruses (AIDS, T-cell leukaemia), arenaviruses (meningitis, Lassa fever), hepadnaviruses (serum hepatitis) and arboviruses (various arthropod-borne illnesses, e.g. encephalitis, yellow fever).

VIRUS FUNCTION AND LIFE HISTORY

As viruses have no metabolic machinery of their own, they have to attach to and penetrate a living host cell – animal, plant or bacterial – and hijack the victim's own metabolic processes to replicate. The first step in this process is facilitated by polypeptide binding sites on the envelope or *capsid* which interact with receptors on the host cell. These 'receptors' are normal membrane constituents, for example receptors for cytokines, neurotransmitters or hormones, ion channels, integral membrane glycoproteins, etc. Some examples are listed in Table 52.1.

Following attachment, the receptor–virus complex enters the cell (often by receptor-mediated endocytosis), during which time the virus coat may be removed by host cell enzymes (often lysosomal in nature). Some viruses bypass this route. Once inside the host cell, the viral nucleic acid then uses the host cell's machinery to synthesise nucleic acids and proteins that are assembled into new virus particles. The actual way in which this occurs differs between DNA and RNA viruses.

Replication of DNA viruses

Viral DNA enters the host cell nucleus, where transcription into mRNA occurs catalysed by the host cell *RNA polymerase*. Translation of the mRNA into virus-specific proteins then takes place. Some of these proteins are enzymes that then synthesise more viral DNA, as well as structural proteins comprising the viral coat and envelope. After assembly of coat proteins around the viral DNA, complete *virions* are released by budding or after host cell lysis.

Replication of RNA viruses

Enzymes within the virion synthesise its mRNA from the viral RNA template, or sometimes the viral RNA serves as its own mRNA. This is translated by the host cell into various enzymes, including *RNA polymerase* (which directs the synthesis of more viral RNA), and also into structural proteins of the virion. Assembly and release of virions occurs as explained above. The host cell nucleus is usually not involved in replication of RNA viruses, although some (e.g. *orthomyxoviruses*) replicate exclusively within the host nuclear compartment.

Replication in retroviruses

The virion in *retroviruses*[1] contains a *reverse transcriptase enzyme* (virus RNA-dependent DNA polymerase), which makes a DNA copy of the viral RNA. This DNA copy is

[1]Viruses that can synthesise DNA from an RNA template – the reverse of the normal situation.

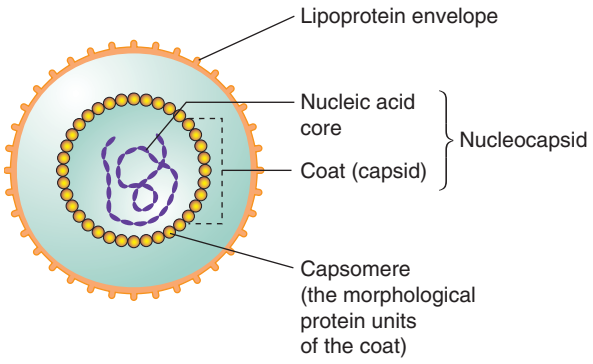

Fig. 52.1 Schematic diagram of the components of a virus particle or virion.

Table 52.1 Some host cell structures that can function as receptors for viruses

Host cell structure[a]	Virus(es)
Helper T lymphocytes CD4 glycoprotein	HIV (causing AIDS)
CCR5 receptor for chemokines MCP-1 and RANTES	HIV (causing AIDS)
CXCR4 chemokine receptor for cytokine SDF-1	HIV (causing AIDS)
Acetylcholine receptor on skeletal muscle	Rabies virus
B lymphocyte complement C3d receptor	Glandular fever virus
T lymphocyte interleukin-2 receptor	T-cell leukaemia viruses
β Adrenoceptors	Infantile diarrhoea virus
MHC molecules	Adenovirus (causing sore throat and conjunctivitis) T-cell leukaemia viruses

MCP-1, monocyte chemoattractant protein-1; MHC, major histocompatibility complex; RANTES, regulated on activation normal T-cell expressed and secreted; SDF-1, stromal cell-derived factor-1.

[a]For more detail on complement, interleukin-2, the CD4 glycoprotein on helper T lymphocytes, MHC molecules, etc., see Chapter 6.

integrated into the genome of the host cell, and it is then termed a *provirus*. The provirus DNA is transcribed into both new viral genome RNA as well as mRNA for translation in the host into viral proteins, and the completed viruses are released by budding. Many retroviruses can replicate without killing the host cell.

The ability of several viruses to remain dormant within, and be replicated together with, the host genome is responsible for the periodic nature of some viral diseases, such as those caused by *herpes labialis* (cold sores) or *varicella zoster* – another type of herpes virus (which causes

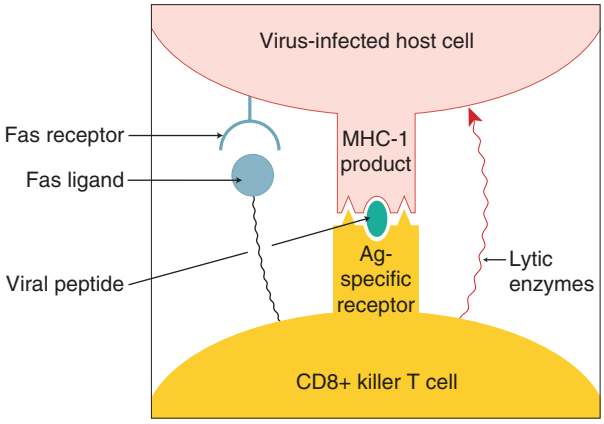

Fig. 52.2 How a CD8⁺ T cell kills a virus-infected host cell. The virus-infected host cell expresses a complex of virus peptides plus major histocompatibility complex class I product (MHC-I) on its surface. This is recognised by the CD8⁺ T cell, which then releases lytic enzymes into the virus-infected cell and also expresses a Fas ligand. This triggers apoptosis in the infected cell by stimulating its Fas 'death receptor'.

chickenpox and shingles), which can recur when viral replication is reactivated by some factor (or when the immune system is compromised in some way). Some RNA retroviruses (e.g. the *Rous sarcoma* virus) can transform normal cells into malignant cells (a serious concern with use of retroviral vectors for gene therapy, see Ch. 59).

THE HOST–VIRUS INTERACTION

HOST DEFENCES AGAINST VIRUSES

The first defence is the simple barrier function of intact skin, which most viruses are unable to penetrate. However, broken skin (e.g. at sites of wounds or insect bites) and mucous membranes are more vulnerable to viral attack. Should the virus gain entry to the body, then the host will deploy both the innate and subsequently the adaptive immune response (Ch. 6) to limit the incursion. The infected cell presents viral peptides, complexed with major histocompatibility complex (MHC) class I molecules on its surface. This is recognised by T lymphocytes, which then kill the infected cell (Fig. 52.2). Killing may be accomplished by the release of lytic proteins (such as *perforins*, *granzymes*) or by triggering the apoptotic pathway in the infected cell by activation of its Fas receptor ('death receptor', see Ch. 5). The latter may also be triggered indirectly through the release of a cytokine such as tumour necrosis factor (TNF)-α. The virus may escape immune detection by cytotoxic lymphocytes by modifying the expression of the peptide–MHC complex (see Ch. 6), but still fall victim to natural killer (NK) cells. This reaction to the absence of normal MHC molecules is called the 'mother turkey' strategy (kill everything that does not sound exactly like a baby turkey; see Ch. 6). Some viruses also have a device for evading NK cells as well.

Within the cell itself, *gene silencing* provides a further level of protection (see Schutze, 2004). Short double-stranded fragments of RNA, such as those that could arise as a result of the virus's attempts to recruit the host's

transcription/translational machinery, actually cause the gene coding for the RNA to be 'silenced' – to be switched off – probably by DNA phosphorylation. This means that the gene is no longer able to direct further viral protein synthesis and replication is halted. This mechanism can be exploited for experimental purposes in many areas of biology, and tailored siRNA (*small- or short-interfering RNA*) is a cheap and useful technique to suppress temporarily the expression of a particular gene of interest. Attempts to harness the technique for viricidal purposes have met with some success (see Barik, 2004), and are beginning to find their way into therapeutics (see Ch. 59).

VIRAL PLOYS TO CIRCUMVENT HOST DEFENCES

Viruses have evolved a variety of strategies to ensure successful infection, some entailing redirection of the host's response for the advantage of the virus (discussed by Tortorella et al., 2000). Some examples are discussed below.

Subversion of the immune response

Viruses can inhibit the synthesis or action of the cytokines, such as interleukin-1, TNF-α and the antiviral interferons (IFNs) that normally coordinate the innate and adaptive immune responses. For example, following infection, some poxviruses express proteins that mimic the extracellular ligand-binding domains of cytokine receptors. These *pseudoreceptors* bind cytokines, preventing them from reaching their natural receptors on cells of the immune system and thus moderating the normal immune response to virus-infected cells. Other viruses that can interfere with cytokine signalling include human cytomegalovirus, Epstein–Barr virus, herpesvirus and adenovirus.

Evasion of immune detection and attack by killer cells

Once within host cells, viruses may also escape immune detection and evade lethal attack by cytotoxic lymphocytes and NK cells in various ways, such as:

- *Interference with the surface protein markers on the infected cells necessary for killer cell recognition and attack.* Some viruses inhibit generation of the antigenic peptide and/or the presentation of MHC–peptide molecules that signals that the cells are infected. In this way, the viruses remain undetected. Examples of viruses that can do this are adenovirus, herpes simplex virus, human cytomegalovirus, Epstein–Barr virus and influenza virus.
- *Interference with the apoptotic pathway.* Some viruses (e.g. adenovirus, human cytomegalovirus, Epstein–Barr virus) can subvert this pathway to ensure their own survival.
- *Adopting the 'baby turkey' ploy.* Some viruses (e.g. cytomegalovirus) get round the mother turkey approach of NK cells by expressing a homologue of MHC class I (the equivalent of a turkey chick's chirping) that is close enough to the real thing to hoodwink NK cells.

It is evident that natural selection has equipped pathogenic viruses with many efficacious tactics for circumventing host defences, and understanding these in more detail is likely to suggest new types of antiviral therapy. Fortunately, the biological arms race is not one-sided, and evolution has also equipped the host with sophisticated countermeasures. In most cases these prevail, and most viral infections eventually resolve spontaneously, except in an immunocompromised host. The situation does not always end happily though; some viral infections, such as Lassa fever and Ebola virus infection, have a high mortality, and we now discuss a further, grave example: the HIV virus. This is appropriate because, whilst the infection develops more slowly than (e.g.) Ebola virus, HIV exhibits many of the features common to other viral infections, and the sheer scale of the global AIDS problem has pushed HIV to the top of the list of antiviral targets.

Viruses

- Viruses are small infective agents consisting of nucleic acid (RNA or DNA) enclosed in a protein coat.
- They are not cells and, having no metabolic machinery of their own, are obligate intracellular parasites, utilising the metabolic processes of the host cell they infect to replicate.
- *DNA viruses* usually enter the host cell nucleus and direct the generation of new viruses.
- *RNA viruses* direct the generation of new viruses usually without involving the host cell nucleus (the influenza virus is an exception).
- *RNA retroviruses* (e.g. HIV, T-cell leukaemia virus) contain an enzyme, reverse transcriptase, which makes a DNA copy of the viral RNA. This DNA copy is integrated into the host cell genome and directs the generation of new virus particles.

HIV AND AIDS

HIV is an RNA retrovirus. Two forms are known. *HIV-1* is the organism responsible for human AIDS. The *HIV-2* organism is similar to the HIV-1 virus in that it also causes immune suppression, but it is less virulent. HIV-1 is distributed around the world, whereas the HIV-2 virus is confined to parts of Africa.

▼ Thanks to increased availability of effective drug therapy the global situation is improving and the number of AIDS-related deaths is falling. Even so, the World Health Organization (2013 report) estimated that almost 34 million people were living with AIDS and that some 1.7 million people die of the disease each year. The epidemic is overwhelmingly centred on sub-Saharan Africa, which accounts for two-thirds of the total global number of infected persons, and where the adult prevalence is over 10 times greater than in Europe. For a review of the pathogenesis (and many other aspects) of AIDS, see Moss (2013).

The interaction of HIV with the host's immune system is complex, and although it involves mainly cytotoxic T lymphocytes (CTLs, $CD8^+$ T cells) and $CD4^+$ helper T lymphocytes ($CD4^+$ cells), other immune cells, such as macrophages, dendritic cells and NK cells, also play a part. Antibodies are produced by the host to various HIV components, but it is the action of the CTLs and $CD4^+$ cells that initially prevents the spread of HIV.

Cytotoxic T lymphocytes directly kill virally infected cells and produce and release antiviral cytokines (Fig. 52.2). The lethal event is lysis of the target cell, but induction of apoptosis by interaction of Fas ligand (see Ch. 5, Fig. 5.5) on the CTL with Fas receptors on the virally infected cell can also play a part. **$CD4^+$ cells** have an important role as helper cells, and may have a direct role (e.g. lysis of target cells), in the control of HIV replication (Norris et al., 2004). It is the progressive loss of these cells that is the defining characteristic of HIV infection (see Fig. 52.4 below).

The priming of naive T cells to become CTLs during the induction phase involves interaction of the T-cell receptor complex with antigenic HIV peptide in association with MHC class I molecules on the surface of antigen-presenting cells (APCs; see Ch. 6, Figs 6.3 and 6.4). Priming also requires the presence and participation of $CD4^+$ cells. It is thought that both types of cell need to recognise antigen on the surface of the same APC (Fig. 6.3).

The CTLs thus generated are effective during the initial stages of the infection but are not able to stop the progression of the disease. It is believed that this is because they become 'exhausted' and unable to maintain their protective function. Different mechanisms may be involved (see Jansen et al., 2004, and Barber et al., 2006, for further details).

▼ The HIV virion cannily attaches to proteins on the host cell surface to gain entry to the cells. The main targets are CD4 (the glycoprotein marker of a particular group of helper T lymphocytes) and CCR5 (a co-receptor for certain chemokines, including monocyte chemoattractant protein-1 and RANTES; see Ch. 6). $CD4^+$ cells normally orchestrate the immune response to viruses, but by entering these cells and using them as virion factories, HIV virtually cripples this aspect of the immune response. Figure 52.3 shows an HIV virion infecting a $CD4^+$ T cell. Such infected activated cells in lymphoid tissue form the major source of HIV production in HIV-infected individuals; infected macrophages are another source.

As for CCR5, evidence from exposed individuals who somehow evade infection indicates that this surface protein has a central role in HIV pathogenesis. Compounds that inhibit the entry of HIV into cells by blocking CCR5 are now available.

When immune surveillance breaks down, other strains of HIV arise that recognise other host cell surface molecules such as CD4 and CXCR4. A surface glycoprotein, gp120, on the HIV envelope binds to CD4 and also to the T-cell chemokine co-receptor CXCR4. Another viral glycoprotein, gp41, then causes fusion of the viral envelope with the plasma membrane of the cell (Fig. 52.3).

Once within the cell, HIV is integrated with the host DNA (the provirus form), undergoing transcription and generating new virions when the cell is activated (Fig. 52.3). In an untreated subject, a staggering 10^{10} new virus particles may be produced each day. Intracellular HIV can remain silent (latent) for a long time.

Viral replication is highly error-prone. Many mutations occur daily at each site in the HIV genome, so HIV soon escapes recognition by the original cytotoxic lymphocytes. Although other cytotoxic lymphocytes arise that recognise the altered virus protein(s), further mutations, in turn, allow escape from surveillance by these cells too. It is suggested that wave after wave of cytotoxic lymphocytes act against new mutants as they arise, gradually depleting a T-cell repertoire already seriously compromised by the loss of $CD4^+$ helper T cells, until eventually the immune response fails.

There is considerable variability in the progress of the disease, but the usual clinical course of an untreated HIV infection is shown in Figure 52.4. An initial acute influenza-like illness is associated with an increase in the number of virus particles in the blood, their widespread dissemination through the tissues and the seeding of lymphoid tissue with the virion particles. Within a few weeks, this *viraemia* is reduced by the action of cytotoxic lymphocytes as specified above.

The acute initial illness is followed by a symptom-free period during which there is reduction in the viraemia accompanied by silent virus replication in the lymph nodes, associated with damage to lymph node architecture and the loss of $CD4^+$ lymphocytes and dendritic cells. Clinical latency (median duration 10 years) comes to an end when the immune response finally fails and the signs and symptoms of AIDS appear – opportunistic infections (e.g. *Pneumocystis* pneumonia or tuberculosis), neurological disease (e.g. confusion, paralysis, dementia), bone marrow depression and cancers. Chronic gastrointestinal infections contribute to the severe weight loss. Cardiovascular and kidney damage can also occur. In an untreated patient, death usually follows within 2 years. The advent of effective drug regimens has greatly improved the prognosis in countries that are able to deploy them and the treated disease is now compatible with a normal life expectancy.

There is evidence that genetic factors play an important role in determining the susceptibility – or resistance – to HIV (see Flores-Villanueva et al., 2003).

ANTIVIRAL DRUGS

Because viruses hijack many of the metabolic processes of the host cell itself, it is difficult to find drugs that are selective for the pathogen. However, there are some enzymes that are virus-specific and these have proved to be useful drug targets. Most currently available antiviral agents are effective only while the virus is replicating. Because the initial phases of viral infection are often asymptomatic, treatment is often not initiated until the infection is well established. As is often the case with infectious diseases, an ounce of prevention is worth a pound of cure.

Antiviral drugs, of which many are now available, may be conveniently grouped according to their mechanisms of action and side effects. Table 52.2 shows the commonest antiviral drugs, classified in this manner together with some of the diseases they are used to treat and common side effects.

REVERSE TRANSCRIPTASE INHIBITORS

The majority are *nucleoside analogues*, typified by **zidovudine**, all of which are phosphorylated by host cell enzymes to give the 5′-trisphosphate derivative. In retroviral replication this moiety competes with the equivalent host cellular trisphosphate substrates for proviral DNA synthesis by viral reverse transcriptase (viral RNA-dependent DNA polymerase). Eventually, the incorporation of the 5′-trisphosphate moiety into the growing viral DNA chain results in chain termination. Mammalian α-DNA polymerase is relatively resistant to the effect. However, γ-DNA polymerase in the host cell mitochondria is more susceptible, and this may be the basis of some unwanted effects. The main utility of these drugs is the treatment of HIV, but a number of them have useful activity against other viruses also (e.g. hepatitis B, which, though not a retrovirus, uses reverse transcriptase for replication).

