51 Antiviral drugs

OVERVIEW

This chapter deals with drugs used to treat infections caused by viruses. We give first some necessary information about viruses: a simple outline of virus structure, a list of the main pathogenic viruses 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 describe the various types of antiviral drugs and their mechanisms of action, with particular reference to the treatment of 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. 51.1). The viral coat, together with the nucleic acid core, is termed the *nucleocapsid*. Some viruses have, in addition, 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 commonly cause disease in humans.

▼ Some important examples of the diseases they cause are as follow:

- *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 (acquired immunodeficiency syndrome [AIDS], T-cell leukaemia), arenaviruses (meningitis, Lassa fever), hepadnaviruses (serum hepatitis) and arboviruses (arthropod-borne encephalitis and various febrile illnesses, e.g. 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*, interacting 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 of host cell receptors utilised by particular viruses are listed in Table 51.1.

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 bypass this route. Once in the host cell, the nucleic acid of the virus 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 in 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 in 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. With these viruses, the host cell nucleus is usually not involved in viral replication, although some RNA viruses (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 integrated into the genome of the host cell, and it is then

[1] A virus that can synthesise DNA from an RNA template—the reverse of the normal situation.

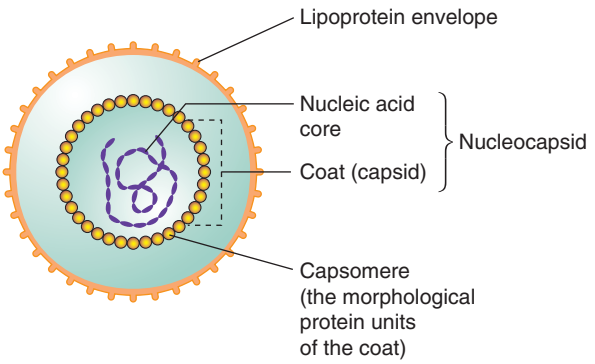

Fig. 51.1 Schematic diagram of the components of a virus particle or virion.

Table 51.1 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

[a]For more detail on complement, interleukin-2, the CD4 glycoprotein on helper T lymphocytes, MHC molecules, etc., see Chapter 6. For SDF-1, see Chapter 25.
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.

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 the *varicella zoster* (chickenpox and shingles) virus, which recur when viral replication is reactivated by some factor (or when the immune system is compromised in some way). Some RNA retroviruses can transform normal cells into malignant cells.

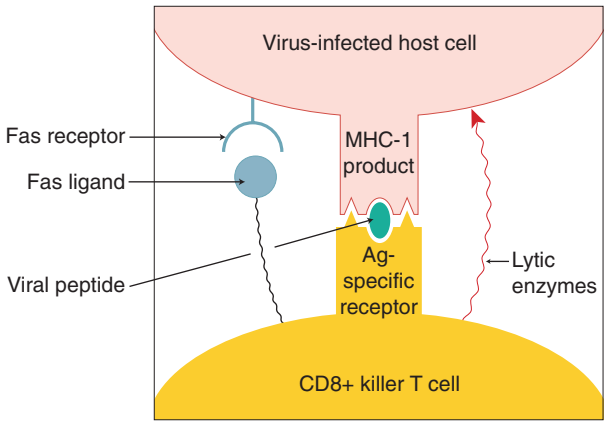

Fig. 51.2 The mechanisms whereby a CD8⁺ T cell kills a virus-infected host cell. The virus-infected host cell expresses a complex of virus peptides plus major histocompatibility complex class I product (MHC-I) on its surface. This is recognised by the CD8⁺ T cell, which then releases lytic enzymes into the virus-infected cell and also expresses a Fas ligand. This triggers apoptosis in the infected cell by stimulating its Fas 'death receptor'.

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, on its surface, viral peptides complexed with major histocompatibility complex (MHC) class I molecules. This complex is recognised by T lymphocytes, which then kill the infected cell (Fig. 51.2). This 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'). The latter may also be triggered indirectly through the release of a cytokine such as tumour necrosis factor (TNF)-α. If the virus escapes immune detection by cytotoxic lymphocytes by modifying the expression of the peptide–MHC complex (see below), it may still fall victim to natural killer (NK) cells. This reaction to the absence of normal MHC molecules might be called the 'mother turkey' strategy (kill everything that does not sound exactly like a baby turkey). But some viruses also have a device for evading NK cells as well (see below).