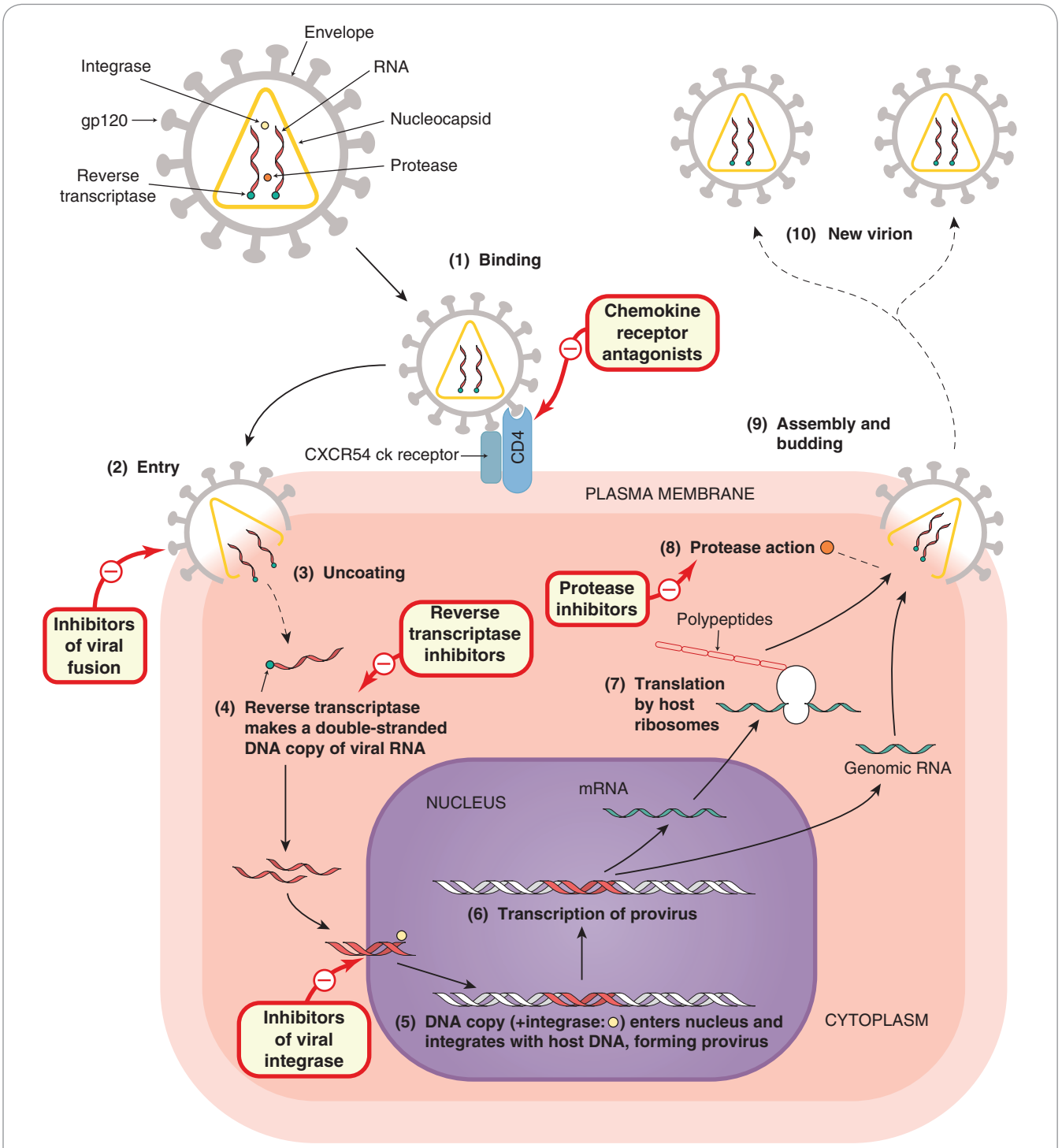

Fig. 52.3 Schematic diagram of infection of a CD4⁺ T cell by an HIV virion, with the sites of action of the two main classes of anti-HIV drugs. The 10 steps of HIV infection, from attachment to the cell to release of new virions, are shown. The virus uses the CD4 co-receptor and the chemokine (ck) receptors CCR5/CXCR4 as binding sites to facilitate entry into the cell, where it becomes incorporated into host DNA (steps 1–5). When transcription occurs (step 6), the T cell itself is activated and the transcription factor nuclear factor κB initiates transcription of both host cell and provirus DNA. A viral protease cleaves the nascent viral polypeptides (steps 7 and 8) into structural proteins and enzymes (integrase, reverse transcriptase, protease) for the new virion. The new virions are assembled and released from the cells, initiating a fresh round of infection (steps 9 and 10). The sites of action of the currently used anti-HIV drugs are shown.

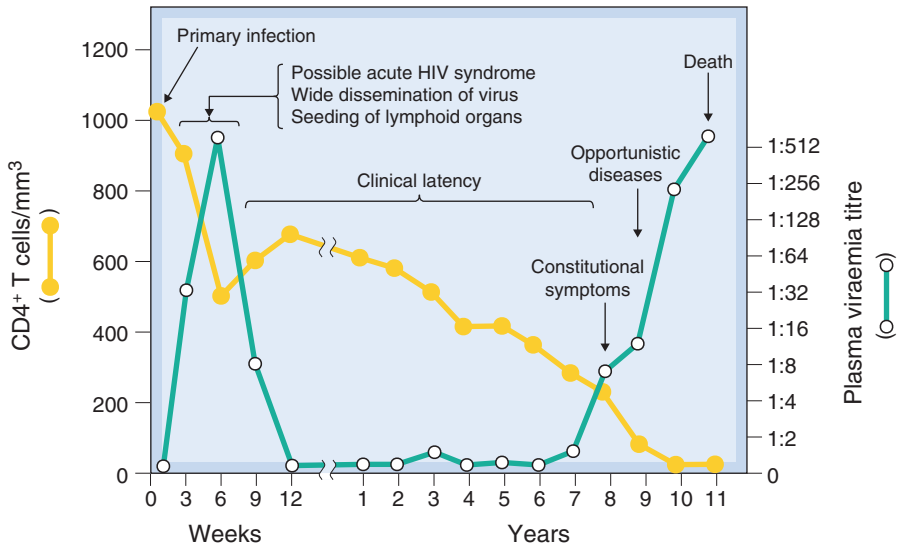

Fig. 52.4 Schematic outline of the course of HIV infection. The $CD4^+$ T-cell titre is often expressed as cells/mm^3. *(Adapted from Pantaleo et al. 1993.)*

Zidovudine

Zidovudine (or **azidothymidine**, AZT) was the first drug to be introduced for the treatment of HIV and retains an important place. It can prolong life in HIV-infected individuals and diminish HIV-associated dementia. Given during pregnancy and labour and then to the newborn infant, it can reduce mother- to-baby transmission by more than 20%. It is generally administered orally 2–3 times each day but can also be given by intravenous infusion. Its plasma half-life is 1 h, but the intracellular half-life of the active trisphosphate is 3 h. The concentration in cerebrospinal fluid (CSF) is 65% of the plasma level. Most of the drug is metabolised to the inactive glucuronide in the liver, only 20% of the active form being excreted in the urine.

Because of rapid mutation, the virus is a constantly moving target, and resistance develops with long-term use of zidovudine, particularly in late-stage disease. Furthermore, resistant strains can be transferred between individuals. Other factors that underlie the loss of efficacy of the drug are decreased activation of zidovudine to the trisphosphate and increased virus load as the host immune response fails.

Unwanted effects include gastrointestinal disturbances (e.g. nausea, vomiting, abdominal pain), blood disorders (sometimes anaemia or neutropenia) and central nervous system (CNS) effects (e.g. insomnia, dizziness, headache) as well as the risk of lactic acidosis in some patients, which are shared by this entire group of drugs to a greater or lesser extent.

Other, currently approved, antiviral drugs in this group include **abacavir**, **adefovir**, **dipivoxil**, **didanosine**, **emtricitabine**, **entecavir**, **lamivudine**, **stavudine**, **telbivudine** and **tenofovir**.

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

Non-nucleoside reverse transcriptase inhibitors are chemically diverse compounds that bind to the reverse transcriptase enzyme near the catalytic site and inactivate it. Most non-nucleoside reverse transcriptase inhibitors are also inducers, substrates or inhibitors, to varying degrees, of the liver cytochrome P450 enzymes (Ch. 9). Currently available drugs include **efavirenz** and **nevirapine**, and the related compounds **etravirine** and **rilpivarine**.

Efavirenz is given orally, once daily, because of its plasma half-life (~50 h). It is 99% bound to plasma albumin, and its CSF concentration is ~1% of that in the plasma. Nevertheless, its major adverse effects are insomnia, bad dreams and sometimes psychotic symptoms. It is teratogenic.

Nevirapine has good oral bioavailability, and penetrates into the CSF. It is metabolised in the liver, and the metabolite is excreted in the urine. Nevirapine can prevent mother-to-baby transmission of HIV.

Unwanted effects common to both of these drugs include rash (common) as well as a cluster of other effects (see Table 52.2).

PROTEASE INHIBITORS

In HIV and many other viral infections, the mRNA transcribed from the provirus is translated into two biochemically inert *polyproteins*. A virus-specific protease then converts the polyproteins into various structural and functional proteins by cleavage at the appropriate positions (see Fig. 52.3). Because this protease does not occur in the host, it is a useful target for chemotherapeutic intervention. HIV-specific protease inhibitors bind to the site where cleavage occurs, and their use, in combination with reverse transcriptase inhibitors, has transformed the therapy of AIDS. Examples of current protease inhibitors are shown in Table 52.2.

Ritonavir, a typical example, binds to and thus inactivates proteases from HIV-1 or HIV-2. It is often given in combination with other protease inhibitors (e.g. **lopinavir**) as it potentiates their action. Ritonavir is given orally, usually twice a day. It is usual to start at a low dose and increase gradually to a maximum over a period of a few days.

Table 52.2 Antiviral drugs

Type	Drug	Common therapeutic indication	Principal unwanted effects
Nucleoside reverse transcriptase inhibitors	Abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zidovudine	Mainly HIV, generally in combination with other retrovirals	Multiple effects including: GI disturbances; CNS and related effects; musculoskeletal and dermatological effects; blood disorders; metabolic effects including pancreatitis, liver damage, lactic acidosis and lipodystrophy
	Adefovir, entecavir, lamivudine, telbivudine, tenvofir	Hepatitis B	
Non-nucleoside reverse transcriptase inhibitors	Efavirenz, etravirine, nevirapine, rilpivirine	HIV, generally in combination with other retrovirals	Multiple effects including: dermatological effects; GI disturbances; CNS and related effects; musculoskeletal and blood disorders; metabolic effects including pancreatitis, liver damage and lipodystrophy Efavirenz is teratogenic
Protease inhibitors	Atazanavir, darunavir, fosamprenavir, indinavir, lopinavir, ritonavir, saquinavir, timpranavir	HIV, generally in combination with other retrovirals	Multiple effects including: GI disturbances; CNS and related effects; musculoskeletal and dermatological effects; blood disorders; metabolic effects including pancreatitis, liver damage and lipodystrophy
	Boceprevir, telaprevir	Hepatitis C	
Viral DNA polymerase inhibitors	Cidofovir, foscarnet, ganciclovir, valganciclovir	Cytomegalovirus	Nephrotoxicity, blood disorders, ocular problems
	Aciclovir, famciclovir, idoxuridine, penciclovir, valaciclovir	Herpes	Mainly GI and dermatological disorders
Inhibitor of HIV fusion with host cells	Enfurvitide	HIV, generally in combination with other retrovirals	CNS, metabolic and GI effects
Inhibitors of viral coat disassembly and neuraminidase inhibitors	Amantadine	Influenza A	GI disturbances, CNS effects
	Oseltamivir	Influenza A and B	GI disturbances, headache
	Zanamivir		Brochospasm (unusual)
Integrase inhibitor	Ratelgravir	HIV (refractory to other treatments)	Mainly GI and metabolic disturbances
Chemokine receptor antagonist (CCR5)	Maraviroc	HIV (CCR5 dependent)	Mainly GI and CNS disturbances
Biopharmaceuticals and immunomodulators	Interferon-α, pegylated interferon-α	Hepatitis B and C	Flu-like symptoms, anorexia and fatigue
	Ribavirin, palivizumab	Respiratory syncytial virus	Fever, some GI effects
	Inosine prabonex	Herpes	Hyperuricaemia, GI effects

CNS, central nervous system; GI, gastrointestinal.

The plasma half-life of ritonavir is 3–5 h but oral absorption may be delayed in the presence of food. The drug is mainly (>80%) excreted in the faeces with some 10% excreted in the urine. A major metabolite accounts for approximately one-third of all excreted drug.

Unwanted effects that are shared among this group include gastrointestinal disturbances (e.g. nausea, vomiting, abdominal pain), blood disorders (sometimes anaemia or neutropenia) and CNS effects (e.g. insomnia, dizziness, headache) as well as the risk of hyperglycaemia.

Drug interactions are numerous, clinically important and unpredictable. As with other antiretroviral drugs it is essential to look up possible interactions before prescribing any other drugs in patients receiving anti-retroviral treatment.

DNA POLYMERASE INHIBITORS

Aciclovir

The development of the landmark drug **aciclovir** launched the era of effective selective antiviral therapy. Typical of

drugs of this type, it is a guanosine derivative that is converted to the monophosphate by viral thymidine kinase, which is very much more effective in carrying out the phosphorylation than the enzyme of the host cell; it is therefore only activated in infected cells. The host cell kinases then convert the monophosphate to the trisphosphate, the active form that inhibits viral DNA polymerase, terminating the nucleotide chain. It is 30 times more potent against the herpes virus enzyme than the host enzyme. Aciclovir trisphosphate is inactivated within the host cells, presumably by cellular phosphatases. Resistance caused by changes in the viral genes coding for thymidine kinase or DNA polymerase has been reported, and aciclovir-resistant herpes simplex virus has been the cause of pneumonia, encephalitis and mucocutaneous infections in immunocompromised patients.

Aciclovir can be given orally, intravenously or topically. When it is given orally, only 20% of the dose is absorbed. The drug is widely distributed, and reaches effective concentrations in the CSF. It is excreted by the kidneys, partly by glomerular filtration and partly by tubular secretion.

Unwanted effects are minimal. Local inflammation can occur during intravenous injection if there is extravasation of the solution. Renal dysfunction has been reported when aciclovir is given intravenously; slow infusion reduces the risk. Nausea and headache can occur and, rarely, encephalopathy.

There are now many other drugs with a similar action to aciclovir (see list in Table 52.2). **Foscarnet** achieves the same effect through a slightly different mechanism.

Clinical uses of drugs for herpes viruses (e.g. aciclovir, famciclovir, valaciclovir)

- *Varicella zoster* infections (chickenpox, shingles):
  - orally in immunocompetent patients
  - intravenously in immunocompromised patients.
- *Herpes simplex* infections (*genital* herpes, *mucocutaneous* herpes and herpes *encephalitis*).
- Prophylactically:
  - patients who are to be treated with immunosuppressant drugs or radiotherapy and who are at risk of herpesvirus infection owing to reactivation of a latent virus
  - in individuals who suffer from frequent recurrences of genital infection with herpes simplex virus.

NEURAMINIDASE INHIBITORS AND INHIBITORS OF VIRAL COAT DISASSEMBLY

Viral neuraminidase is one of three transmembrane proteins coded by the influenza genome. Infection with these RNA viruses begins with the attachment of the viral haemaglutinin to neuraminic (sialic) acid residues on host cells. The viral particle then enters the cell by endocytosis. The endosome is acidified following influx of H^+ through another viral protein, the *M2 ion channel*. This facilitates the disassembly of the viral structure, allowing the RNA to enter the host nucleus, thus initiating a round of viral replication. Newly replicated virions escape from the host cell by budding from the cell membrane. Viral neuraminidase promotes this by severing the bonds linking the particle coat and host sialic acid.

The neuraminidase inhibitors **zanamivir** and **oseltamivir** are active against both influenza A and B viruses, and are licensed for use at early stages in the infection or when use of the vaccine is impossible. Zanamivir is available as a powder for inhalation, and oseltamivir as an oral preparation. Though oseltamivir has been 'stockpiled' by governments when flu pandemics (e.g. 'swine' flu – H1N1) are forecast, clinical trials suggest that its efficacy in reducing disease severity is very limited.

Unwanted effects of both include gastrointestinal symptoms (nausea, vomiting, dyspepsia and diarrhoea), but these are less frequent and severe in the inhaled preparation.

Amantadine,[2] quite an old drug (1966) and seldom recommended today, effectively blocks viral M2 ion channels, thus inhibiting disassembly. It is active against influenza A virus (an RNA virus) but has no action against influenza B virus. Given orally, amantadine is well absorbed, reaches high levels in secretions (e.g. saliva) and most is excreted unchanged via the kidney. Aerosol administration is feasible.

Unwanted effects are relatively infrequent, occurring in 5–10% of patients, and are not serious. Dizziness, insomnia and slurred speech are the most common adverse effects.

DRUGS ACTING THROUGH OTHER MECHANISMS

Enfurvitide inhibits the fusion of HIV with host cells. It is generally given by subcutaneous injection in combination with other drugs to treat HIV when resistance becomes a problem or when the patient is intolerant of other anti-retroviral drugs.

Unwanted effects include flu-like symptoms, central effects such as headache, dizziness, alterations in mood, gastrointestinal effects and sometimes hypersensitivity reactions.

Ratelgravir acts by inhibiting HIV DNA integrase, the enzyme that splices viral DNA into the host genome when forming the provirus. It is used for the treatment of HIV as part of combination therapy, and is generally reserved for cases that are resistant to other antiretroviral agents.

MARAVIROC

CCR5, together with CXCR4, are cell surface chemokine receptors that have been exploited by some strains of HIV to gain entry to the cell. In patients who harbour 'R5' strains, the chemokine receptor antagonist **maraviroc** may be used, in combination with more conventional antiretroviral drugs. Maraviroc – a novel concept in HIV therapy (see Dhami et al., 2009) – is the only drug of its type currently available. Its use, in combination with other antiretroviral drugs, is currently restricted to CCR5-tropic HIV infection in patients previously treated with other antiretrovirals.

[2]Also used for its mildly beneficial effects in Parkinson's disease (see Ch. 40).

BIOPHARMACEUTICAL ANTIVIRAL DRUGS

Biopharmaceuticals that have been recruited in the fight against virus infections include immunoglobulin preparations, interferons (IFNs) and monoclonal antibodies.

Immunoglobulin

Pooled immunoglobulin contains antibodies against various viruses present in the population. The antibodies are directed against the virus envelope and can 'neutralise' some viruses and prevent their attachment to host cells. If used before the onset of signs and symptoms, it may attenuate or prevent measles, German measles, infectious hepatitis, rabies or poliomyelitis. *Hyperimmune* globulin, specific against particular viruses, is used against hepatitis B, varicella zoster and rabies.

Palivisumab

Related in terms of its mechanism of action to immunoglobulins is **palivisumab**, a monoclonal antibody (see Chs 18 and 59) directed against a glycoprotein on the surface of respiratory syncytial virus. It is used as an intramuscular injection, under specialist supervision, in children at high risk to prevent infection by this organism.

Interferons

IFNs are a family of inducible proteins synthesised by mammalian cells and now generally produced commercially by recombinant DNA technology. There are at least three types, α, β and γ, constituting a family of hormones involved in cell growth and regulation and the modulation of immune reactions. IFN-γ, termed *immune interferon*, is produced mainly by T lymphocytes as part of an immunological response to both viral and non-viral antigens, the latter including bacteria and their products, rickettsiae, protozoa, fungal polysaccharides and a range of polymeric chemicals and other cytokines. IFN-α and IFN-β are produced by B and T lymphocytes, macrophages and fibroblasts in response to the presence of viruses and cytokines. The general actions of the IFNs are described briefly in Chapter 18.

The IFNs bind to specific ganglioside receptors on host cell membranes. They induce, in host cell ribosomes, the production of enzymes that inhibit the translation of viral mRNA into viral proteins, thus halting viral replication. They have a broad spectrum of action and inhibit the replication of most viruses *in vitro*. Given intravenously, IFNs have a half-life of 2–4 h. They do not cross the blood–brain barrier.

IFN-α-2a is used for treatment of hepatitis B infections and AIDS-related Kaposi sarcomas; **IFN-α-2b** is used for hepatitis C (a chronic viral infection which can progress insidiously in apparently healthy people, leading to end-stage liver disease or liver cancer). There are reports that IFNs can prevent reactivation of herpes simplex after trigeminal root section in animals and can prevent spread of herpes zoster in cancer patients. Preparations of IFNs conjugated with polyethylene glycol (pegylated IFNs) have a longer lifetime in the circulation.

Unwanted effects are common and resemble the symptoms of influenza (which are mediated by cytokine release) including fever, lassitude, headache and myalgia. Repeated injections cause chronic malaise. Bone marrow depression, rashes, alopecia and disturbances in cardiovascular, thyroid and hepatic function can also occur.

OTHER AGENTS

Immunomodulators are drugs that act by moderating the immune response to viruses or use an immune mechanism to target a virus or other organism. **Inosine pranobex** may interfere with viral nucleic acid synthesis but also has immunopotentiating actions on the host. It is sometimes used to treat herpes infections of mucosal tissues or skin.

Tribavirin (**ribavirin**) is a synthetic nucleoside, similar in structure to guanosine. It is thought to act either by altering virus nucleotide pools or by interfering with the synthesis of viral mRNA. While it inhibits a wide range of DNA and RNA viruses, including many that affect the lower airways, it is mainly used in aerosol or tablet form to treat infections with *respiratory syncytial virus* (an RNA paramyxovirus). It has also been shown to be effective in hepatitis C as well as Lassa fever, an extremely serious *arenavirus* infection. When given promptly to victims of the latter disease, it has been shown to reduce to fatality rates (usually about 76%) by approximately 8-fold.

Antiviral drugs

Most antiviral drugs generally fall into the following groups:

- *Nucleoside analogues* that inhibit the viral reverse transcriptase enzyme, preventing replication (e.g. **lamivudine**, **zidovudine**).
- *Non-nucleoside analogues* that have the same effect (e.g. **efavirenz**).
- *Inhibitors of proteases* that prevent viral protein processing (e.g. **saquinavir**, **indinavir**).
- *Inhibitors of viral DNA polymerase* that prevent replication (e.g. **aciclovir**, **famciclovir**).
- *Inhibitors of viral capsule disassembly* (e.g. **amantidine**).
- *Inhibitors of neuraminidase* that prevent viral escape from infected cells (e.g. **oseltamivir**).
- *Inhibitors of HIV integrase* that prevent the incorporation of viral DNA into the host genome (**ratelgravir**).
- *Inhibitors of viral entry* that block the use of host cell surface receptors, which are used as entry points by viruses (**maraviroc**).
- *Immunomodulators* that enhance host defences (e.g. interferons and **inosine pranobex**).
- *Immunoglobulin and related preparations* that contain neutralising antibodies to various viruses.

COMBINATION THERAPY FOR HIV

Two main classes of antiviral drugs are used to treat HIV: reverse transcriptase inhibitors and protease inhibitors. As they have different mechanisms of action (Fig. 52.3), they can usefully be deployed in combinations and this has dramatically improved the prognosis of the disease. The combination treatment is known as **h**ighly **a**ctive **a**nti**r**etroviral **t**herapy (HAART). A typical HAART three- or four-drug combination would involve two nucleoside

reverse transcriptase inhibitors with either a non-nucleoside reverse transcriptase inhibitor or one or two protease inhibitors.

Using a HAART protocol, HIV replication is inhibited, the presence in the plasma of HIV RNA is reduced to undetectable levels and patient survival is greatly prolonged. But the regimen is complex and has many unwanted effects. Compliance is difficult and lifelong treatment is necessary. The virus is not eradicated but lies latent in the host genome of memory T cells, ready to reactivate if therapy is stopped.

Unwelcome interactions can occur between the component drugs of HAART combinations, and there may be inter-individual variations in absorption. Metabolic and cardiovascular complications attend the usage of these drugs and pose a problem to patients who may require lifelong therapy (see Hester, 2012). Some drugs penetrate poorly into the brain, and this could lead to local proliferation of the virus. So far there is no cross-resistance between the three groups of drugs, but it needs to be borne in mind that the virus has a high mutation rate – so resistance could be a problem in the future. The AIDS virus has certainly not yet been outsmarted. Even with full compliance – which is often not achieved for long periods, given the complexity of the regimen and side effects – the virus can only be kept in check, not eliminated.

The choice of drugs to treat pregnant or breastfeeding women is difficult. The main aims are to avoid damage to the fetus and to prevent transmission of the disease to the neonate. Therapy with zidovudine alone is often used in these cases. Another area that requires special consideration is prophylaxis for individuals who may have been exposed to the virus accidentally. Specific guidelines have been developed for such cases, but they are beyond the scope of this chapter.

Other drugs such as enfurvitide, maraviroc and raltegravir are used in combination therapy regimens and are seldom deployed alone.

PROSPECTS FOR NEW ANTIVIRAL DRUGS

At the beginning of the 1990s there were only five drugs available to treat viral infections; 20 years later, this number has increased some 10-fold. Our understanding of the biology of pathogenic viruses and their action on, and in, host cells has grown enormously (see for example, Stevenson, 2012). New strategies could, if vigorously implemented, have the potential to target the viruses causing most viral diseases (see de Clercq, 2002). One such example has been the recent introduction of drugs that prevent CCR5 from serving as an entry portal for HIV. Work is under way to develop CXCR4 inhibitors for similar purposes, as are other approaches to disrupting this function of CCR5 (reviewed by Dhami et al., 2009).

However, the ultimate weapon in the fight against HIV would be vaccination. This has proved to be highly effective in the past against diseases such as polio and smallpox, and more recently against influenza (both types) and hepatitis B. Unfortunately, despite some encouraging results in animal models (and even some very modest success with one human trial) the prospect of a vaccine against HIV (and sadly many other viruses) still seems rather remote (Girard et al., 2011). Part of the problem is *antigenic drift*, a process whereby the virus mutates, thus presenting different antigenic structures and minimising the chance of an effective and long-lasting immune response or the production of a vaccine.. The problem of HIV vaccines is the subject of numerous reviews (see Kaufman & Barouch, 2009; Rhee & Barouch, 2009; Girard et al., 2011).

Drugs for HIV infections

- Reverse transcriptase inhibitors (RTIs):
  - *nucleoside RTIs* are phosphorylated by host cell enzymes to give the 5′-trisphosphate, which competes with the equivalent host cellular trisphosphates that are essential substrates for the formation of proviral DNA by viral reverse transcriptase (examples are **zidovudine** and **abacavir**); they are used in combination with protease inhibitors
  - *non-nucleoside RTIs* are chemically diverse compounds that bind to the reverse transcriptase near the catalytic site and denature it; an example is **nevirapine**.
- Protease inhibitors inhibit cleavage of the nascent viral protein into functional and structural proteins. They are often used in combination with reverse transcriptase inhibitors. An example is **saquinavir**.
- Combination therapy is essential in treating HIV; this characteristically comprises two nucleoside RTIs with either a non-nucleoside RTI or one or two protease inhibitors. Other drugs, such as the HIV integrase inhibitor **ratelgravir**, the chemokine receptor antagonist **maraviroc** and the HIV fusion inhibitor **enfurvitide**, may also be used in such combination therapy regimens.

Treatment of HIV/AIDS

A consensus on the use of retroviral therapy in AIDS has emerged based on the following principles:

- Monitor plasma viral load and $CD4^+$ cell count.
- Start treatment before immunodeficiency becomes evident.
- Aim to reduce plasma viral concentration as much as possible for as long as possible.
- Use combinations of at least three drugs (e.g. two reverse transcriptase inhibitors and one protease inhibitor).
- Change to a new regimen if plasma viral concentration increases.

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Books

Pisani, E., 2008. The Wisdom of Whores. Granta Books, London. (*An entertaining and informative account of efforts made to pioneer HIV programmes in developing countries and the many bureaucratic and other obstacles that had to be overcome. See also* www.wisdomofwhores.com/. *Highly recommended*)

Useful Web resources

<www.aidsinfo.nih.gov/>. (*The official HIV/AIDS site of the US National Institutes of Health. Authoritative and up-to-date information on every aspect of this disease and its treatment, including data on drugs and drug action as well as the results of recent clinical trials and the latest progress in developing a vaccine. Superb*)

<www.unaids.org/en/default.asp>. (*The official site of the United Nations Programme on HIV/AIDS. It focuses on the demographics of the epidemic with various resources that bring home the enormous problems in dealing with this disease. Prepare to be appalled*)

53 Antifungal drugs

OVERVIEW

Fungal infections (*mycoses*) are widespread in the population. In temperate climates, such as the UK, they are generally associated with the skin (e.g. 'athlete's foot') or mucous membranes (e.g. 'thrush').[1] In otherwise healthy people, these infections are mainly minor, being more of a nuisance than a threat. However, they become a more serious problem when the immune system is compromised or when the organism gains access to the systemic circulation. When this occurs, fungal infections can be fatal. In this chapter, we will briefly review the main types of fungal infections and discuss the drugs that can be used to treat them.

FUNGI AND FUNGAL INFECTIONS

Fungi are non-motile eukaryotic cells. Unlike plants, they cannot photosynthesise and many are parasitic or saprophytic in nature. Thousands of species have been characterised. Many are of economic importance, either because they are edible (e.g. mushrooms), useful in manufacturing other products (e.g. yeast in brewing and in the production of antibiotics) or because of the damage they cause to other animals, crops or to foodstuffs.

Approximately 50 species are pathogenic in humans. These organisms are present in the environment or may co-exist with humans as *commensals* without causing any overt risks to health. However, since the 1970s there has been a steady increase in the incidence of serious secondary systemic fungal infections. One of the contributory factors has been the widespread use of broad-spectrum antibiotics, which eradicate the non-pathogenic bacterial populations that normally compete with fungi for nutritional resources. Other causes include the spread of AIDS and the use of immunosuppressant or cancer chemotherapy agents. The result has been an increased prevalence of *opportunistic infections*, i.e. infections that rarely cause disease in healthy individuals. Older people, diabetics, pregnant women and burn wound victims are particularly at risk of fungal infections such as *candidiasis*. Primary fungal infections, once rare in the temperate world, are also now encountered more often because of increased international travel.

Clinically important fungi may be classified into four main types on the basis of morphological and other characteristics. Of particular taxonomic significance is the presence of *hyphae* – filamentous projections that can knit together to form a complex *mycelium*, a mat-like structure that is responsible for the characteristic appearance of moulds. Fungi are remarkably specific in their choice of preferred location. The main groups are:

- yeasts (e.g. *Cryptococcus neoformans*)
- yeast-like fungi that produce a structure resembling a mycelium (e.g. *Candida albicans*)
- filamentous fungi with a true mycelium (e.g. *Aspergillus fumigatus*)
- 'dimorphic' fungi that, depending on nutritional constraints, may grow as either yeasts or filamentous fungi (e.g. *Histoplasma capsulatum*).

Another organism, *Pneumocystis carinii* (also known as *P. jirovecii*), described in Ch. 54, shares characteristics of both protozoa and fungi; it is an important opportunistic pathogen in patients with compromised immune systems (e.g. those suffering from AIDS), but is not susceptible to antifungal drugs.

Drugs vary in their efficacy between the different fungal groups. Table 53.1 gives examples of each type of organism and lists some of the diseases they cause and the most common choice of drug.

Superficial fungal infections can be classified into the *dermatomycoses* and *candidiasis*. Dermatomycoses include infections of the skin, hair and nails (*onychomycosis*). They are most commonly caused by *Trichophyton*, *Microsporum* or *Epidermophyton*, giving rise to various types of 'ringworm' (not to be confused with genuine helminth infections; see Ch. 54) or tinea. *Tinea capitis* affects the scalp; *Tinea cruris*, the groin ('dhobie itch'); *Tinea pedis*, the feet ('athlete's foot'); and *Tinea corporis*, the body. In superficial candidiasis, the yeast-like organism may infect the mucous membranes of the mouth or vagina (thrush), or the skin. Secondary bacterial infections may complicate the course and treatment of these conditions.

Systemic (or 'disseminated') fungal diseases are much more serious than superficial infections. The commonest in the UK is candidiasis. Other serious conditions are cryptococcal meningitis, endocarditis, pulmonary aspergillosis, and rhinocerebral mucormycosis. Invasive pulmonary aspergillosis is now a leading cause of death in recipients of bone marrow transplants or those with neutropenia. Colonisation by *Aspergillus* of the lungs of patients with asthma or cystic fibrosis can lead to a condition termed allergic *bronchopulmonary aspergillosis*.

In other parts of the world, systemic fungal infections include blastomycosis, histoplasmosis (which is quite common as an asymptomatic finding usually of characteristic calcifications on chest X-ray in the American midwest), coccidiomycosis and paracoccidiomycosis; these are often primary infections, i.e. they are not secondary to reduced immunological function or altered commensal microorganisms.

[1]However, they may also 'infect' buildings too and contribute to the 'sick building syndrome'.

Table 53.1 Some clinically significant fungal infections and a typical first choice of antifungal drug therapy

Organism(s) responsible		Principal disease(s)	Common drug treatments
Yeasts	*Cryptococcus neoformans*	Meningitis	Amphotericin, flucytosine, fluoconazole
Yeast-like fungus	*Candida albicans*	Thrush (and other superficial infection)	Fluconazole, itraconazole
		Systemic candidiasis	Echinocandins, fluconazole, amphotericin, other azoles
Filamentous fungi	*Trichophyton* spp. *Epidermophyton floccosum* *Microsporum* spp.	All these organisms cause skin and nail infections and are referred to as tinea or 'ringworm'	Itraconazole, terbinafine, griseofulvin
	Aspergillus fumigatus	Pulmonary aspergillosis	Voriconazole, amphotericin, capsofungin, other azoles
Dimorphic fungi	*Histoplasma capsulatum*	Histoplasmosis	Itraconazole, amphotericin
	Coccidioides immitis	Coccidiomycosis	
	Blastomyces dermatides	Blastomycosis	

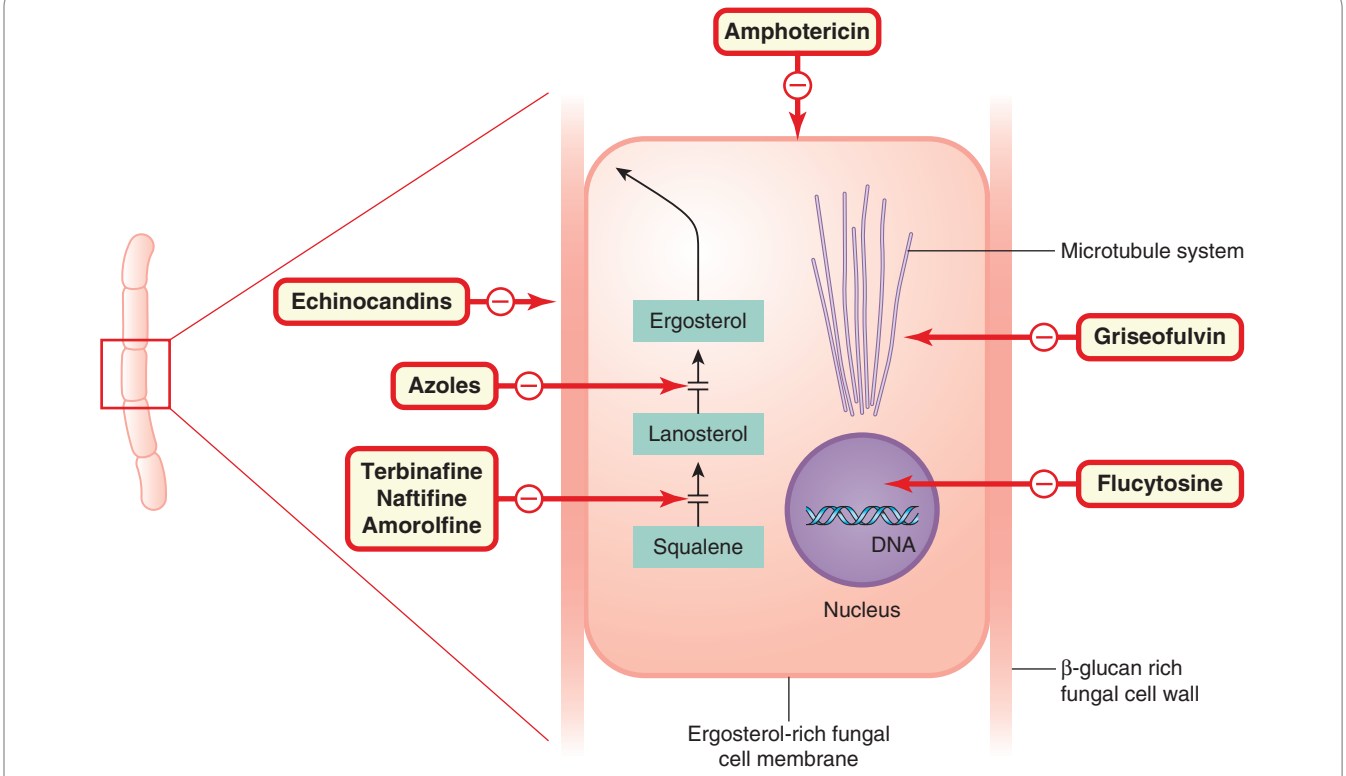

Fig. 53.1 Sites of action of common antifungal drugs. Fungi are morphologically very diverse organisms, and this diagram of a 'typical' fungus is not intended to be technically accurate. The principal sites of action of the main antifungal agents mentioned in this chapter (in red-bordered boxes) are indicated as shown.

DRUGS USED TO TREAT FUNGAL INFECTIONS

The current therapeutic agents can be broadly classified into two groups: first, the naturally occurring antifungal antibiotics such as the *polyenes* and *echinocandins*, and second, synthetic drugs including *azoles* and *fluorinated pyrimidines*. Because many infections are superficial, there are many topical preparations. Many antifungal agents are quite toxic, and when systemic therapy is required this is generally undertaken under strict medical supervision.

Figure 53.1 shows sites of action of common antifungal drugs.

ANTIFUNGAL ANTIBIOTICS

Amphotericin

Amphotericin (also called **amphotericin B**) is a mixture of antifungal substances derived from cultures of *Streptomyces*. Structurally, these are very large ('macrolide')

molecules belonging to the polyene group of antifungal agents.

Like other polyene antibiotics (see Ch. 50), the site of amphotericin action is the fungal cell membrane, where it interferes with permeability and with transport functions by forming large pores in the membrane. The hydrophilic core of the doughnut-shaped molecule creates a transmembrane ion channel, causing gross disturbances in ion balance including the loss of intracellular K^+. Amphotericin has a selective action, binding avidly to the membranes of fungi and some protozoa, less avidly to mammalian cells and not at all to bacteria. The basis of this relative specificity is the drug's greater avidity for *ergosterol*, a fungal membrane sterol that is not found in animal cells (where cholesterol is the principal sterol). Amphotericin is active against most fungi and yeasts, and is the gold standard for treating disseminated infections caused by organisms including *Aspergillus* and *Candida*. Amphotericin also enhances the antifungal effect of **flucytosine**, providing a useful synergistic combination.