Within the cell itself, *gene silencing* may also provide 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, thus interrupting the replication cycle. 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).

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).

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. Following infection, for example, 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 the following:

- *Interference with the surface protein markers on the infected cells essential for killer cell attack.* Some viruses inhibit generation of the antigenic peptide and/or the presentation of MHC–peptide molecules. This turns off the signal that the cells are infected, enabling the viruses to 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 evolution 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 of this group: the HIV virus. This is appropriate because 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 in that it does involve the host cell nucleus).
- *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.

▼ The global situation is improving but even so, in 2007, the World Health Organization estimated that almost 33 million people were living with AIDS, and that women and children constituted approximately half that total number. During the same year, some 2 million people died of the disease (including 0.27 million children under 15 years), and there were a further 2.7 million new cases of AIDS infection reported. 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 20 times greater than in Europe. For a review of the pathogenesis of AIDS, see Mindel & Tenant-Flowers (2001).

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. 51.2). The lethal event is lysis of the target cell, but induction of apoptosis by interaction of Fas ligand (see 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 it is the progressive loss of these cells that is the defining characteristic of HIV infection (see Fig. 51.4). Recent work suggests that $CD4^+$ cells may themselves have a direct role (e.g. lysis of target cells) in the control of HIV replication (Norris et al., 2004).

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 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 the CTLs have become 'exhausted' and dysfunctional, thus losing 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 [**r**egulated on **a**ctivation **n**ormal **T**-cell **e**xpressed and **s**ecreted]). $CD4^+$ 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 51.3 shows an HIV virion infecting a $CD4^+$ T cell. Infected activated CD4 T 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 (see below).

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. 51.3).

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. 51.3). 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 error prone, and there are a large number of mutations 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 51.4. 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, the *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 damage 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.

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 delayed 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 available, fall into a few groups with similar mechanisms of action and side effects. Table 51.2 shows the commonest antiviral drugs, classified according to their mechanisms of action, some of the diseases they are used to treat and common side effects.

REVERSE TRANSCRIPTASE INHIBITORS

The main group are *nucleoside analogues*, typified by **zidovudine**, all of which are phosphorylated by host cell enzymes to give the 5′-trisphosphate derivative. 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).

Zidovudine

Zidovudine (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 to the parturient mother and then to the newborn infant, it can reduce mother-to-baby transmission by more than 20%. It is generally

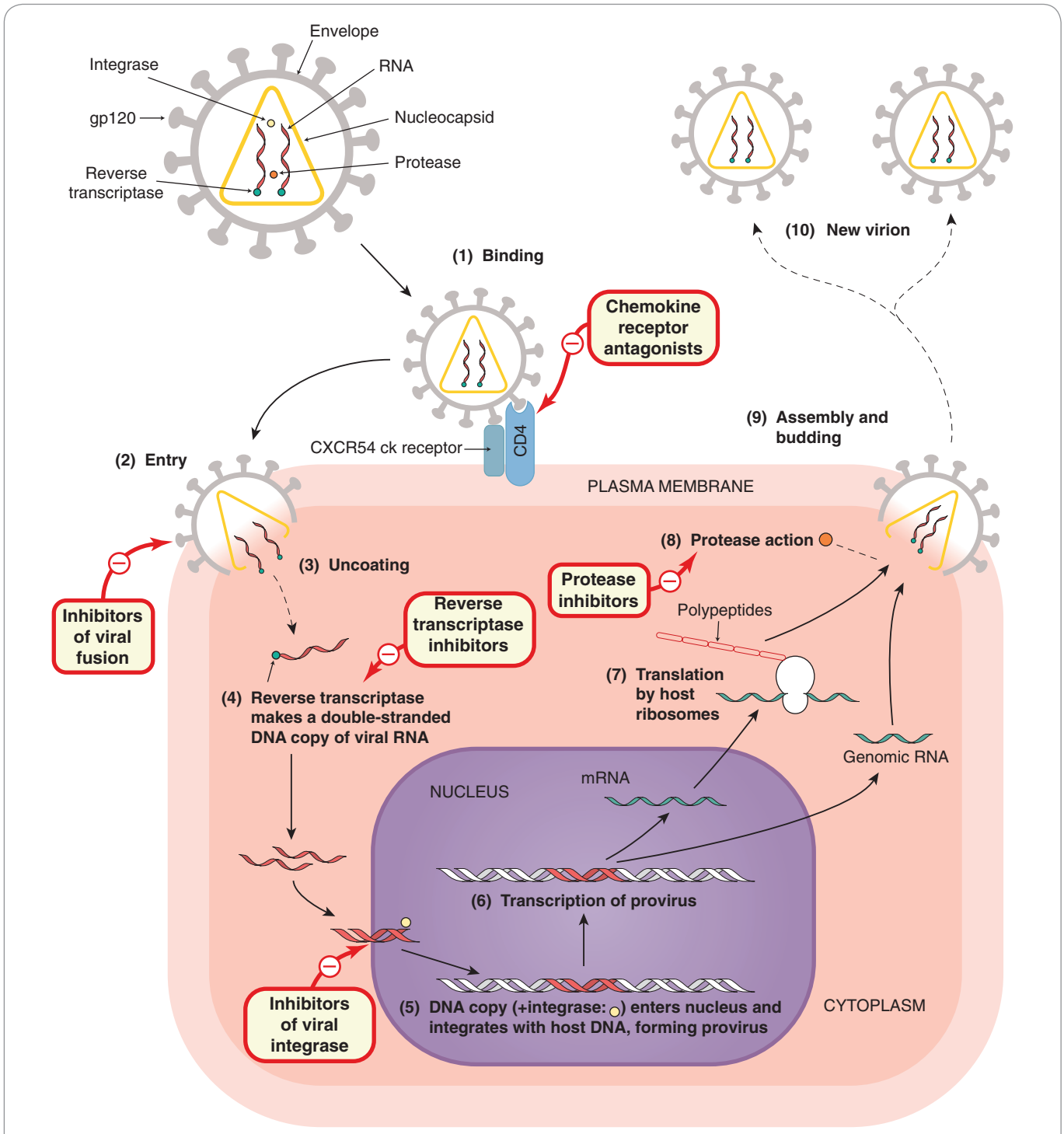

Fig. 51.3 Schematic diagram of infection of a CD4⁺ T cell by an HIV virion, with the sites of action of the two main classes of anti-HIV drugs. The 10 steps of HIV infection, from attachment to the cell to release of new virions, are shown. The virus uses the CD4 co-receptor and the chemokine (ck) receptors 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.

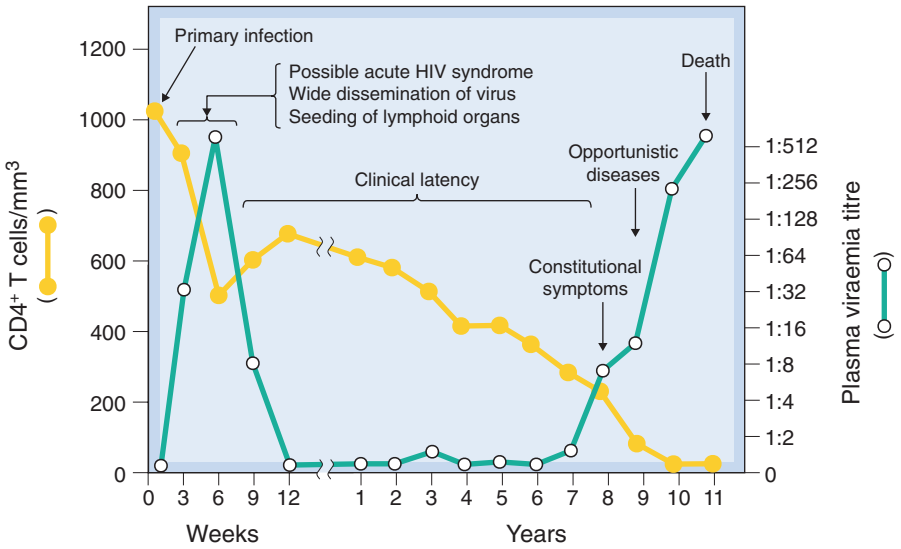

Fig. 51.4 Schematic outline of the course of HIV infection. The CD4+ T-cell titre is often expressed as cells/mm^3. (Adapted from Pantaleo et al. 1993 N Engl J Med 328: 327–335.)