Pharmacokinetic aspects

Amphotericin is very poorly absorbed when given orally, and this route is used only for treating fungal infections of the upper gastrointestinal tract. It can be used topically, but for systemic infections it is generally administered, formulated in liposomes or other lipid-containing preparations, by slow intravenous infusion. This improves the pharmacokinetics and reduces the considerable burden of side effects.

Amphotericin is very highly protein-bound. It penetrates tissues and membranes (such as the blood–brain barrier) poorly, although it is found in fairly high concentrations in inflammatory exudates and may cross the blood–brain barrier more readily when the meninges are inflamed. Intravenous amphotericin is essential in the treatment of cryptococcal meningitis, often with flucytosine. It is excreted very slowly via the kidney, traces being found in the urine for 2 months or more after administration has ceased.

Unwanted effects

The commonest (indeed almost invariable) adverse effect of amphotericin is a reaction with rigors, fever, chills and headache during drug infusion; hypotension and anaphylactoid reactions occur in more severely affected individuals. The (considerably more expensive) liposome-encapsulated and lipid-complexed preparations have no greater efficacy than the native drug but cause much less frequent and less severe infusion reactions.

The most serious and common unwanted effect of amphotericin is renal toxicity. Some reduction of renal function occurs in more than 80% of patients receiving the drug; although this generally improves after treatment is stopped, some impairment of glomerular filtration may remain. Hypokalaemia occurs in 25% of patients, due to the primary action of the drug on fungi spilling over into renal tubular cells, causing potassium loss, which often requires potassium chloride supplementation. Hypomagnesaemia also occurs for the same reason. Acid-base disturbance and anaemia can be further problems. Other unwanted effects include impaired hepatic function and thrombocytopenia,. The drug is irritant to the endothelium of the veins, and can cause local thrombophlebitis. Intrathecal injections can cause neurotoxicity, and topical applications cause a skin rash.

Nystatin

Nystatin (also called **fungicidin**) is a polyene macrolide antibiotic similar in structure to amphotericin and with the same mechanism of action. It is given orally, but is not absorbed through mucous membranes or skin, and its use is mainly limited to *Candida* infections of the skin, mucous membranes and the gastrointestinal tract. *Unwanted effects* may include nausea, vomiting and diarrhoea.

Griseofulvin

Griseofulvin is a narrow-spectrum antifungal agent isolated from cultures of *Penicillium griseofulvum*. It interferes with mitosis by binding to fungal microtubules. It can be used to treat dermatophyte infections of skin or nails when local administration is ineffective, but treatment needs to be prolonged. It has largely been superseded by other drugs.

Pharmacokinetic aspects

Griseofulvin is given orally. It is poorly soluble in water, and absorption varies with the type of preparation, in particular with particle size. It is taken up selectively by newly formed skin and concentrated in the keratin. The plasma half-life is 24 h, but it is retained in the skin for much longer. It potently induces cytochrome P450 enzymes and causes several clinically important drug interactions.

Unwanted effects

Unwanted effects with griseofulvin use are infrequent, but the drug can cause gastrointestinal upsets, headache and photosensitivity. Allergic reactions (rashes, fever) may also occur. The drug should not be given to pregnant women.

Echinocandins

Echinocandins comprise a ring of six amino acids linked to a lipophilic side-chain. All drugs in this group are synthetic modifications of **echinocandin B**, which is found naturally in *Aspergillus nidulans*. As a group, the echinocandins are fungicidal for *Candida* and fungistatic for *Aspergillus*. The drugs inhibit the synthesis of 1,3-β-glucan, a glucose polymer that is necessary for maintaining the structure of fungal cell walls. In the absence of this polymer, fungal cells lose integrity and lyse. Resistance genes have been identified in *Candida* (Chen et al., 2011).

Caspofungin is active *in vitro* against a wide variety of fungi, and it has proved effective in the treatment of candidiasis and forms of invasive aspergillosis that are refractory to amphotericin. Oral absorption is poor, and it is given intravenously, once daily. **Anidulafungin** is used mainly for invasive candidiasis; again it is given intravenously. The principal side effects of both drugs include nausea, vomiting and diarrhoea, and skin rash. The relatively new **micafungin** is also mainly used for treating invasive candidiasis. It shares many of the side effects of the group but may also cause serious hepatotoxicity.

SYNTHETIC ANTIFUNGAL DRUGS

AZOLES

The azoles are a group of synthetic fungistatic agents with a broad spectrum of antifungal activity. **Clotrimazole**, **econazole**, **fenticonazole**, **ketoconazole**, **miconazole**, **tioconazole** and **sulconazole** (not UK) are based on the imidazole nucleus and **itraconazole**, **posaconazole**, **voriconazole** and **fluconazole** are triazole derivatives.

The azoles inhibit the fungal cytochrome P450 3A enzyme, lanosine 14α-demethylase, which is responsible for converting lanosterol to ergosterol, the main sterol in the fungal cell membrane. The resulting depletion of ergosterol alters the fluidity of the membrane, and this interferes with the action of membrane-associated enzymes. The net effect is an inhibition of replication. Azoles also inhibit the transformation of candidal yeast cells into hyphae – the invasive and pathogenic form of the parasite. Depletion of membrane ergosterol reduces the binding of amphotericin.

Ketoconazole

Ketoconazole was the first azole that could be given orally to treat systemic fungal infections. It is effective against several different types of organism (see Table 53.1). It is, however, toxic, and relapse is common after apparently successful treatment. It is well absorbed from the gastrointestinal tract. It is distributed widely throughout the tissues and tissue fluids but does not reach therapeutic concentrations in the central nervous system unless high doses are given. It is inactivated in the liver and excreted in bile and in urine. Its half-life in the plasma is 8 h.

Unwanted effects

The main hazard of ketoconazole is liver toxicity, which is rare but can prove fatal. Liver function is monitored before and during treatment. Other side effects that occur are gastrointestinal disturbances and pruritus. Inhibition of adrenocortical steroid and testosterone synthesis has been recorded with high doses, the latter resulting in gynaecomastia in some male patients. There may be adverse interactions with other drugs. **Ciclosporin** and **astemizole** all interfere with cytochrome P450 drug-metabolising enzymes, causing increased plasma concentrations of ketoconazole or the interacting drug, or both. **Rifampicin**, histamine H_2-receptor antagonists and antacids decrease the absorption of ketoconazole.

Fluconazole

Fluconazole is well absorbed and can be given orally or intravenously. It reaches high concentrations in the cerebrospinal fluid and ocular fluids, and is used to treat most types of fungal meningitis. Fungicidal concentrations are also achieved in vaginal tissue, saliva, skin and nails. It has a half-life of ~25 h, and is mainly excreted unchanged in the urine.

Unwanted effects

Unwanted effects, which are generally mild, include nausea, headache and abdominal pain. However, exfoliative skin lesions (including, on occasion, Stevens–Johnson syndrome[2]) have been seen in some individuals – primarily in AIDS patients who are being treated with multiple drugs. Hepatitis has been reported, although this is rare, and fluconazole, in the doses usually used, does not inhibit steroidogenesis and hepatic drug metabolism to the same extent as occurs with ketoconazole.

Itraconazole

Itraconazole is active against a range of dermatophytes. It may be given orally but, after absorption (which is variable) undergoes extensive hepatic metabolism. It is highly lipid-soluble (and water-insoluble), and a formulation in which the drug is retained within pockets of β-cyclodextrin is available. In this form, itraconazole can be administered intravenously, thereby overcoming the problem of variable absorption from the gastrointestinal tract. Administered orally, its half-life is about 36 h, and it is excreted in the urine. It does not penetrate the cerebrospinal fluid.

Unwanted effects

The most serious are hepatoxicity and Stevens–Johnson syndrome. Gastrointestinal disturbances, headache and allergic skin reactions can occur. Inhibition of steroidogenesis has not been reported. Drug interactions as a result of inhibition of cytochrome P450 enzymes occur (similar to ketoconazole).

Miconazole

Miconazole is generally used topically (often as a gel) for oral and other infections of the gastrointestinal tract or for skin or mucosal fungal infection. If significant systemic absorption occurs, drug interactions can present a problem.

Other azoles

Clotrimazole, econazole, tioconazole and sulconazole are used only for topical application. Clotrimazole interferes with amino acid transport into the fungus by an action on the cell membrane. It is active against a wide range of fungi, including candidal organisms. These drugs are sometimes combined with anti-inflammatory glucocorticoids (see Ch. 26). Posacanazole and voriconazole are used mainly for the treatment of invasive life-threatening infections such as aspergillosis.

OTHER ANTIFUNGAL DRUGS

Flucytosine is a synthetic, orally active antifungal agent that is effective against a limited range (mainly yeasts) of systemic fungal infections. If given alone, drug resistance commonly arises during treatment, so it is usually combined with amphotericin for severe systemic infections such as candidiasis and cryptococcal meningitis.

Flucytosine is converted to the antimetabolite 5-fluorouracil in fungal but not human cells. 5-Fluorouracil inhibits thymidylate synthetase and thus DNA synthesis (see Chs 5 and 56). Resistant mutants may emerge rapidly, so this drug should not be used alone.

Flucytosine is usually given by intravenous infusion (because such patients are often too ill to take medicine by mouth) but can also be given orally. It is widely distributed throughout the body fluids, including the cerebrospinal fluid. About 90% is excreted unchanged via the kidneys, and the plasma half-life is 3–5 h. The dosage should be reduced if renal function is impaired.

Unwanted effects include gastrointestinal disturbances, anaemia, neutropenia, thrombocytopenia and alopecia (possibly due to formation of fluorouracil [Ch. 56] from flucytosine by gut bacteria), but these are usually manageable. Uracil is reported to decrease the toxic effects on the bone marrow without impairing the antimycotic action. Hepatitis has been reported but is rare.

Terbinafine is a highly lipophilic, keratinophilic fungicidal compound active against a wide range of skin pathogens. It is particularly useful against nail infections.

[2]This is a severe and sometimes fatal condition involving blistering of the skin, mouth, gastrointestinal tract, eyes and genitalia, often accompanied by fever, polyarthritis and kidney failure.

It acts by selectively inhibiting the enzyme *squalene epoxidase*, which is involved in the synthesis of ergosterol from squalene in the fungal cell wall. The accumulation of squalene within the cell is toxic to the organism.

When used to treat ringworm or fungal infections of the nails, it is given orally. The drug is rapidly absorbed and is taken up by skin, nails and adipose tissue. Given topically, it penetrates skin and mucous membranes. It is metabolised in the liver by the cytochrome P450 system, and the metabolites are excreted in the urine.

Unwanted effects occur in about 10% of individuals and are usually mild and self-limiting. They include gastrointestinal disturbances, rashes, pruritus, headache and dizziness. Joint and muscle pains have been reported and, more rarely, hepatitis.

Naftifine is similar in action to terbinafine. Among other developments, a morpholine derivative, **amorolfine**, which interferes with fungal sterol synthesis, is available as a nail lacquer, being effective against onchomycoses.

FUTURE DEVELOPMENTS

Increasing numbers of fungal strains are becoming resistant to the current antifungal drugs (fortunately, drug resistance is not transferable in fungi), and toxicity and low efficacy also contribute to the need for better antifungal drugs. An additional problem is that new strains of commensal-turned-pathogenic fungi have emerged. Fungal infections are also on the rise because of the prevalence of cancer chemotherapy and transplant-associated immunosuppression.

Encouragingly, new compounds are in development, some with novel mechanisms of action. The development of new inhibitors of β-glucan has been reviewed by Hector and Bierer (2011), new targets such as V-ATPase are being assessed (Zhang & Rao, 2012) while the prospect of discovering new naturally occurring antifungals (like the antibiotic drugs already mentioned) continues to attract attention (Dhankhar et al., 2012). The prospect of using combination therapies has been explored in more depth (see Lupetti et al., 2003) and several groups have identified resistance genes that may improve the design and use of new drugs in the future (Chen et al., 2011; Hadrich et al., 2012; Noel, 2012).

Because fungal infections are often secondary to compromised host defence, attempts have been made to boost this by administration of the cytokine *granulocyte macrophage colony stimulating factor* (GM–CSF, see Ch. 18) and other factors that increase host leukocyte numbers or function (see also Lupetti et al., 2003). Finally, the possibility of developing an antifungal vaccine, first mooted in the 1960s, has recently met with limited success in animals (see Torosantucci et al., 2005 for an account of a *Candida* vaccine). It is hoped that such advances will soon find their way into clinical practice.

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Zhang, Y., Rao, R., 2012. The V-ATPase as a target for antifungal drugs. Curr. Protein Peptide Sci. 13, 134–140. (*The title is self explanatory*)

Useful Web resources

<www.doctorfungus.org> (*This is an excellent site sponsored by a consortium of pharmaceutical companies. It covers all aspects of fungal infections and drug therapy, and has many compelling images and some video clips. Highly recommended – and fun!*)

54 Antiprotozoal drugs

OVERVIEW

Protozoa are motile, unicellular eukaryotic organisms that have colonised virtually every habitat and ecological niche. They may be conveniently classified into four main groups on the basis of their mode of locomotion: *amoebas*, *flagellates* and *sporozoa* are easily characterised but the final group comprises *ciliates* and other organisms of uncertain affiliation, such as the *Pneumocystis jirovecii* mentioned in the last chapter. Protozoa have diverse feeding behaviour, with some being parasitic. Many have extremely complex life cycles, sometimes involving several hosts, reminiscent of the helminths discussed in Chapter 55.

As a group, the protozoa are responsible for an enormous burden of illness in humans as well as domestic and wild animal populations. Table 54.1 lists some of these clinically important organisms, together with the diseases that they cause and an overview of anti-infective drugs. In this chapter we will first discuss some general features of protozoa–host interactions and then discuss the therapy of each group of diseases in turn. In view of its global importance, malaria is the main topic.

HOST–PARASITE INTERACTIONS

Mammals have developed very efficient mechanisms for defending themselves against invading parasites, but many parasites have, in turn, evolved sophisticated evasion tactics. One common parasite ploy is to take refuge within the cells of the host, where antibodies cannot reach them. Most protozoa do this, for example *Plasmodium* species take up residence in red cells, *Leishmania* species infect macrophages exclusively, while *Trypanosoma* species invade many other cell types. The host deals with these intracellular fugitives by deploying cytotoxic $CD8^+$ T cells and T helper (Th)1 pathway cytokines, such as interleukin (IL)-2, tumour necrosis factor (TNF)-α and interferon-γ. These cytokines (see Ch. 18) activate macrophages, which can then kill intracellular parasites.

As we explained in Chapter 6, the Th1 pathway responses can be downregulated by Th2 pathway cytokines (e.g. transforming growth factor-β, IL-4 and IL-10). Some intracellular parasites have exploited this fact by stimulating the production of Th2 cytokines thus reducing their vulnerability to Th1-driven activated macrophages. For example, the invasion of macrophages by *Leishmania* species induces transforming growth factor-β, IL-10, inactivates complement pathways, and downregulates many other intracellular defence mechanisms (Singh et al., 2012). Similar mechanisms operate during worm infestations (see Ch. 55).

Toxoplasma gondii has evolved a different gambit – *upregulation* of host defence responses. The definitive (i.e. where sexual recombination occurs) host of this protozoon is the cat, but humans can inadvertently become intermediate hosts, harbouring the asexual form of the parasite. In humans, *T. gondii* infects numerous cell types and has a highly virulent replicative stage. To ensure that its host survives, it stimulates production of interferon-γ, modulating the host's cell-mediated responses to promote encystment of the parasite in the tissues. The use of cytokine analogues and/or antagonists to treat disease caused by protozoa is a promising area for the development of new anti-parasite drugs (see Odeh, 2001).

MALARIA AND ANTIMALARIAL DRUGS

Malaria[1] is caused by parasites belonging to the genus *Plasmodium*. Four main species infect humans: *P. vivax, P. falciparum, P. ovale* and *P. malariae*. A related parasite that infects monkeys, *P. knowlesi*, can also infect humans and is causing increasing concern in some regions, such as South-East Asia. The insect vector in all cases is the female *Anopheles* mosquito. This breeds in stagnant water and the disease it spreads is one of the major killers on our planet.

Largely because of a massive increase in spending on public health campaigns such as the *Roll Back Malaria* programme (which is sponsored by a partnership of transnational organisations including the World Health Organization, WHO), the global malaria mortality rate has fallen by approximately a quarter over the last decade, but even so, the overall statistics make gloomy reading. According to the 2012 WHO report, malaria is a significant public health problem in more than 100 countries. In 2010, there were an estimated 219 million cases and some 660 000 deaths from the disease. More than 90% of these occur in sub-Saharan Africa, and most of the victims are children. Even those who survive may suffer from lasting mental impairment. Other high-risk groups include pregnant women, refugees and labourers entering endemic regions. Malaria also imposes a huge economic burden on countries where the disease is rife.

Also of concern is the fact that malaria has gained a foothold in other countries where it is not normally endemic.[2] The WHO recorded over 100 000 such cases in over 90 countries between 2001 and 2010. This phenomenon is partly due to international travel, partly due to immigration from countries where the disease is endemic and (possibly) partly caused by global warming.

The symptoms of malaria include fever, shivering, pain in the joints, headache, repeated vomiting, generalised

[1]The disease was once considered to arise from marshy land, hence the Latin name '*mal aria*', meaning bad or poisonous air.
[2]This is usually referred to as 'imported malaria'. 'Airport malaria' is caused by infected mosquitoes in aircraft arriving from areas where the disease is endemic; 'baggage malaria' is caused by their presence in luggage arriving from such areas; and 'runway malaria' has been contracted by passengers who have stopped in endemic areas, but have not even left the aircraft.

Table 54.1 Principal protozoal infections and common drug treatments

Organism	Disease	Common drug treatments
Amoeba		
Entamoeba histolytica	Amoebic dysentery	Metronidazole, tinidazole, diloxanide
Flagellates		
Trypanosoma brucei rhodesiense *Trypanosoma brucei gambiense*	Sleeping sickness	Suramin, pentamidine, melarpasol, eflornithine, nifurtimox
Trypanosoma cruzi	Chagas' disease	Nifurtimox, benzindazole
Leishmania tropica *Leishmania donovani* *Leishmania Mexicana* *Leishmania braziliensis*	'Kala-azar' 'Chiclero's ulcer' 'Espundia' 'Oriental sore'	Sodium stibogluconate, amphotericin, pentamidine isetionate
Trichomonas vaginalis	Vaginitis	Metronidazole, tinidazole
Giardia lamblia	Diarrhoea, steatorrhoea	Metronidazole, tinidazole, mepacrine
Sporozoa		
Plasmodium falciparum[a] *Plasmodium vivax* *Plasmodium ovale* *Plasmodium malariae*	Malignant tertian malaria Benign tertian malaria Benign tertian malaria Quartan malaria	Artemether, atovaquone, chloroquine, clindamycin, dapsone, doxycycline, lumefantrine, mefloquine, primaquine, proguanil, pyrimethamine, quinine, sulfadoxine
Toxoplasma gondii	Encephalitis, congenital malformations, eye disease	Pyrimethamine-sulfadiazine
Ciliates and others		
Pneumocystis carinii[b]	Pneumonia	Co-trimoxazole, atovaquone, pentamidine isetionate

[a]See also Table 54.2.
[b]This organism is of uncertain classification. See text for details and Chapter 53 for further comments.