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. Chemically, zidovudine is an analogue of thymidine. 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 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, 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 are **nevirapine** and **efavirenz**.

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 if given to the parturient mother and the neonate.

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 also teratogenic if used in early pregnancy.

Unwanted effects common to both of these drugs include rash (common) as well as a cluster of other effects (see Table 51.2).

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. 51.3). 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 51.2 and are exemplified by drugs such as **amprenavir**, **atazanavir**, **darunavir**, **fosamprenavir** (prodrug of amprenavir), **indinavir**, **lopinavir**, **nelfinavir**, **ritonavir**, **saquinavir** and **timpranavir**.

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.

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

Table 51.2 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 Lamivudine is also used in the treatment of hepatitis B	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 dipivoxil, entecavir, telbivudine	Hepatitis B	
Non-nucleoside reverse transcriptase inhibitors	Efavirenz, nevirapine	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	Amprenavir, atazanavir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, 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
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.

neutropenia) and CNS effects (e.g. insomnia, dizziness, headache) as well as the risk of hyperglycaemia.

DNA POLYMERASE INHIBITORS

Aciclovir

The era of effective selective antiviral therapy began with **aciclovir**, a guanosine derivative that is typical of drugs of this type.

Aciclovir 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 adequately 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 herpesvirus enzyme than the host enzyme. Aciclovir trisphosphate is fairly rapidly broken down 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.

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.

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 including **cidofovir**, **famciclovir** (prodrug of penciclovir), **ganciclovir**, **idoxuridine**, **penciclovir**, **valaciclovir** (prodrug of aciclovir) and **valganciclovir** (prodrug of ganciclovir). **Foscarnet** achieves the same effect through a slightly different mechanism as does idoxuridine, which is sometimes used topically to treat herpes infections of the skin.

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 an endocytic process. 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. At the time of writing, governments around the world are stockpiling this latter drug in the expectation that it will mitigate the effects of the anticipated 'swine' (H1N1) flu pandemic.

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. The closely related **rimantadine** is similar in its effects.

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 BY OTHER MECHANISMS

Enfurvirtide inhibits the fusion of HIV with host cells. The drug is generally given by subcutaneous injection in combination with others to treat HIV when resistance becomes a problem or when the patient is intolerant of other antiretroviral 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 is a chemokine receptor antagonist—a novel concept in HIV therapy (see Dhami et al., 2009) and is the only such drug currently available.

CCR5, together with CXCR4, are cell surface chemokine receptors that have been hijacked by some strains of HIV to gain entry to the cell. In patients who are demonstrated to harbour 'R5' strains, maraviroc may be used, in combination with more conventional antiretroviral drugs. Its use in the UK is currently restricted. A similar compound, **vicriviroc**, is in clinical development.

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.

[2]Also used for its mildly beneficial effects in Parkinson's disease (see Ch. 39).

Palivisumab

Related in terms of its mechanism of action to immunoglobulins is **palivisumab**, a monoclonal antibody (see Chs 17 and 59) directed against a glycoprotein on the surface of respiratory syncytial virus. It is used (as an intramuscular injection) in infants to prevent infection by this organism.

Interferons

IFNs are a family of inducible proteins synthesised by mammalian cells and now generally produced commercially using 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 17.

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. 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 include 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 in mucosal tissues or on the skin.

Tribavirin 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 9% a case fatality rate previously 76%.

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* block the use of host cell surface receptors that 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. 51.3), they can usefully be deployed in combinations and this technique 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 3- or 4-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 interindividual variations in absorption. 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.

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.

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 **ratelgravir** 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. New strategies—based on the growing understanding of the biology of pathogenic viruses and their action on, and in, host cells—could well, 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 underway 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 the virus is 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. However, while there has been no shortage of candidate vaccines (some 40 have been trialled in thousands of volunteers), the prospect of a vaccine against HIV (and sadly many other viruses) still seems rather remote. 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 way forward is not totally clear, but the issue has stimulated research into the interface between the innate and adaptive immune systems in a quest to boost the effectiveness of vaccine design. The whole problem of HIV vaccines is the subject of numerous reviews (see Girard et al., 2006; Kaufman & Barouch, 2009; Rhee & Barouch, 2009).

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Useful Web resources

http://www.aidsinfo.nih.gov/ (*The official HIV/AIDS site of the US National Institutes of Health. This comprehensive Web site carries authoritative and completely 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*)

http://www.unaids.org/en/default.asp (*This is the official site of the United Nations Programme on HIV/AIDS. It deals with a wide range of issues but focuses on the demographics of the epidemic. It carries photographs, maps, slides, movies and statistics, as well as other resources that bring home the enormous problems faced by the international community in dealing with this disease. Prepare to be appalled*)

52 Antifungal drugs

OVERVIEW

Fungal infections (*mycoses*) are widespread in the population; they are generally associated with the skin (e.g. 'athlete's foot') or mucous membranes (e.g. 'thrush').[1] In temperate climates such as the UK, and in otherwise healthy people, they are mainly benign, being more of a nuisance than a threat. However, they become a more serious problem when the immune system is compromised or when they gain 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 in nature. Many 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 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. 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, rare in many parts of the temperate world, are also now encountered more often because of the increase in international travel.

Clinically important fungi may be classified into four main types on the basis of their morphological and other characteristics. Of particular taxonomic significance is the presence of *hyphae*—filamentous projections that may knit together to form a complex *mycelium*, a mat-like structure giving 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 kown as *P. jirovecii*), shares characteristics of both protozoa (see Ch. 53) and fungi; however, it is not susceptible to antifungal drugs and will not be considered here even though it is an important opportunistic pathogen in patients with compromised immune systems (e.g. those suffering from AIDS).

Drugs vary in their efficacy between the different fungal groups. Table 52.1 gives examples of each type of organism and lists some of the diseases caused by these agents and the most common choice of drug classes.

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.

In the UK, the commonest *systemic* (or 'disseminated') fungal disease is candidiasis. Other more 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 similar condition termed *allergic bronchopulmonary aspergillosis*.

In other parts of the world, the commonest systemic fungal infections include blastomycosis, histoplasmosis, coccidiomycosis and paracoccidiomycosis; these are often primary infections, i.e. they are not secondary to reduced immunological function or altered commensal microorganisms.

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*

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

Table 52.1 Some common fungal infections and their sensitivity to various classes of antifungal drugs

Organism	Principal disease(s)	Most common treatment					
		Polyenes	Echinocandins	Azoles/triazoles	Flucytosine[a]	Griseofulvin	Terbinafine
Yeasts							
Cryptococcus neoformans	Meningitis	+++	–	+	+	–	–
Yeast-like fungus							
Candida albicans	Thrush, systemic candidiasis	++	Rarely	++	–	–	–
Filamentous fungi							
Trichophyton spp. *Epidermophyton floccosum* *Microsporum* spp.	All these organisms cause skin and nail infections and are referred to as tinea or 'ringworm'	–	–	+++	–	+++	+++
Aspergillus fumigatus	Pulmonary aspergillosis	++	+	+	–	–	–
Dimorphic fungi							
Histoplasma capsulatum	Histoplasmosis	++	–	++	–	–	–
Coccidioides immitis	Coccidiomycosis	++	–	++	–	–	–
Blastomyces dermatides	Blastomycosis	++	–	+	–	–	–

[a] Generally used as an adjunct to amphotericin.

pyrimidines. Because many infections are superficial, there are many topical preparations. Many antifungal agents are quite toxic, and when systemic therapy is required these agents must often be used under strict medical supervision.