Malaria

- Malaria is caused by various species of plasmodia, which are carried by the female *Anopheles* mosquito. Sporozoites (the asexual form of the parasite) are introduced into the host following insect bite and these develop in the liver into:
  - schizonts (the pre-erythrocytic stage), which liberate merozoites – these infect red blood cells, forming motile trophozoites, which, after development, release another batch of erythrocyte-infecting merozoites, causing fever; this constitutes the erythrocytic cycle
  - dormant hypnozoites, which may liberate merozoites later (the exoerythrocytic stage).
- The main malarial parasites causing tertian ('every third day') malaria are:
  - *P. vivax*, which causes benign tertian malaria
  - *P. falciparum*, which causes malignant tertian malaria; unlike *P. vivax*, this plasmodium has no exoerythrocytic stage.
- Some merozoites develop into gametocytes, the sexual forms of the parasite. When ingested by the mosquito, these give rise to further stages of the parasite's life cycle within the insect.

convulsions and coma. Symptoms become apparent only 7–9 days after being bitten by an infected mosquito. By far the most dangerous parasite is *P. falciparum*.

Malaria was eradicated from most temperate countries in the 20th century, and the WHO attempted to eradicate malaria elsewhere using the powerful 'residual' insecticides and the highly effective antimalarial drugs, such as **chloroquine**, which had, by then, become available. By the end of the 1950s, the incidence of malaria had dropped dramatically. However, it was clear by the 1970s that the attempt at eradication had failed, largely because of the increasing resistance of the mosquito to the insecticides, and of the parasite to the drugs. Sadly, malaria has now re-emerged in several countries where it was previously under control or eradicated.

THE LIFE CYCLE OF THE MALARIA PARASITE

The life cycle of the parasite consists of a sexual cycle, which takes place in the female *Anopheles* mosquito, and

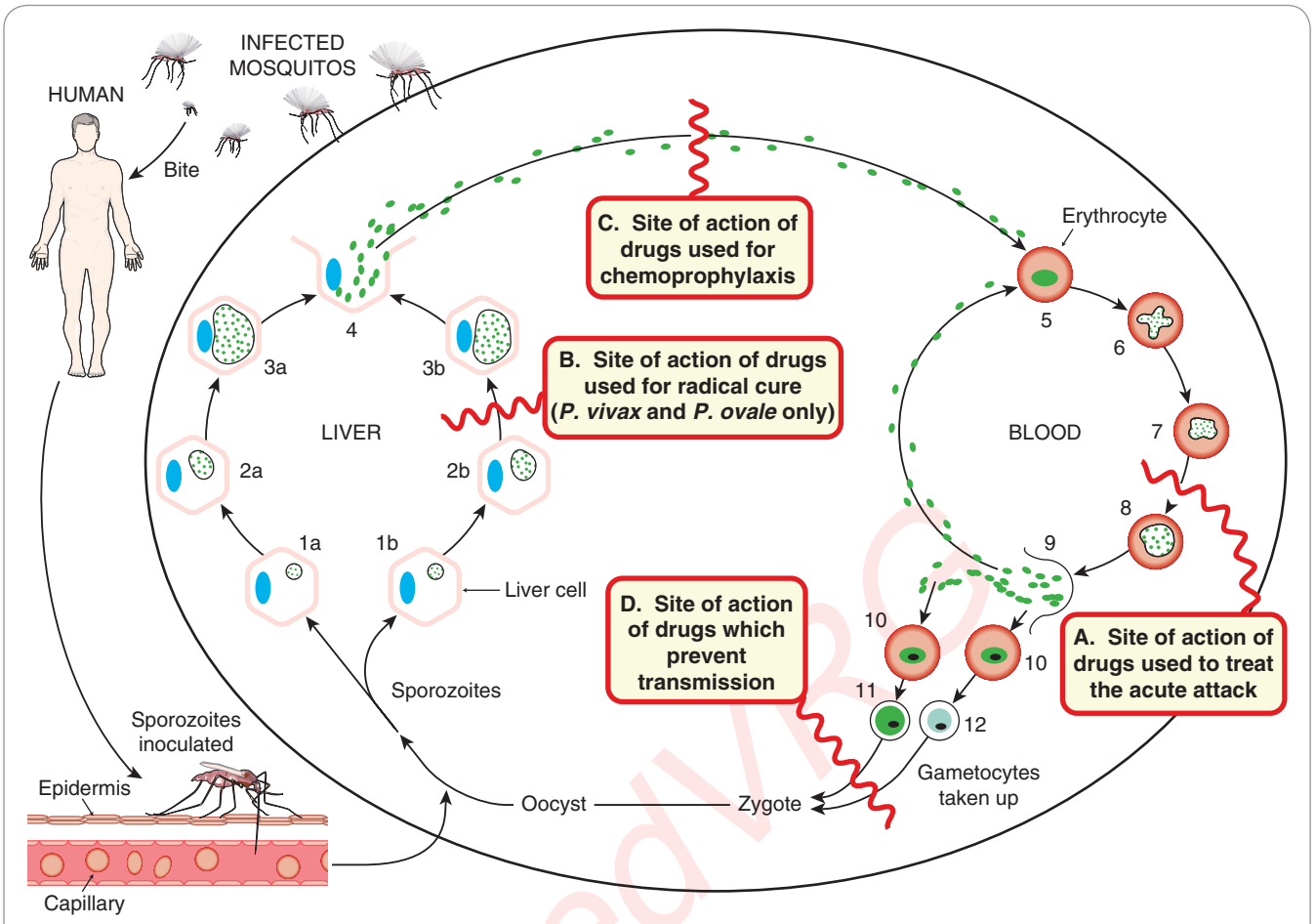

Fig. 54.1 The life cycle of the malarial parasite and the site of action of antimalarial drugs. The infection is initiated by the bite of an infected female *Anopheles* mosquito, which introduces the parasite into the blood. This then enters a pre- or exoerythrocytic cycle in the liver and an erythrocytic cycle in the blood: (**1a**) from the blood stream the sporozoite enters into liver cells (the parasite is shown as a small circle containing dots, and the liver cell nucleus as a blue oval); (**2a** and **3a**) the schizont develops in the liver cells; (**4**) these eventually rupture releasing merozoites (some may enter further liver cells and become resting forms of the parasite, hypnozoites). (**5**) Merozoites enter into red cells and form motile trophozoites (**6**); following division and multiplication (**7** and **8**) schizonts develop in red cells that eventually (**9**) rupture releasing further merozoites, most of which parasitise other red cells. Sometimes (**10–12**) merozoites develop into male and female gametocytes in red cells. These can constitute a fresh source of infective material if the blood is then consumed by another mosquito. (**1b**) Resting form of parasite in liver (hypnozoite). (**2b** and **3b**) Growth and multiplication of hypnozoites.

Sites of drug action are as follows. (**A**) Drugs used to treat the acute attack (also called 'blood schizonticidal agents' or 'drugs for suppressive or clinical cure'). (**B**) Drugs that affect the exoerythrocytic hypnozoites and result in a 'radical' cure of *P. vivax* and *P. ovale*. (**C**) Drugs that block the link between the exoerythrocytic stage and the erythrocytic stage; they are used for chemoprophylaxis (also termed causal prophylactics) and prevent the development of malarial attacks. (**D**) Drugs that prevent transmission and thus prevent increase of the human reservoir of the disease.

an asexual cycle, which occurs in humans (Fig. 54.1 and the 'Malaria' box). Therefore, the mosquito, not the human, is the definitive host for plasmodia. Indeed, it has been said that the only function of humans is to enable the parasite to infect more mosquitoes so that further sexual recombination can occur.

▼ The cycle in the mosquito involves fertilisation of the female *gametocyte* by the male gametocyte, with the formation of a *zygote*, which develops into an *oocyst* (*sporocyst*). A further stage of division and multiplication takes place, leading to rupture of the sporocyst with release of *sporozoites*, which then migrate to the mosquito's salivary glands and a few enter the human host with the mosquito's bite.

When sporozoites enter the human host they disappear from the bloodstream within 30 min and enter the parenchymal cells of the liver where, during the next 10–14 days, they undergo a *pre-erythrocytic* stage of development and multiplication. The parasitised liver cells then rupture, and a host of fresh *merozoites* are released. These bind to and enter erythrocytes and form motile intracellular parasites termed *trophozoites*. During the erythrocytic stage the parasite remodels the host cell, inserting parasite proteins and phospholipids into the red cell membrane. The host's haemoglobin is transported to the parasite's food vacuole, where it is digested, providing a source of amino acids. Free haem, which would be toxic to the plasmodium, is rendered harmless by polymerisation to *haemozoin*. Some antimalarial drugs act by inhibiting the haem polymerase enzyme responsible for this step.

▼ Following mitotic replication, the parasite in the red cell is termed a schizont, and its rapid growth and division, *schizogony*. Another phase of multiplication results in the production of further merozoites, which are released when the red cell ruptures. These

merozoites then bind to and enter fresh red cells, and the erythrocytic cycle begins again. In certain forms of malaria, some sporozoites entering the liver cells form *hypnozoites*, or 'sleeping' forms of the parasite, which can be reactivated months or years later to continue an *exoerythrocytic* cycle of multiplication.

Malaria parasites can multiply in the body at a phenomenal rate – a single parasite of *P. vivax* can give rise to 250 million merozoites in 14 days. To appreciate the action required of an antimalarial drug, note that destruction of 94% of the parasites every 48 h will serve only to maintain equilibrium and will not further reduce their number or their propensity for proliferation. Some merozoites, on entering red cells, differentiate into male and female gametocytes. These can complete their cycle only when taken up by the mosquito, when it sucks the blood from the infected host.

The periodic episodes of fever that characterise malaria result from the synchronised rupture of red cells with release of merozoites and cell debris. The rise in temperature is associated with a rise in the concentration of TNF-α in the plasma. Relapses of malaria are likely to occur with those forms of malaria that have an exoerythrocytic cycle, because the dormant hypnozoite form in the liver may emerge after an interval of weeks or months to start the infection again.

▼ The characteristic presentations of the different forms of human malaria are as follows (see Fig. 54.1 for details):

- *P. falciparum*, which has an erythrocytic cycle of 48 h in humans, produces *malignant tertian malaria* – 'tertian' because the fever was believed to recur every third day (actually it varies), 'malignant' because it is the most severe form of malaria and can be fatal. The plasmodium induces adhesion molecules on the infected cells, which then stick to uninfected red cells, forming clusters (rosettes), and also adhere to and pack the vessels of the microcirculation, interfering with tissue blood flow and causing organ dysfunction including renal failure and encephalopathy (cerebral malaria). *P. falciparum* does not have an exoerythrocytic stage, so if the erythrocytic stage is eradicated, relapses do not occur.
- *P. vivax* produces *benign tertian malaria*, less severe than falciparum malaria and rarely fatal. Exoerythrocytic forms may persist for years and cause relapses.
- *P. ovale*, which has a 48 h cycle and an exoerythrocytic stage, is the cause of a rare form of malaria.
- *P. malariae* has a 72 h cycle, causes *quartan malaria* and has no exoerythrocytic cycle.

Individuals living in areas where malaria is endemic may acquire a natural immunity, but this may be lost if the individual is absent from the area for more than 6 months. The best way to deal with malaria is to prevent mosquito bites by suitable clothing, insect repellents and bed nets. Bed nets sprayed with insecticides such as permethrin can be very effective.

ANTIMALARIAL DRUGS

Some drugs can be used prophylactically to prevent malaria (see Table 54.2), while others are directed towards treating acute attacks. In general, antimalarial drugs are classified in terms of the action against the different stages of the life cycle of the parasite (Fig. 54.1).

The use of drugs for the treatment of malaria has changed considerably during the last half-century mainly because resistance developed to chloroquine and other successful early drug combinations (see Butler et al., 2010). Monotherapy has largely been abandoned in favour of **artemisinin**-based combination therapy (ACT; see Table 54.3). Only antimalarial drugs in common use are described in this chapter. For a brief summary of currently recommended treatment regimens, see the 'Antimalarial drugs' box and Table 54.1. Na-Bangchang and Karbwang (2009) give a more detailed coverage of current therapeutic options and their use in the treatment of malaria around the world.

Antimalarial therapy and the parasite life cycle

Drugs used in the treatment of malaria may have several sites of action:

- Drugs used to treat the acute attack of malaria act on the parasites in the blood; they can cure infections with parasites (e.g. *P. falciparum*) that have no exoerythrocytic stage.
- Drugs used for prophylaxis act on merozoites emerging from liver cells.
- Drugs used for radical cure are active against parasites in the liver.
- Some drugs act on gametocytes and prevent transmission by the mosquito.

Table 54.2 Examples of drug treatment and chemoprophylaxis of malaria[a]

To treat …	Typical drug choices
Infection with *P. falciparum* or with unknown or mixed organisms	Quinine + doxycycline or clindamycin; or Proguanil + atovoquone;[b] or Artemether + lumefantrine[c]
Infection with *P. malariae*, *P. vivax* or *P. ovale*	Chloroquine, possibly followed by primaquine in the case of *P. vivax* or *P. ovale*
Chemoprophylaxis (short-term)	Proguanil + atovoquone[b] or doxycycline
Chemoprophylaxis (long-term)	Chloroquine + proguanil; mefloquine or doxycycline

[a]It must be appreciated that this is only a summary, not a definitive guide to prescription, as the recommended drug combinations vary depending on the patient, the area visited, the overall risk of infection, the presence of resistant forms of the disease and so on. This information is based on current UK recommendations (source: British National Formulary 2013).

[b]Malarone is a proprietary combination of atovoquone and proguanil hydrochloride.

[c]Riamet is a proprietary combination of artemether and lumefantrine.

Drugs used to treat the acute attack

Blood schizonticidal agents (Fig. 54.1, site A) are used to treat the acute attack but also produce a 'suppressive' or 'clinical' cure. They act on the erythrocytic forms of the plasmodium. In the case of *P. falciparum* or *P. malariae*, which have no exoerythrocytic stage, these drugs effect a cure; however, with *P. vivax* or *P. ovale*, the drugs suppress the actual attack but exoerythrocytic forms can re-emerge later to cause relapses.

This group of drugs includes:

- artemisinin and related compounds derived from the Chinese herb *qinghao*, which are usually used in combination with other drugs
- the quinoline–methanols (e.g. **quinine** and **mefloquine**) and various 4-aminoquinolines (e.g. chloroquine)
- agents that interfere either with the synthesis of folate (e.g. **dapsone**) or with its action (e.g. **pyrimethamine** and **proguanil**)
- **atovaquone**, which affects mitochondrial function.

Combinations of these agents are frequently used. Some antibiotics, such as the tetracycline **doxycycline** (see Ch. 51), have proved useful when combined with the above agents. They have an antiparasite effect in their own right but also control other concomitant infections.

Drugs that effect a radical cure

Tissue schizonticidal agents effect a 'radical' cure by eradicating *P. vivax* and *P. ovale* parasites in the liver (Fig. 54.1, site B). Only the 8-aminoquinolines (e.g. **primaquine** and **tafenoquine**) have this action. These drugs also destroy gametocytes and thus reduce the spread of infection.

Drugs used for chemoprophylaxis

Drugs used for chemoprophylaxis (also known as *causal prophylactic drugs*: see Table 54.2) block the link between the exoerythrocytic stage and the erythrocytic stage, and thus prevent the development of malarial attacks. True causal prophylaxis – the prevention of infection by the killing of the sporozoites on entry into the host – is not feasible with present drugs, although it may be feasible in the future with vaccines. Clinical attacks can be prevented by chemoprophylactic drugs that kill the parasites when they emerge from the liver after the pre-erythrocytic stage (Fig. 54.1, site C). The drugs used for this purpose are mainly artemisinin derivatives, chloroquine, **lumefantrine**, mefloquine, proguanil, pyrimethamine, dapsone and doxycycline. They are often used in combinations.

▼ Chemoprophylactic agents are given to individuals who intend travelling to an area where malaria is endemic. Administration should start at least 1 week before entering the area and should be continued throughout the stay and for at least a month afterwards. No chemoprophylactic regimen is 100% effective, and unwanted effects may occur. A further problem is the complexity of the regimens, which require different drugs to be taken at different times, and the fact that different agents may be required for different travel destinations. For a brief summary of currently recommended regimens of chemoprophylaxis, see Table 54.2.

Drugs used to prevent transmission

Some drugs (e.g. primaquine, proguanil and pyrimethamine) can also destroy gametocytes (Fig. 54.1, site D), preventing transmission by the mosquito and thus diminishing the human reservoir of the disease, although they are rarely used for this action alone.

Table 54.3 summarises what is known about the molecular targets of these drugs and Figure 54.2 shows chemical structures of some significant drugs.

CHLOROQUINE

The 4-aminoquinoline chloroquine dates from the 1940s but is still widely used as a blood schizonticidal agent (Fig. 54.1, site A), effective against the erythrocytic forms of all four plasmodial species (where resistance is not an issue), but it does not have any effect on sporozoites, hypnozoites or gametocytes. It is uncharged at neutral pH and can therefore diffuse freely into the parasite lysosome. At the acid pH of the lysosome, it is converted to a protonated, membrane-impermeable form and is 'trapped' inside the parasite. Its chief antimalarial action derives from an inhibition of *haem polymerase*, the enzyme that polymerises toxic free haem to haemozoin. This poisons the parasite and prevents it from utilising the amino acids from haemoglobin proteolysis. Chloroquine is also used as a disease-modifying antirheumatoid drug (Ch. 26) and also has some quinidine-like actions on the heart (Ch. 21).

Resistance

P. falciparum is now resistant to chloroquine in most parts of the world. Resistance appears to result from enhanced efflux of the drug from parasitic vesicles as a result of mutations in plasmodia transporter genes (Baird, 2005). Resistance of *P. vivax* to chloroquine is also a growing problem in many parts of the world.

Table 54.3 Drug targets of antimalarial drugs

Parasite organelle	Target	Chemical class	Drugs
Cytosolic compartment	Inhibit or antagonise folic acid metabolism	Diaminopyridines	Pyrimethamine
		Biguanides	Proguanil
		Sulfones	Dapsone
		Sulfonamides	Sulfadoxine
Mitochondrion	Block electron transport energy production	Hydroxynapthoquinones	Atovaquone, tafenoquine, pyridones
Apicoplast	Block protein synthetic machinery	Tetracyclines and others	Azithromycin, doxycycline, clindamycin other antibiotics
Digestive vacuole	Inhibit the detoxification of haem	Quinolones	Chloroquine, amodiaquine, mefloquine, quinine
		Aryl amino alcohols	Lumefantrine
Membranes?	Inhibition of Ca^{2+}-dependent ATPase	Sesquiterpene lactones	Artemisinin derivatives

After Fidock et al. 2004.