Figure 52.1 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 membranes, where it interferes with permeability and with transport functions. Its most important property is probably its ability to form 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 several organisms including *Aspergillus* and *Candida*. Amphotericin also enhances the antifungal effect of **flucytosine** (see below), 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 by slow intravenous injection complexed with liposomes or other lipid-containing preparations. This improves the pharmacokinetics and reduces the considerable burden of side effects. Long-circulating or so-called 'stealth' liposomes containing amphotericin have been used to good effect.

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, and intravenous amphotericin is used with flucytosine to treat cryptococcal meningitis. It is excreted very slowly via the kidney, traces being found in the urine for 2 months or more after administration has ceased.

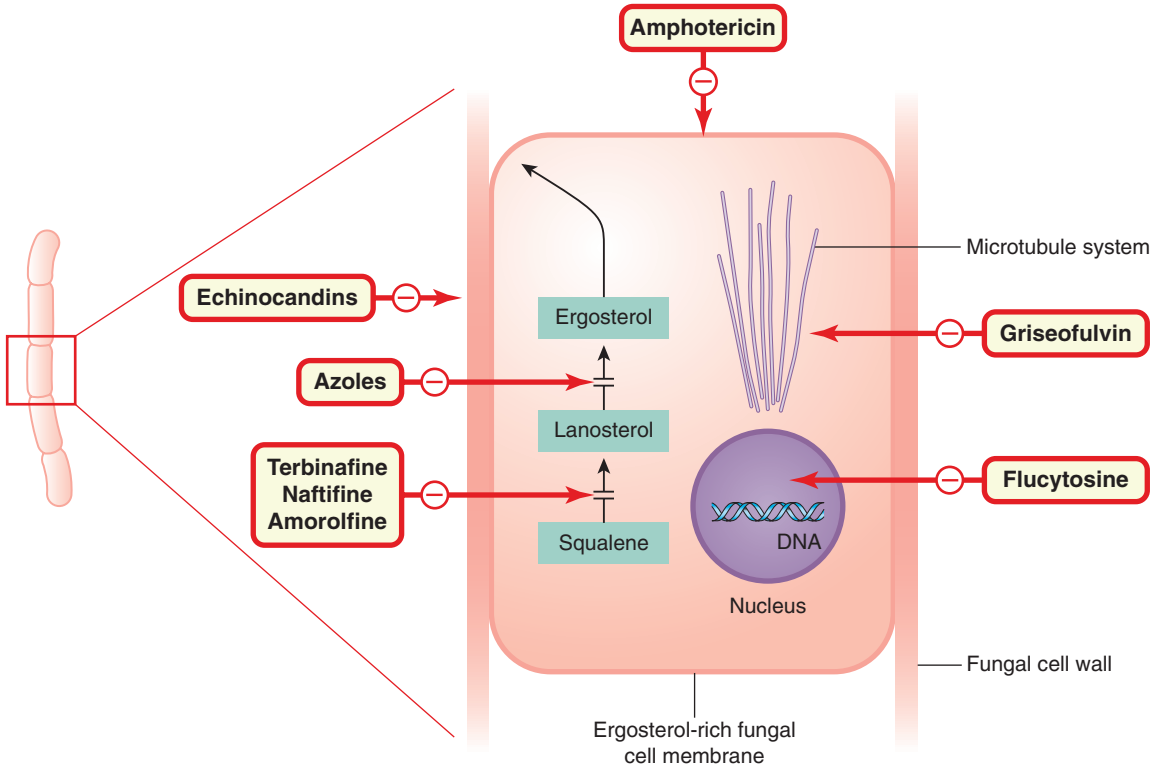

Fig. 52.1 Sites of action of common antifungal drugs. Fungi are morphologically very diverse organisms, and this diagram of a 'typical' fungus is not intended to be technically accurate. The principal sites of action of the main antifungal agents mentioned in this chapter (in red-bordered boxes) are indicated as shown.

Unwanted effects

The commonest and most serious unwanted effect of amphotericin is renal toxicity. Some degree of reduction of renal function occurs in more than 80% of patients receiving the drug; although this generally recovers after treatment is stopped, some impairment of glomerular filtration may remain. Hypokalaemia occurs in 25% of patients, requiring potassium chloride supplementation. Hypomagnesaemia also occurs, and anaemia can be a further problem. Other unwanted effects include impaired hepatic function, thrombocytopenia and anaphylactic reactions. Injection frequently results initially in chills, fever, tinnitus and headache, and about one in five patients vomits. The drug is irritant to the endothelium of the veins, and local thrombophlebitis is sometimes seen after intravenous injection. Intrathecal injections can cause neurotoxicity, and topical applications cause a skin rash. The (considerably more expensive) liposome-encapsulated and lipid-complexed preparations have no greater efficacy than the native drug but cause fewer adverse reactions.

Nystatin (also called **fungicidin**) is a polyene macrolide antibiotic similar in structure to amphotericin and with the same mechanism of action. It 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 treatment is ineffective, but treatment needs to be very 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 based

on the structure of **echinocandin B**, which is found naturally in *A. nidulans*. The echinocandins 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.

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.

SYNTHETIC ANTIFUNGAL DRUGS

AZOLES

The azoles are a group of synthetic fungistatic agents with a broad spectrum of activity based on the imidazole (**clotrimazole**, **econazole**, **fenticonazole**, **ketoconazole**, **miconazole**, **tioconazole** and **sulconazole**) or triazole nucleus (**itraconazole**, **voriconazole** and **fluconazole**).

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 52.1). It is, however, toxic (see below), 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 produce the inhibition of hepatic drug metabolism and of steroidogenesis that 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

Though rare, the most serious are hepatoxicity and Stevens–Johnson syndrome (see above). 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 those described above for ketoconazole).

Miconazole

Miconazole is given topically for oral and other infections of the gastrointestinal tract. It has a short plasma half-life and needs to be given every 8 h. It reaches therapeutic concentrations in bone, joints and lung tissue but not in the central nervous system, and it is inactivated in the liver.

Unwanted effects

Unwanted effects are relatively infrequent, those most commonly seen being gastrointestinal disturbances, but pruritus, blood dyscrasias and hyponatraemia are also reported. There are isolated reports of liver damage, and it should not be given to patients with impaired hepatic function.

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). **Poscanazole** and **voriconazole** are used mainly for the treatment of invasive life-threatening infections such as aspergillosis.

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

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 55). Resistant mutants may emerge rapidly, so this drug should not be used alone.

Flucytosine is usually given by intravenous infusion 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 are infrequent. Gastrointestinal disturbances, anaemia, neutropenia, thrombocytopenia and alopecia have occurred (possibly due to formation of fluorouracil by gut bacteria), but these are usually mild (but may be more significant in AIDS patients) and are easily reversed when therapy ceases. 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. 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 and the prospect of using combination therapies has been explored in more depth (see Lupetti et al., 2003).

At the time of writing, a new echinocandin, **micafungin**, has just been introduced into the UK for treating invasive candidiasis. Unwanted effects are mild, and their incidence less than that seen with amphotericin. Several 'new-generation' triazoles are also in prospect (see Boucher et al., 2004).

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* (GMCSF, see Ch. 17) 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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infection and examines novel strategies for antifungal therapy drawing on these data)

Vermes, A., Guchelaar, H.J., Dankert, J., 2000. Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. J. Antimicrob. Chemother. 46, 171–179. (*The title is self-explanatory!*)

Useful Web resources

http://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!*)

53 Antiprotozoal drugs

OVERVIEW

Protozoa are motile, unicellular eukaryotic organisms that have colonised virtually every habitat and ecological niche. They may be conveniently classified, on the basis of their method of locomotion, into four main groups: *amoebas*, *flagellates* and *sporozoa* together with a further group comprising *ciliates* and other organisms of uncertain affiliation, such as the *Pneumocystis jirovecii* mentioned in the last chapter. The 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 54.

As a group, the protozoa are responsible for an enormous burden of disease among humans as well as domestic and wild animal populations. Table 53.1 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, a discussion of malaria will occupy much of the chapter.

HOST–PARASITE INTERACTIONS

Mammals have developed very efficient mechanisms for dealing with invading parasites but many parasites have, in turn, evolved clever tactics to evade these defensive responses. 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 *Plasmodia* 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-α and interferon-γ. These cytokines (see Ch. 17) activate macrophages, which can then kill intracellular parasites.