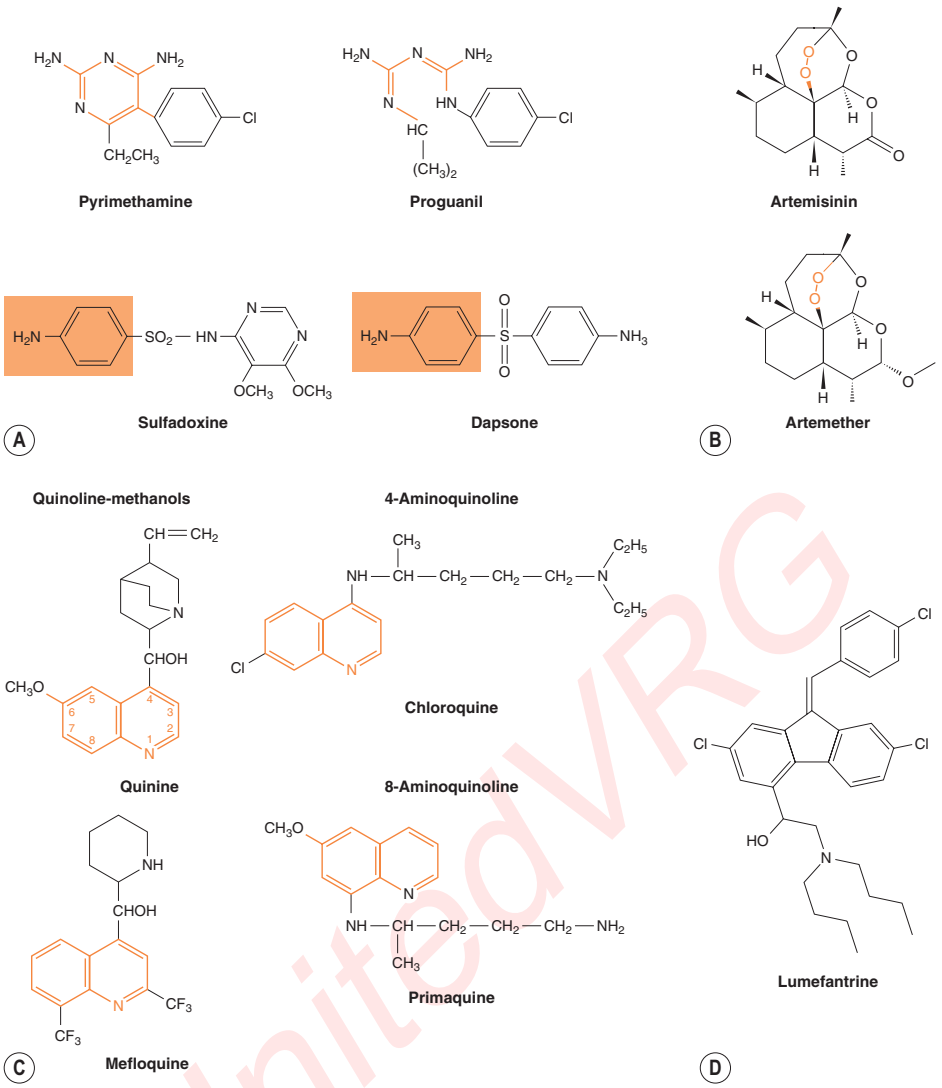

Fig. 54.2 Structures of some significant antimalarial drugs. [**A**] Drugs that act on the folic acid pathway of the plasmodia. Folate antagonists (pyrimethamine, proguanil) inhibit dihydrofolate reductase; the relationship between these drugs and the pteridine moiety is shown in orange. Sulfones (e.g. dapsone) and sulfonamides (e.g. sulfadoxine) compete with p-aminobenzoic acid for dihydropteroate synthetase (relationship shown in orange box; see also Ch. 50). [**B**] Artemisinin and a derivative artemether. Note the endoperoxide bridge structure (in orange) that is crucial to their action. [**C**] Some quinolone antimalarials. The quinoline moiety is shown in orange. [**D**] The aryl amino alcohol lumefantrine.

Administration and pharmacokinetic aspects

Chloroquine is generally administered orally, but severe falciparum malaria may be treated by frequent intramuscular or subcutaneous injection of small doses, or by slow continuous intravenous infusion. Following oral dosing, it is completely absorbed, extensively distributed throughout the tissues and concentrated in parasitised red cells. Release from tissues and infected erythrocytes is slow. The drug is metabolised in the liver and excreted in the urine, 70% as unchanged drug and 30% as metabolites. Elimination is slow, the major phase having a half-life of 50 h, and a residue persists for weeks or months.

Unwanted effects

Chloroquine has few adverse effects when given for chemoprophylaxis. However, unwanted effects, including nausea and vomiting, dizziness and blurring of vision, headache and urticarial symptoms, can occur when larger doses are administered to treat acute attacks of malaria. Large doses have also sometimes resulted in retinopathies and hearing loss. Bolus intravenous injections of chloroquine may cause hypotension and, if high doses are used, fatal dysrhythmias. Chloroquine is considered to be safe for use by pregnant women.

Amodiaquine has very similar action to chloroquine. It was withdrawn several years ago because of the risk of agranulocytosis, but has now been reintroduced in several areas of the world where chloroquine resistance is endemic.

QUININE

Quinine, derived from cinchona bark, has been used for the treatment of 'fevers' since the 16th century, when Jesuit missionaries brought the bark to Europe from Peru. It is a blood schizonticidal drug effective against the

erythrocytic forms of all four species of plasmodium (Fig. 54.1, site A), but it has no effect on exoerythrocytic forms or on the gametocytes of *P. falciparum*. Its mechanism of action is the same as that of chloroquine, but quinine is not so extensively concentrated in the plasmodium as chloroquine, so other mechanisms could also be involved. With the emergence and spread of chloroquine resistance, quinine is now the main chemotherapeutic agent for *P. falciparum* in certain parts of the world. Pharmacological actions on host tissue include a depressant action on the heart, a mild oxytocic effect on the uterus in pregnancy, a slight blocking action on the neuromuscular junction and a weak antipyretic effect.

Some degree of resistance to quinine has developed because of increased expression of plasmodial drug efflux transporters.

Pharmacokinetic aspects

Quinine is well absorbed and is usually administered orally as a 7-day course, but it can also be given by slow intravenous infusion for severe *P. falciparum* infections and in patients who are vomiting. A loading dose may be required, but bolus intravenous administration is contraindicated because of the risk of cardiac dysrhythmias. The half-life of the drug is 10 h; it is metabolised in the liver and the metabolites are excreted in the urine within about 24 h.

Unwanted effects

Quinine has a bitter taste, and oral compliance is often poor.[3] It is irritant to the gastric mucosa and can cause nausea and vomiting. 'Cinchonism' – characterised by nausea, dizziness, tinnitus, headache and blurring of vision – is likely to occur if the plasma concentration exceeds 30–60 µmol/l. Excessive plasma levels may also cause hypotension, cardiac dysrhythmias and severe CNS disturbances such as delirium and coma.

Other, infrequent, unwanted reactions that have been reported are bone marrow depression (mainly thrombocytopenia) and hypersensitivity reactions. Quinine can stimulate insulin release. Patients with marked falciparum parasitaemia can have low blood sugar for this reason and also because of glucose consumption by the parasite. This can make a differential diagnosis between a coma caused by cerebral malaria and hypoglycaemia difficult. A rare result of treating malaria with quinine, or of erratic and inappropriate use of quinine, is Blackwater fever, a severe and often fatal condition in which acute haemolytic anaemia is associated with renal failure.

MEFLOQUINE

Mefloquine (Fig. 54.2) is a blood schizonticidal compound active against *P. falciparum* and *P. vivax* (Fig. 54.1, site A); however, it has no effect on hepatic forms of the parasites, so treatment of *P. vivax* infections should be followed by a course of primaquine to eradicate the hypnozoites. Mefloquine acts in the same way as quinine, and is frequently combined with pyrimethamine.

Resistance to mefloquine has occurred in *P. falciparum* in some areas – particularly in South-East Asia – and is thought to be caused, as with quinine, by increased expression in the parasite of drug efflux transporters.

Pharmacokinetic aspects and unwanted effects

Mefloquine is given orally and is rapidly absorbed. It has a slow onset of action and a very long plasma half-life (up to 30 days), which may be the result of enterohepatic cycling or tissue storage.

When mefloquine is used for treatment of the acute attack, about 50% of subjects complain of gastrointestinal disturbances. Transient CNS side effects – giddiness, confusion, dysphoria and insomnia – can occur, and there have been a few reports of aberrant atrioventricular conduction and serious, but rare, skin diseases. Rarely, mefloquine may provoke severe neuropsychiatric reactions. Mefloquine is contraindicated in pregnant women or in those liable to become pregnant within 3 months of stopping the drug, because of its long half-life and uncertainty about its teratogenic potential. When used for chemoprophylaxis, the unwanted actions are usually milder, but the drug should not be used in this way unless there is a high risk of acquiring chloroquine-resistant malaria.

LUMEFANTRINE

This aryl amino alcohol drug is related to an older compound, halofantrine, which is now seldom used. Lumefantrine is never used alone but in combination with **artemether**. Its mode of action is probably to prevent parasite detoxification of haem. The pharmacokinetics of the combination is complex and the reader is referred to Ezzet et al. (1998) for more details. Unwanted effects of the combination may include gastrointestinal and central nervous system (CNS) symptoms.

DRUGS AFFECTING FOLATE METABOLISM

Sulfonamides and sulfones, used as antibacterial drugs (see Ch. 51), inhibit the synthesis of folate in plasmodia by competing with p-aminobenzoic acid. Pyrimethamine and proguanil inhibit dihydrofolate reductase, which prevents the utilisation of folate in DNA synthesis. Used together, they block the folate pathway at different points, and thus act synergistically.

The main sulfonamide used in malaria treatment is **sulfadoxine**, and the only sulfone used is dapsone. Details of these drugs are given in Chapter 51. The sulfonamides and sulfones are active against the erythrocytic forms of *P. falciparum* but are less active against those of *P. vivax*; they have no activity against the sporozoite or hypnozoite forms of the plasmodia. Pyrimethamine–sulfadoxine has been extensively used for chloroquine-resistant malaria, but resistance to this combination has developed in many areas.

Pyrimethamine is similar in structure to the antibacterial drug **trimethoprim** (see Ch. 51). Proguanil has a slightly different structure but its (active) metabolite can assume a similar configuration. Both drugs have a greater affinity for the plasmodium enzyme than for the human enzyme. They have a slow action against the erythrocytic forms of the parasite (Fig. 54.1, site A), and proguanil is believed to have an additional effect on the initial hepatic stage (1a to 3a in Fig. 54.1) but not on the hypnozoites of *P. vivax* (Fig. 54.1, site B). Pyrimethamine is used only in combination with either a sulfone or a sulfonamide.

Pharmacokinetic aspects

Both pyrimethamine and proguanil are given orally and are well, although slowly, absorbed. Pyrimethamine has

[3]Hence the invention of palatable drinks containing the drug, including, of course, the famous 'tonic' drunk together with gin and other beverages.

a plasma half-life of 4 days, and effective 'suppressive' plasma concentrations may last for 14 days; it is taken once a week. The half-life of proguanil is 16 h. It is a prodrug and is metabolised in the liver to its active form, cycloguanil, which is excreted mainly in the urine. It must be taken daily.

Unwanted effects

These drugs have few untoward effects in therapeutic doses. Larger doses of the pyrimethamine–dapsone combination can cause serious reactions such as haemolytic anaemia, agranulocytosis and lung inflammation. The pyrimethamine–sulfadoxine combination can cause serious skin reactions, blood dyscrasias and allergic alveolitis; it is no longer recommended for chemoprophylaxis. In high doses, pyrimethamine may inhibit mammalian dihydrofolate reductase and cause a *megaloblastic anaemia* (see Ch. 25) and folic acid supplements should be given if this drug is used during pregnancy. Resistance to antifolate drugs arises from single-point mutations in the genes encoding parasite dihydrofolate reductase.

PRIMAQUINE

Primaquine is an 8-aminoquinoline drug, which is (almost uniquely among clinically available antimalarial drugs) active against liver hypnozoites (see Fig. 54.2). **Etaquine** and tafenoquine are more active and slowly metabolised analogues of primaquine. These drugs can effect a radical cure of *P. vivax* and *P. ovale* malaria in which the parasites have a dormant stage in the liver. Primaquine does not affect sporozoites and has little if any action against the erythrocytic stage of the parasite. However, it has a gametocidal action and is the most effective antimalarial drug for preventing transmission of all four species of plasmodia. It is almost invariably used in combination with another drug, usually chloroquine. Resistance to primaquine is rare, although evidence of a decreased sensitivity of some *P. vivax* strains has been reported. The pharmacology of primaquine and similar drugs has been reviewed by Shanks et al. (2001).

Pharmacokinetic aspects

Primaquine is given orally and is well absorbed. Its metabolism is rapid, and very little drug is present in the body after 10–12 h. The half-life is 3–6 h. Tafenoquine is metabolised much more slowly and therefore has the advantage that it can be given on a weekly basis.

Unwanted effects

Primaquine has few unwanted effects in most patients when used in normal therapeutic dosage. Dose-related gastrointestinal symptoms can occur, and large doses may cause methaemoglobinaemia with cyanosis.

Primaquine can cause haemolysis in individuals with the X chromosome-linked genetic metabolic condition, *glucose 6-phosphate dehydrogenase deficiency*, in red cells (Ch. 11). When this deficiency is present, the red cells are not able to regenerate NADPH, which is depleted by the oxidant metabolic derivatives of primaquine. As a consequence, the metabolic functions of the red cells are impaired and haemolysis occurs. The deficiency of the enzyme occurs in up to 15% of black males and is also fairly common in some other ethnic groups. Glucose 6-phosphate dehydrogenase activity should be estimated before giving primaquine.

ARTEMISININ AND RELATED COMPOUNDS

The importance of this group is that they are often the only drugs that can effectively treat resistant *P. falciparum*. These sesquiterpene lactones are derived from the herb *qinghao*, a traditional Chinese remedy for fevers. The scientific name, conferred on the herb by Linnaeus, is *Artemisia*.[4] Artemisinin, a poorly soluble chemical extract from *Artemisia*, is a fast-acting blood schizonticide effective in treating the acute attack of malaria (including chloroquine-resistant and cerebral malaria). Derivatives of artemisinin, which include **artesunate** (a water-soluble derivative available in some countries) and **artemether**, have higher activity and are better absorbed. The compounds are concentrated in parasitised red cells. The mechanism of action is probably through inhibition of a parasite Ca^{2+}-dependent ATPase (Eckstein-Ludwig et al., 2003) and it is likely that the unusual 'endoperoxide bridge' of this drug (see Fig. 54.2) has to be 'activated' in the presence of intracellular iron before it can exert its effects. These drugs are without effect on liver hypnozoites. Artemisinin can be given orally, intramuscularly or by suppository, artemether orally or intramuscularly, and artesunate intramuscularly or intravenously. They are rapidly absorbed and widely distributed, and are converted in the liver to the active metabolite dihydroartemisinin. The half-life of artemisinin is about 4 h, of artesunate, 45 min and of artemether, 4–11 h.

There are few unwanted effects. Transient heart block, decrease in blood neutrophil count and brief episodes of fever have been reported. In animal studies, artemisinin causes an unusual injury to some brain stem nuclei, particularly those involved in auditory function; however, there have been no reported incidences of neurotoxicity in humans. So far, resistance has not been a problem, but recent reports suggest that the parasite in some areas of the world is becoming less sensitive to these drugs. In rodent studies, artemisinin potentiated the effects of mefloquine, primaquine and tetracycline, was additive with chloroquine and antagonised the sulfonamides and the folate antagonists. For this reason, artemisinin derivatives are frequently used in combination with other antimalarial drugs; for example, artemether is often given in combination with lumefantrine.

In randomised trials, artemisinins have cured attacks of malaria, including cerebral malaria, more rapidly and with fewer unwanted effects than other antimalarial agents. Artemisinin and derivatives are effective against multidrug-resistant *P. falciparum* in sub-Saharan Africa and, combined with mefloquine, against multidrug-resistant *P. falciparum* in South-East Asia.

ATAVOQUONE

Atavoquone is a hydroxynaphthoquinone drug used prophylactically to prevent malaria, and to treat cases resistant to other drugs. It acts primarily to inhibit the parasite's mitochondrial electron transport chain,

[4]Having been used for thousands of years in China as a herbal extract for treating 'fevers', the active compound artemisinin was isolated by Chinese chemists in 1972. This was ignored in the West for more than 10 years, until the WHO recognised its importance and, in 2002, placed it on the WHO 'essential drugs' list for malaria treatment. The herbs are noted for their extreme bitterness, and their name derives from *Artemisia*, wife and sister of the 4th-century king of Halicarnassus; her sorrow on his death led her to mix his ashes with whatever she drank to make it bitter.

possibly by mimicking the natural substrate ubiquinone. Atavoquone is usually used in combination with the antifolate drug proguanil, because they act synergistically. The mechanism underlying this synergism is not known, but it is specific for this particular pair of drugs, because other antifolate drugs or electron transport inhibitors have no such synergistic effect. When combined with proguanil, atavoquone is highly effective and well tolerated. Few unwanted effects of such combination treatment have been reported, but abdominal pain, nausea and vomiting can occur. Pregnant or breastfeeding women should not take atavoquone. Resistance to atavoquone alone is rapid and results from a single point mutation in the gene for cytochrome b. Resistance to combined treatment with atavoquone and proguanil is less common.

POTENTIAL NEW ANTIMALARIAL DRUGS

Malaria has been dubbed a 're-emerging disease' largely because of the ongoing development of resistant strains of the parasite. The quest for new drugs is urgent and there has been some progress in this area both in the search for new entities (see Muregi et al., 2012, and Tschan et al., 2012) as well as a better understanding of the pharmacokinetic aspects of current drugs (Na-Bangchang & Karbwang, 2009), enabling better treatment regimes. But perhaps the most significant advance has come through the application of synthetic biology to solve the problem of artemisinin production. Artemisinin is notoriously difficult to synthesise by conventional chemical techniques and awkward to harvest in large amounts. Using genetically modified yeast transfected with genes from *Artemisia* it has been possible to produce large amounts of the precursor *artemisinic acid*, which can be easily converted into artemisinin (Paddon et al., 2013). This breakthrough technique should relieve the desperate shortage of the drug.

The prospects for an effective malaria vaccine have increased dramatically over the last decade and some candidate vaccines are undergoing field trials. Discussion is beyond the scope of this chapter but the reader is referred to Schwarz et al. (2012) and Epstein and Richie (2013) for more information.

AMOEBIASIS AND AMOEBICIDAL DRUGS

The main organism in this group to concern us here is *Entamoeba histolytica*, the causative agent of amoebiasis, which may manifest as a severe colitis (dysentery) and, sometimes, liver abscesses.

▼ The infection is encountered around the world, but more often in warmer climates. Approximately 500 million people are thought to harbour the disease, with 40 000–100 000 deaths occurring each year as a result (Stanley, 2003). It is considered to be the second leading cause of death from parasitic diseases worldwide.

The organism has a simple life cycle, and humans are the chief hosts. Infection, generally spread by poor hygiene, follows the ingestion of the mature cysts in water or food that is contaminated with human faeces. The infectious cysts pass into the colon, where they develop into trophozoites. These motile organisms adhere to colonic epithelial cells, utilising a galactose-containing lectin on the host cell membrane. Here, the trophozoites feed, multiply, encyst and eventually pass out in the faeces, thus completing their life cycle. Some individuals are symptomless 'carriers' and harbour the parasite without developing overt disease, but cysts are present in their faeces and they can infect other individuals. The cysts can survive outside the body for at least a week in a moist and cool environment.

Antimalarial drugs

- **Chloroquine** is a blood schizonticide that is concentrated in the parasite and inhibits the haem polymerase. Orally active; half-life 50 h. *Unwanted effects*: gastrointestinal disturbances, dizziness and urticaria. Bolus intravenous injections can cause dysrhythmias. Resistance is now common.
- **Quinine** is a blood schizonticide. It may be given orally or intravenously; half-life 10 h. *Unwanted effects*: gastrointestinal tract disturbances, tinnitus, blurred vision and, in large doses, dysrhythmias and central nervous system disturbances. It is usually given in combination therapy with:
  - **pyrimethamine**, a folate antagonist that acts as a slow blood schizonticide (orally active; half-life 4 days), and either
  - **dapsone**, a sulfone (orally active; half-life 24–48 h), or
  - **sulfadoxine**, a long-acting sulfonamide (orally active; half-life 7–9 days).
- **Proguanil**, a folate antagonist, is a slow blood schizonticide with some action on the primary liver forms of *P. vivax*. Orally active; half-life 16 h.
- **Mefloquine** is a blood schizonticidal agent active against *P. falciparum* and *P. vivax*, and acts by inhibiting the parasite haem polymerase. Orally active; half-life 30 days. The onset of action is slow. *Unwanted effects*: gastrointestinal disturbances, neurotoxicity and psychiatric problems.
- **Primaquine** is effective against the liver hypnozoites and is also active against gametocytes. Orally active; half-life 36 h. *Unwanted effects*: gastrointestinal tract disturbances and, with large doses, methaemoglobinaemia. Erythrocyte haemolysis in individuals with genetic deficiency of glucose 6-phosphate dehydrogenase.
- **Artemisinin** derivatives are now widely used particularly in combination with other drugs such as **lumefantrine**. They are fast-acting blood schizonticidal agents that are effective against both *P. falciparum* and *P. vivax*.
- **Artesunate** is water-soluble and can be given orally or by intravenous, intramuscular or rectal administration. Side effects are rare. Resistance is so far uncommon.
- **Atavoquone** (in combination with **proguanil**) is used for prevention, and for the treatment of, acute uncomplicated *P. falciparum* malaria. The drug combination is effective orally. It is given at regular intervals over 3 to 4 days. *Unwanted effects*: diarrhoea, nausea and vomiting. Resistance to **atavoquone** develops rapidly if it is given alone.

The trophozoite lyses the colonic mucosal cells (hence 'histolytica') using proteases, *amoebapores* (peptides that form pores in cell membranes) or by inducing host cell apoptosis. The organism then invades the submucosa, where it secretes factors to modify the host response, which would otherwise prove lethal to the parasite. It is this process that produces the characteristic bloody diarrhoea and abdominal pain, although a chronic intestinal infection may be

present in the absence of dysentery. In some patients, an *amoebic granuloma* (amoeboma) may be present in the intestinal wall. The trophozoites may also migrate through the damaged intestinal tissue into the portal blood and hence the liver, giving rise to the most common extra-intestinal symptom of the disease – amoebic liver abscesses.

The use of drugs to treat this condition depends largely on the site and type of infection. The drugs of choice for the various forms of amoebiasis are:

- **metronidazole** (or **tinidazole**) followed by **diloxanide** for acute invasive intestinal amoebiasis resulting in acute severe amoebic dysentery
- diloxanide for chronic intestinal amoebiasis
- metronidazole followed by diloxanide for hepatic amoebiasis
- diloxanide for the carrier state.

These agents are often used in combination.

METRONIDAZOLE

Metronidazole kills the trophozoites of *E. histolytica* but has no effect on the cysts. It is the drug of choice for invasive amoebiasis of the intestine or the liver, but it is less effective against organisms in the lumen of the gut. Metronidazole is activated by anaerobic organisms to a compound that damages DNA, leading to parasite apoptosis.

Metronidazole is usually given orally and is rapidly and completely absorbed. Rectal and intravenous preparations are also available. It is distributed rapidly throughout the tissues, reaching high concentrations in the body fluids, including the cerebrospinal fluid. Some is metabolised, but most is excreted in urine.

Unwanted effects are mild. The drug has a metallic, bitter taste in the mouth but causes few unwanted effects in therapeutic doses. Minor gastrointestinal disturbances have been reported, as have CNS symptoms (dizziness, headache, sensory neuropathies). Metronidazole causes a disulfiram-like reaction to alcohol (see Ch. 49), which should be strictly avoided. Metronidazole should not be used in pregnancy.

Tinidazole is similar to metronidazole in its mechanism of action and unwanted effects, but is eliminated more slowly, having a half-life of 12–14 h.

DILOXANIDE

Diloxanide or, more commonly, an insoluble ester, diloxanide furoate, are the drugs of choice for the asymptomatic infected patient, and are often given as a follow-up after the disease has been reversed with metronidazole. Both drugs have a direct amoebicidal action, affecting the parasites before encystment. Diloxanide furoate is given orally, and acts without being absorbed. Unwanted gastrointestinal or other effects may be seen but it has an excellent safety profile.

Other drugs that are sometimes used include the antibiotic **paromomycin**.

Drugs used in amoebiasis

Amoebiasis is caused by infection with *E. histolytica*, which causes dysentery and liver abscesses. The organism may be present in motile invasive form or as a cyst. The main drugs are:

- **metronidazole** given orally (half-life 7 h). Active against the invasive form in gut and liver but not the cysts. Unwanted effects (rare); gastrointestinal disturbances and central nervous system symptoms. **Tinidazole** is similar.
- **diloxanide** is given orally with no serious unwanted effects. It is active, while unabsorbed, against the non-invasive form in the gastrointestinal tract.

TRYPANOSOMIASIS AND TRYPANOCIDAL DRUGS

Trypanosomes belong to the group of pathogenic flagellate protozoa. Two subtypes of *Trypanosoma brucei* (*rhodesiense* and *gambiense*) cause sleeping sickness in Africa (also called *HAT* – human African trypanosomiasis). In South America, another species *Trypanosoma cruzi*, causes *Chagas' disease* (also known as American trypanosomiasis). Almost eliminated by 1960, HAT has re-emerged. In 2009 WHO estimated about 30000 cases, with about 70 million people at risk of contracting sleeping sickness. The disease caused by *T. b. rhodesiense* is the more aggressive form. Civil unrest, famine and AIDS encourage the spread of the disease by reducing the chances of distributing medication or because patients are immunocompromised, but despite this the incidence appears to be dropping. Related trypanosome infections also pose a major risk to livestock and thus have a secondary impact on human health and well-being.

▼ The vector of HAT is the tsetse fly. In both types of disease, there is an initial local lesion at the site of entry, which may (in the case of *T. b. rhodesiense*) develop into a painful *chancre* (ulcer or sore). This is followed by bouts of parasitaemia and fever as the parasite enters the haemolymphatic system. The parasites and the toxins they release during the second phase of the disease cause organ damage. This manifests as 'sleeping sickness' when parasites reach the CNS causing somnolence and progressive neurological breakdown. Left untreated, such infections are fatal.

T. cruzi is spread through other blood-sucking insects, including the 'kissing bugs'. The initial phases of the infection are similar but parasites damage the heart, muscles and sometimes liver, spleen, bone and intestine. Many people harbour chronic infections but the cure rate is good if treatment begins immediately after infection.

The main drugs used for HAT are **suramin**, with **pentamidine** as an alternative, in the haemolymphatic stage of the disease, and the arsenical **melarsoprol** for the late stage with CNS involvement and **eflornithine** (see Burchmore et al., 2002; Burri & Brun, 2003). All have toxic side effects. **Nifurtimox**, eflornithine and **benznidazole** are used in Chagas' disease: however, there is no totally effective treatment for this form of trypanosomiasis.

SURAMIN

Suramin was introduced into the therapy of trypanosomiasis in 1920. The drug binds firmly to host plasma proteins, and the complex enters the trypanosome by endocytosis, then liberated by lysosomal proteases. It inhibits key parasite enzymes inducing gradual destruction of organelles, such that the organisms are cleared from the circulation after a short interval.

The drug is given by slow intravenous injection. The blood concentration drops rapidly during the first few hours and then more slowly over the succeeding days. A residual concentration remains for 3–4 months. Suramin

tends to accumulate in mononuclear phagocytes, and in the cells of the proximal tubule in the kidney.

Unwanted effects are common. Suramin is relatively toxic, particularly in a malnourished patient, the main organ affected being the kidney. Many other slowly developing adverse effects have been reported, including optic atrophy, adrenal insufficiency, skin rashes, haemolytic anaemia and agranulocytosis. A small proportion of individuals have an immediate idiosyncratic reaction to suramin injection that may include nausea, vomiting, shock, seizures and loss of consciousness.

PENTAMIDINE

Pentamidine has a direct trypanocidal action *in vitro*. It is rapidly taken up in the parasites by a high-affinity energy-dependent carrier and is thought to interact with their DNA. The drug is administered intravenously or by deep intramuscular injection, usually daily for 10–15 days. After absorption from the injection site, it binds strongly to tissues (especially the kidney) and is eliminated slowly, only 50% of a dose being excreted over 5 days. Fairly high concentrations of the drug persist in the kidney, the liver and the spleen for several months, but it does not penetrate the blood–brain barrier. It is also active in *Pneumocystis* pneumonia (Ch. 51). Its usefulness is limited by its unwanted effects – an immediate decrease in blood pressure, with tachycardia, breathlessness and vomiting, and later serious toxicity, such as kidney damage, hepatic impairment, blood dyscrasias and hypoglycaemia.

MELARPROSOL

▼ This is an organic arsenical compound that is used mainly when the CNS is involved. It is given intravenously and enters the CNS in high concentrations, where it is able to kill the parasite. It is a highly toxic drug that produces many unwanted effects including encephalopathy and, sometimes, immediate fatality. As such, it is only administered under strict supervision.

EFLORNITHINE

▼ Eflornithine inhibits the parasite ornithine decarboxylase enzyme. It shows good activity against *T. b. gambiense* and is used as a back-up for melarsoprol, although unfortunately it has limited activity against *T. b. rhodesiense*. Side effects are common and may be severe, but are readily reversed when treatment is discontinued.

There is an urgent need for new agents to treat trypanasome infections, partly because of the toxicity of existing drugs and partly because of developing drug resistance. There is some cause for optimism and new agents, and new treatment modalities, may be forthcoming in the medium term (Barrett, 2010; Brun et al., 2011).

OTHER PROTOZOAL INFECTIONS AND DRUGS USED TO TREAT THEM

LEISHMANIASIS

Leishmania organisms are flagellate protozoa and *leishmaniasis*, the infection that they cause, is spread by the sandfly. According to the WHO (2013 figures) the incidence of the disease is increasing with some 1.3 million new cases and 20 000–30 000 deaths recorded each year. With increasing international travel, leishmaniasis is being imported into new areas and opportunistic infections are now being reported (particularly in AIDS patients).

▼ The vector is the female sandfly. The parasite exists as a flagellated form (promastigote) in the gut of the infected insect, and a non-flagellated intracellular form (amastigote) in the mononuclear phagocytes of the infected mammalian host. Within these cells, the parasites thrive in modified phagolysosomes. By deploying an array of countermeasures (Singh et al., 2012), they promote the generation of Th2 cytokines and subvert the macrophage's microbiocidal systems to ensure their survival. The amastigotes multiply, and eventually the infected cell releases a new crop of parasites into the haemolymphatic system, where they can infect further macrophages and possibly other cells.

Different species of *Leishmania* exist in different geographical areas and cause distinctive clinical manifestations (see Table 54.1). Typical presentations include:

- a *cutaneous form*, which presents as an unpleasant chancre ('oriental sore', 'Chiclero's ulcer' and other names) that may heal spontaneously but can leave scarring. This is the most common form and is found in the Americas, some Mediterranean countries and parts of central Asia;
- a *mucocutaneous form* ('espundia' and other names), which presents as large ulcers of the mucous membranes of the mouth, nose and throat; most cases are seen in South America;
- a serious *visceral form* ('kala-azar' and other names), where the parasite spreads through the bloodstream and causes hepatomegaly, splenomegaly, anaemia and intermittent fever. This manifestation is encountered mainly in the Indian subcontinent and West Africa.

The main drugs used in visceral leishmaniasis are pentavalent antimony compounds such as **sodium stibogluconate** and pentamidine as well as **amphotericin** (see Ch. 53), which is sometimes used as a follow-up treatment. **Miltefosine**, an antitumour drug, is also used in some countries (not UK), as is **meglumine antimoniate**.

Sodium stibogluconate is given intramuscularly or by slow intravenous injection in a 10-day course. It is rapidly eliminated in the urine, 70% being excreted within 6 h. More than one course of treatment may be required.

Unwanted effects include anorexia, vomiting, bradycardia and hypotension. Coughing and substernal pain may occur during intravenous infusion. Reversible hepatitis and pancreatitis are common. The mechanism of action of sodium stibogluconate is not clear, but the drug may increase production of toxic oxygen free radicals in the parasite.

Miltefosine (hexadecylphosphocholine) is also effective in the treatment of both cutaneous and visceral leishmaniasis. The drug may be given orally and is well tolerated. Side effects are mild and include nausea and vomiting. *In vitro*, the drug induces DNA fragmentation and apoptosis in the parasites

Other drugs, such as antibiotics and antifungals, may be given concomitantly with the above agents. They may have some action on the parasite in their own right, but their main utility is to control the spread of secondary infections.

Resistance to current drugs, particularly the pentavalent antimonials (possibly caused by increased expression of an antimonial efflux pump), is a serious problem and there is no immediate prospect of a vaccine. The pharmacology of current drugs and prospects for new agents is reviewed by Singh et al. (2012).

TRICHOMONIASIS

The principal *Trichomonas* organism that produces disease in humans is *T. vaginalis*. Virulent strains cause inflammation of the vagina and sometimes of the urethra in males. The main drug used in therapy is metronidazole (Ch. 51), although resistance to this drug is on the increase.

High doses of tinidazole are also effective, with few side effects.

GIARDIASIS

Giardia lamblia colonises the upper gastrointestinal tract in its trophozoite form, and the cysts pass out in the faeces. Infection is then spread by ingestion of food or water contaminated with faecal matter containing the cysts. It is encountered worldwide, and epidemics caused by bad sanitation are not uncommon. Metronidazole is the drug of choice, and treatment is usually very effective. Tinidazole or **mepacrine** may be used as an alternative.

TOXOPLASMOSIS

The cat is the definitive host of *Toxoplasma gondii*, a pathogenic member of this group of organisms (i.e. it is the only host in which the sexual cycle can occur). It expels the infectious cysts in its faeces; humans can inadvertently become intermediate hosts, harbouring the asexual form of the parasite. Ingested oocysts develop into sporozoites, then to trophozoites, and finally encyst in the tissues. In most individuals, the disease is asymptomatic or self-limiting, although intrauterine infections can severely damage the developing fetus and it may cause fatal generalised infection in immunosuppressed patients or those with AIDS, in whom toxoplasmic encephalitis may occur. In humans, *T. gondii* infects numerous cell types and has a highly virulent replicative stage.

The treatment of choice is pyrimethamine–sulfadiazine (to be avoided in pregnant patients); trimethoprim–**sulfamethoxazole** (co-trimoxazole, see Ch. 51) or combinations of pyrimethamine with **clindamycin**, **clarithromycin** or **azithromycin** (see Ch. 51) have shown promise.

PNEUMOCYSTIS

First recognised in 1909, *Pneumocystis carinii* (now known as *P. jirovecii*; see also Ch. 53) shares structural features with both protozoa and fungi, leaving its precise classification uncertain. Previously considered to be a widely distributed but largely innocuous microorganism, it is now recognised as an important cause of opportunistic infections in immunocompromised patients. It is common in AIDS, where *P. carinii* pneumonia is often the presenting symptom as well as a leading cause of death.

High-dose **co-trimoxazole** (Ch. 50) is the drug of choice in serious cases, with parenteral pentamidine as an alternative. Treatment of milder forms of the disease (or prophylaxis) can be effected with atovaquone, trimethoprim–dapsone, or clindamycin–primaquine combinations.

FUTURE DEVELOPMENTS

This field is a huge global challenge, with each species posing its own distinct problems to the would-be designer of new antiprotozoal drugs.

Transnational initiatives (e.g. Medicines for Malaria Venture and Institute for OneWorld Health) are now major players in the development of new medicines for protozoal diseases. But it is not simply a lack of new drugs that is the problem: for economic reasons, the countries and populations most affected often lack an efficient infrastructure for the distribution and safe administration of the drugs that we already possess. Cultural attitudes, civil wars, famine, the circulation of counterfeit or defective drugs, drought and natural disasters also exacerbate this problem.

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Pneumocystis pneumonia

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Useful Web resources

<http://malaria.who.int/> (*The WHO home page containing the major information on malaria – a terrific starting point for further investigation. Other who.int sites cover trypanosomiasis, leishmaniasis and other important protozoal diseases*)

<www.mmv.org/> (*The Web page of the Medicines for Malaria Venture, a private–public partnership established to bring together funding and expertise from a number of sources to tackle malaria*)

<www.oneworldhealth.org> (*The Web page of the visionary 'non-profit pharmaceutical company', with details of their current programmes dealing with global health issues*)

55 Anthelmintic drugs

OVERVIEW

Some 2 billion people around the world suffer from *helminthiasis* – infection with various species of parasitic *helminths* (worms). Inhabitants of tropical or subtropical low-income countries are most at risk; children often become infected at birth and may remain so throughout their lives and polyparasitaemia is common. Helminthiasis is often co-endemic with malaria, TB and HIV/AIDS, adding to the disease burden as well as interfering with vaccination campaigns. The clinical consequences of helminthiasis vary: for example, threadworm infections mainly cause discomfort but infection with *schistosomiasis* (*bilharzia*) or hookworm is associated with serious morbidity. Worm infections are an even greater cause for concern in veterinary medicine, affecting both domestic pets and farm animals. In some parts of the world, *fascioliasis* is associated with significant loss of livestock. Because of its prevalence and economic significance, the pharmacological treatment of helminthiasis is therefore of great practical therapeutic importance.

HELMINTH INFECTIONS

The helminths comprise two major groups: the *nemathelminths* (nematodes, roundworms) and the *platyhelminths* (flatworms). The latter group is subdivided into the *trematodes* (flukes) and the *cestodes* (tapeworms). Almost 350 species of helminths have been found in humans, and most colonise the gastrointestinal tract. The global range and occurrence of helminthiasis has been reviewed by Lustigman et al. (2012).

Helminths have a complex life cycle, often involving several host species. Infection may occur in many ways, with poor hygiene a major contributory factor. Many enter by the mouth in unpurified drinking water or in undercooked flesh from infected animals or fish. However, species can enter through the skin following a cut, an insect bite or even after swimming or walking on infected soil. Humans are generally the *primary* (or *definitive*) host for helminth infections, in the sense that they harbour the sexually mature reproductive form. Eggs or larvae then pass out of the body and infect the *secondary* (*intermediate*) host. In some cases, the eggs or larvae may persist in the human host and become *encysted*, covered with granulation tissue, giving rise to *cysticercosis*. Encysted larvae may lodge in the muscles and viscera or, more seriously, in the eye or the brain.

Approximately 20 helminth species are considered to be clinically significant and these fall into two main categories – those in which the worm lives in the host's alimentary canal, and those in which the worm lives in other tissues of the host's body.

The main examples of intestinal worms are:

- *Tapeworms*: *Taenia saginata*, *Taenia solium*, *Hymenolepis nana* and *Diphyllobothrium latum*. Some 85 million people in Asia, Africa and parts of America harbour one or other of these tapeworm species. Only the first two are likely to be seen in the UK. Cattle and pigs are the usual intermediate hosts of the most common tapeworms (*T. saginata* and *T. solium*). Humans become infected by eating raw or undercooked meat containing the larvae, which have encysted in the animals' muscle tissue. *H. nana* may exist as both the adult (the intestinal worm) and the larval stage in the same host, which may be human or rodent, although some insects (fleas, grain beetles) can also serve as intermediate hosts. The infection is usually asymptomatic. *D. latum* has two sequential intermediate hosts: a freshwater crustacean and a freshwater fish. Humans become infected by eating raw or incompletely cooked fish containing the larvae.
- *Intestinal roundworms*: *Ascaris lumbricoides* (common roundworm), *Enterobius vermicularis* (threadworm, called pinworm in the USA), *Trichuris trichiura* (whipworm), *Strongyloides stercoralis* (threadworm in the USA), *Necator americanus* and *Ancylostoma duodenale* (hookworms). Again, undercooked meat or contaminated food is an important cause of infection by roundworm, threadworm and whipworm, whereas hookworm is generally acquired when their larvae penetrate the skin. Intestinal blood loss is a common cause of anaemia in regions where hookworm is endemic.

The main examples of worms that live elsewhere in host tissues are:

- *Flukes*: *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum*. These cause schistosomiasis (bilharzia). The adult worms of both sexes live and mate in the veins or venules of the bladder or the gut wall. The female lays eggs that pass into the bladder or gut triggering inflammation in these organs. This results in haematuria in the former case and, occasionally, loss of blood in the faeces in the latter. The eggs hatch in water after discharge from the body and thus enter the secondary host – a particular species of snail. After a period of development in this host, free-swimming *cercariae* emerge. These are capable of infecting humans by penetration of the skin. About 200 million people are infected with one or other of the schistosomes.
- *Tissue roundworms*: *Trichinella spiralis*, *Dracunculus medinensis* (*guinea worm*) and the *filariae*, which include *Wuchereria bancrofti*, *Loa loa*, *Onchocerca volvulus* and *Brugia malayi*. The adult filariae live in

the lymphatics, connective tissues or mesentery of the host and produce live embryos or *microfilariae*, which find their way into the bloodstream and may be ingested by mosquitoes or other biting insects. After a period of development within this secondary host, the larvae pass to the mouth parts of the insect and thus infect the next victim. Major filarial diseases are caused by *Wuchereria* or *Brugia*, which cause obstruction of lymphatic vessels, producing *elephantiasis* – hugely swollen legs. Other related diseases are *onchocerciasis* (in which the presence of microfilariae in the eye causes 'river blindness' – a leading preventable cause of blindness in Africa and Latin America) and *loiasis* (in which the microfilariae cause inflammation in the skin and other tissues). *Trichinella spiralis* causes trichinosis; the larvae from the viviparous female worms in the intestine migrate to skeletal muscle, where they become encysted. In *guinea worm disease*,[1] larvae of *D. medinensis* released from crustaceans in wells and waterholes are ingested and migrate from the intestinal tract to mature and mate in the tissues; the gravid female then migrates to the subcutaneous tissues of the leg or the foot, and may protrude through an ulcer in the skin. The worm may be up to a metre in length and has to be removed surgically or by slow mechanical winding of the worm on to a stick over a period of days.

- *Hydatid tapeworm*. These are cestodes of the *Echinococcus* species for which dogs are the primary hosts, and sheep the intermediate hosts. The primary, intestinal stage does not occur in humans, but under certain circumstances humans can function as the intermediate host, in which case the larvae develop into *hydatid cysts* within the tissues, sometimes with fatal consequences.

Some nematodes that generally live in the gastrointestinal tract of animals may infect humans and penetrate tissues. A skin infestation, termed *creeping eruption* or *cutaneous larva migrans*, is caused by the larvae of dog and cat hookworms which often enter through the foot. Visceral larva migrans is caused by larvae of cat and dog roundworms of the *Toxocara* genus.

ANTHELMINTIC DRUGS

The first effective anthelmintic drugs were discovered in the 20th century and incorporated toxic metals such as arsenic (*atoxyl*) or antimony (*tartar emetic*). They were used to treat trypanosome and schistosome infestations.

Current anthelmintic drugs act by paralysing the parasite (e.g. by preventing muscular contraction), by damaging the worm such that the host immune system can eliminate it, or by altering parasite metabolism (e.g. by affecting microtubule function). Because the metabolic requirements of these parasites vary greatly from one species to another, drugs that are highly effective against one type of worm may be ineffective against others. To bring about its action, the drug must penetrate the tough exterior cuticle of the worm or gain access to its alimentary tract. This may present difficulties, because some worms are exclusively *haemophagous* ('blood-eating'), while others are best described as 'tissue grazers'. A further complication is that many helminths possess active drug efflux pumps that reduce the concentration of the drug in the parasite. The route of administration and dose of anthelmintic drugs are therefore important. In a reversal of the normal order of things, several anthelmintic drugs used in human medicine were originally developed for veterinary use.

Some individual anthelmintic drugs are described briefly below and indications for their use are given in Table 55.1. Several of these drugs (e.g. **albendazole**, **ivermectin**, **levamisole**) are unlicensed in the UK and used on a 'named patient' basis.[2]

BENZIMIDAZOLES

This group includes **mebendazole**, **tiabendazole** and **albendazole,** which are widely used broad-spectrum anthelmintics. They are thought to act by inhibiting the polymerisation of helminth β-tubulin, thus interfering with microtubule-dependent functions such as glucose uptake. They have a selective inhibitory action, being 250–400 times more effective in producing this effect in helminth, than in mammalian, tissue. However, the effect takes time to develop and the worms may not be expelled for several days. Cure rates are generally between 60% and 100% with most parasites.

Only 10% of mebendazole is absorbed after oral administration, but a fatty meal increases absorption. It is rapidly metabolised, the products being excreted in the urine and the bile within 24–48 h. It is generally given as a single dose for threadworm, and twice daily for 3 days for hookworm and roundworm infestations. Tiabendazole is rapidly absorbed from the gastrointestinal tract, very rapidly metabolised and excreted in the urine in conjugated form. It may be given twice daily for 3 days for guinea worm and *Strongyloides* infestations, and for up to 5 days for hookworm and roundworm infestations. Albendazole is also poorly absorbed but, as with mebendazole, absorption is increased by food, especially fats. It is metabolised extensively by presystemic metabolism to sulfoxide and sulfone metabolites. The former is likely to be the pharmacologically active species.

Unwanted effects are few with albendazole or mebendazole, although gastrointestinal disturbances can occasionally occur. Unwanted effects with tiabendazole are more frequent but usually transient, the commonest being gastrointestinal disturbances, although headache, dizziness and drowsiness have been reported and allergic reactions (fever, rashes) may also occur. Mebendazole is considered unsuitable for pregnant women or children less than 2 years old.

PRAZIQUANTEL

Praziquantel is a highly effective broad-spectrum anthelmintic drug that was introduced over 20 years ago. It is the drug of choice for all forms of schistosomiasis and is the agent generally used in large-scale schistosome eradication programmes. It is also effective in cysticercosis. The drug affects not only the adult schistosomes

[1]Now, happily, eliminated from many parts of the world.

[2]A situation in which the physician seeks access to an unlicensed drug from a pharmaceutical company to use in a named individual. The drug is either a 'newcomer' that has shown particular promise in clinical trials but has not yet been licensed or, as in these instances, an established drug that has not been licensed because the company has not applied for a product license for this indication (possibly for commercial reasons).

Table 55.1 Principal drugs used in helminth infections and some common indications

	Helminth	Principal drug(s) used
Threadworm (pinworm)	*Enterobius vermicularis*	Mebendazole, piperazine
	Strongyloides stercoralis (threadworm in the USA)	Albendazole
Common roundworm	*Ascaris lumbricoides*	Levamisole, mebendazole, piperazine
Other roundworm (filariae)	Lymphatic filariasis 'elephantiasis'. (*Wuchereria bancrofti*, *Brugia malayi*)	Diethylcarbamazine, ivermectin
	Subcutaneous filariasis 'eyeworm' (*Loa loa*)	Diethylcarbamazine
	Onchocerciasis 'river blindness' (*Onchocerca volvulus*)	Ivermectin
	Guinea worm (*Dracunculus medinensis*)	Praziquantel, mebendazole
	Trichiniasis (*Trichinella spiralis*)	Tiabendazole, mebendazole
	Cysticercosis (infection with larval *Taenia solium*)	Praziquantel, albendazole
	Tapeworm (*Taenia saginata*, *Taenia solium*)	Praziquantel, niclosamide
	Hydatid disease (*Echinococcus granulosus*)	Albendazole
	Hookworm (*Ancylostoma duodenale*, *Necator americanus*)	Mebendazole, albendazole
	Whipworm (*Trichuris trichiura*)	Mebendazole, albendazole, diethylcarbamazine
Blood flukes (*Schistosoma* spp.)	Bilharziasis: *S. haematobium*, *S. mansoni*, *S. japonicum*	Praziquantel
Cutaneous larva migrans	*Ancylostoma caninum*	Albendazole, tiabendazole, ivermectin
Visceral larva migrans	*Toxocara canis*	Albendazole, tiabendazole, diethylcarbamazine

but also the immature forms and the cercariae – the form of the parasite that infects humans by penetrating the skin.

It disrupts Ca^{2+} homeostasis in the parasite by binding to consensus protein kinase C-binding sites in a β subunit of schistosome voltage-gated calcium channels (Greenberg, 2005). This induces an influx of Ca^{2+}, a rapid and prolonged contraction of the musculature, and eventual paralysis and death of the worm. Praziquantel also disrupts the tegument of the parasite, unmasking novel antigens, and as a result it may become more susceptible to the host's normal immune responses.

Given orally, praziquantel is well absorbed; much of the drug is rapidly metabolised to inactive metabolites on first passage through the liver, and the metabolites are excreted in the urine. The plasma half-life of the parent compound is 60–90 min.

Praziquantel has minimal side effects in therapeutic dosage. Such unwanted effects as do occur are usually transitory and rarely of clinical importance. Effects may be more marked in patients with a heavy worm load because of products released from the dead worms. Praziquantel is considered safe for pregnant and lactating women, an important property for a drug that is commonly used in national disease control programmes. Some resistance has developed to the drug.

PIPERAZINE

Piperazine can be used to treat infections with the common roundworm (*A. lumbricoides*) and the threadworm (*E. vermicularis*). It reversibly inhibits neuromuscular transmission in the worm, probably by mimicking GABA (Ch. 38), at GABA-gated chloride channels in nematode muscle. The paralysed worms are expelled alive by normal intestinal peristaltic movements. It is administered with a stimulant laxative such as **senna** (Ch. 30) to facilitate expulsion of the worms.

Piperazine is given orally and some, but not all, is absorbed. It is partly metabolised, and the remainder is eliminated, unchanged, via the kidney. The drug has little pharmacological action in the host. When used to treat roundworm, piperazine is effective in a single dose. For threadworm, a longer course (7 days) at lower dosage is necessary.

Unwanted effects may include gastrointestinal disturbances, urticaria and bronchospasm. Some patients experience dizziness, paraesthesias, vertigo and incoordination. The drug should not be given to pregnant patients or to those with compromised renal or hepatic function.

NICLOSAMIDE

Niclosamide is widely used for the treatment of tapeworm infections together with praziquantel. The *scolex* (the head of the worm that attaches to the host intestine) and a proximal segment are irreversibly damaged by the drug, such that the worm separates from the intestinal wall and is expelled. For *T. solium*, the drug is given in a single dose after a light meal, usually followed by a purgative 2 h later in case the damaged tapeworm segments release ova, which are not affected by the drug. For other tapeworm infections, this precaution is not necessary. There is negligible absorption of the drug from the gastrointestinal tract.

Unwanted effects: nausea, vomiting, pruritus and lightheadedness may occur but generally such effects are few, infrequent and transient.

DIETHYLCARBAMAZINE

Diethylcarbamazine is a piperazine derivative that is active in filarial infections caused by *B. malayi*, *W. bancrofti* and *L. loa*. Diethylcarbamazine rapidly removes the microfilariae from the blood circulation and has a limited effect on the adult worms in the lymphatics, but it has little action on microfilariae *in vitro*. It may act by changing the parasite such that it becomes susceptible to the host's normal immune responses. It may also interfere with helminth arachidonate metabolism.

The drug is absorbed following oral administration and is distributed throughout the cells and tissues of the body, excepting adipose tissue. It is partly metabolised, and both the parent drug and its metabolites are excreted in the urine, being cleared from the body within about 48 h.

Unwanted effects are common but transient, subsiding within a day or so even if the drug is continued. Side effects from the drug itself include gastrointestinal disturbances, joint pain, headache and a general feeling of weakness. Allergic side effects referable to the products of the dying filariae are common and vary with the species of worm. In general, these start during the first day's treatment and last 3–7 days; they include skin reactions, enlargement of lymph glands, dizziness, tachycardia, and gastrointestinal and respiratory disturbances. When these symptoms disappear, larger doses of the drug can be given without further problem. The drug is not used in patients with onchocerciasis, in whom it can have serious unwanted effects.

LEVAMISOLE

Levamisole is effective in infections with the common roundworm (*A. lumbricoides*). It has a nicotine-like action (Ch. 13), stimulating and subsequently blocking the neuromuscular junctions. The paralysed worms are then expelled in the faeces. Ova are not killed. The drug is given orally, is rapidly absorbed and is widely distributed. It crosses the blood–brain barrier. It is metabolised in the liver to inactive metabolites, which are excreted via the kidney. Its plasma half-life is 4 h. It has immunomodulatory effects and has in the past been used to treat various solid tumours.

It can cause gastrointestinal disturbance but also more serious effects, notably agranulocytosis, and it has been withdrawn from North American markets..

IVERMECTIN

First introduced in 1981 as a veterinary drug, **ivermectin** is a safe and highly effective broad-spectrum antiparasitic in humans. It is frequently used in global public health campaigns,[3] and is the first choice of drug for the treatment of many filarial infections. It has also given good results against *W. bancrofti*, which causes elephantiasis. A single dose kills the immature microfilariae of *O. volvulus* but not the adult worms. Ivermectin is also the drug of choice for onchocerciasis, which causes river blindness and reduces the incidence of this disease by up to 80%. It is also active against some roundworms: common roundworms, whipworms, and threadworms of both the UK (*E. vermicularis*) and the US variety (*S. stercoralis*), but not hookworms.

Chemically, ivermectin is a semisynthetic agent derived from a group of natural substances, the *avermectins*, obtained from an actinomycete organism. The drug is given orally and has a half-life of 11 h. It is thought to kill the worm either by opening glutamate-gated chloride channels (found only in invertebrates) and increasing Cl^- conductance; by binding to a novel allosteric site on the acetylcholine nicotinic receptor to cause an increase in transmission, leading to motor paralysis; or by binding to GABA receptors.

Unwanted effects include skin rashes and itching but in general the drug is very well tolerated. One interesting exception in veterinary medicine is the central nervous system (CNS) toxicity seen in Collie dogs.[4]

RESISTANCE TO ANTHELMINTIC DRUGS

Resistance to anthelmintic drugs is a widespread and growing problem affecting not only humans but also the animal health market. During the 1990s, helminth infections in sheep (and, to a lesser extent, cattle) developed varying degrees of resistance to a number of different drugs. Parasites that develop such resistance pass this ability on to their offspring, leading to treatment failure. The widespread use of anthelmintic agents in farming has been blamed for the spread of resistant species.

There are probably several molecular mechanisms that contribute to drug resistance. The presence of the P-glycoprotein transporter (Ch. 9) in some species of nematode has already been mentioned, and agents such as **verapamil** that block the transporter in trypanosomes can partially reverse resistance to the benzimidazoles. However, some aspects of benzimidazole resistance may be attributed to alterations in their high-affinity binding to parasite β-tubulin. Likewise, resistance to levamisole is associated with changes in the structure of the target acetylcholine nicotinic receptor.

Of great significance is the way in which helminths evade the host's immune system. Even though they may reside in immunologically exposed sites such as the lymphatics or the bloodstream, many are long-lived and may co-exist with their hosts for many years without seriously affecting their health, or in some cases without even being noticed. It is striking that the two major families of helminths, while evolving separately, deploy similar strategies to evade destruction by the immune system. Clearly, this must be of major survival value for the species.

▼ It appears that many helminths can actually exploit this mechanism by steering the immune system away from a local Th1 response (see Ch. 6), which would be potentially more damaging to the parasite, and promoting instead a modified systemic Th2 type of response. This is associated with the production of 'anti-inflammatory' cytokines such as interleukin-10 favourable to, or at least better tolerated by, the parasites. The immunology underlying this is complex (see Pearce & MacDonald, 2002; Maizels et al., 2004; Harris, 2011).

Ironically, the ability of helminths to modify the host immune response in this way may confer some survival value on the hosts themselves. For example, in addition to the local anti-inflammatory effect exerted by helminth infections, rapid wound healing is also seen. Clearly, this is of advantage to parasites that must penetrate tissues without killing the host, but may also be beneficial to the host as well. It has been proposed that helminth infections may

[3]Ivermectin is supplied by the manufacturers free of charge in countries where river blindness is endemic. Because the worms develop slowly, a single annual dose of ivermectin is sufficient to prevent the disease.

[4]A multi-drug-resistance (MDR) gene (see Chs 3 and 51) coding for a transporter that expels ivermectins from the CNS, is mutated to an inactive form in Collie dogs.

mitigate some forms of malaria and other diseases, possibly conferring survival advantages in populations where these diseases are endemic. Indeed, ingestion of helminths by patients has been evaluated as an (admittedly unappealing) strategy to induce remission of Crohn's disease (see Ch. 30; Hunter & McKay, 2004; Reddy & Fried, 2007). On the negative side, helminth infections may undermine the efficacy of tuberculosis vaccination programmes that depend upon a vigorous Th1 response (Elias et al., 2006).

On the basis that Th2 responses reciprocally inhibit the development of Th1 diseases, it has also been hypothesised that the comparative absence of Crohn's disease, as well as some other autoimmune diseases, in the developing world may be associated with the high incidence of parasite infection, and that the rise of these disorders in the West is associated with superior sanitation and reduced helminth infection! This type of argument is generally known as the 'hygiene hypothesis'.

VACCINES AND OTHER NOVEL APPROACHES

Despite the enormity of the clinical problem, there are few novel anthelmintic drugs in development. New candidates such as **tribendimidine** are being assessed in a range of human infections and some new veterinary drugs (e.g. **derquantel**) also tested in humans (see Prichard et al., 2012).

The sequencing of the genomes of several helminths may facilitate the creation of a transgenic species that expresses mutations found in resistant parasitic worms, thus providing insights into the mechanisms underlying resistance. Such databases may also reveal new drug targets, as well as opening the way for other types of anthelmintic agent, such as those based on antisense DNA or small interfering RNA.

Ambitious research agendas have been published enumerating the steps that would be required to eliminate helminth infections (see Boatin et al., 2012, for example) and vaccines are often prominent on the list of essential objectives. Efficacious helminth vaccines would have major benefits. Protein antigens on the surface of the (highly infectious) larval stage have been cloned and used as immunogens, and considerable success has also been achieved in the veterinary field with vaccines to organisms such as *T. ovis* and *E. granulosus* (in sheep) as well as *T. saginata* (in cattle) and *T. solium* (in pigs), with cure rates of 90–100% often reported (see Dalton & Mulcahy, 2001; Garcia, 2007). Qualified success has also been obtained with vaccines to other helminth species (see Capron et al., 2005; McManus & Loukas, 2008). Looking further ahead, it may be possible to develop DNA vaccines rather than protein immunogens to control these organisms.

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Anthelmintic drugs

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Anthelmintic vaccines

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