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 evolved mechanisms for manipulating the Th1/Th2 balance to their own advantage by stimulating the production of Th2 cytokines. For example, the invasion of macrophages by *Leishmania* species induces transforming growth factor-β, while the invasion of T cells, B cells and macrophages by trypanosomes induces IL-10 (see Handman & Bullen, 2002, and Sacks & Toben-Trauth, 2002, for further details). Similar mechanisms operate during worm infestations (see Ch. 54).

Toxoplasma gondii has evolved a different ploy: upregulation of some host 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.

Improved understanding of host–protozoon relationships has opened up new vistas for the development of antiprotozoal agents. The possibility of using cytokine analogues and/or antagonists to treat disease caused by protozoa is already being investigated (for review, see Odeh, 2001).

MALARIA AND ANTIMALARIAL DRUGS

Malaria[1] is caused by parasites belonging to the genus *Plasmodium*. Four main species of plasmodia infect humans: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale* and *Plasmodium malariae*. The insect vector is the female *Anopheles* mosquito, which breeds in stagnant water, and the disease it spreads is one of the major killers on our planet.

The statistics are staggering. According to the (2008) World Health Organization (WHO) report, malaria is a significant public health problem in more than 90 countries inhabited by about 50% of the world's population. In 2006, the disease caused an estimated 880 million acute illnesses each year and nearly 1 million deaths. More than 90% of these occur in sub-Saharan Africa, and it is estimated that the disease kills an African child every 30 seconds. 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.

The symptoms of malaria include fever, shivering, pain in the joints, headache, repeated vomiting, generalised 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**, that had become available. By the end of the 1950s, the incidence of malaria had dropped dramatically. However, during the 1970s it became clear that the attempt at eradication had failed, largely owing to the increasing resistance of the mosquito to the insecticides and of the parasite to the drugs. Sadly, malaria has now re-emerged

[1]The disease was once considered to arise from marshy land, hence the Latin name 'mal aria', meaning bad or poisonous air.

Table 53.1 Principal protozoal infections and common drug treatments

Organism	Disease	Common drug treatment
Amoeba		
Entamoeba histolytica	Amoebic dysentery	Metronidazole, tinidazole, diloxanide
Flagellates		
Trypanosoma rhodesiense *Trypanosoma 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[b] *Plasmodium vivax* *Plasmodium ovale* *Plasmodium malarariae* *Toxoplasma gondii*	Malignant tertian malaria Benign tertian malaria Benign tertian malaria Quartan malaria Encephalitis, congenital malformations, eye disease	Amodiaquine, artemisinin and derivatives, atovaquone, chloroquine, clindamycin, dapsone, doxycycline, lumefantrine, mefloquine, primaquine, proguanil, pyrimethamine, quinine, sulfadoxine, tafenoquine and tetracycline Pyrimethamine-sulfadiazine
Ciliates and others		
Pneumocystis carinii[a]	Pneumonia	Co-trimoxazole, atovaquone, pentamidine isetionate

[a] This organism is of uncertain classification. See text for details and Chapter 52 for further comments. [b] See also Table 53.2.

in several countries where it was previously under control or eradicated. International air travel is responsible for sporadic cases in Western Europe and the USA, where the actual risk of transmission is negligible.[2] 1999 saw the initiation of the *Roll Back Malaria* programme sponsored by a partnership of transnational organisations including the WHO. While it is unlikely that this programme will achieve all its goals, one encouraging trend has been that the disease has actually begun to decline in some parts of the world following aggressive public health campaigns.

THE LIFE CYCLE OF THE MALARIA PARASITE

The mosquito, not the human, is the definitive host for plasmodia, and 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 life cycle of the parasites consists of a sexual cycle, which takes place in the female *Anopheles* mosquito, and an asexual cycle, which occurs in humans (Fig. 53.1 and the *Malaria* box).

▼ The cycle in the mosquito involves fertilisation of the female gametocyte by the male gametocyte, with the formation of a *zygote*, which

[2] WHO reported 87 cases of 'airport malaria' in 12 countries between 1969 and 1999. 'Weekend malaria', which occurs when city dwellers in Africa spend weekends in the countryside, is becoming more of a problem.

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.

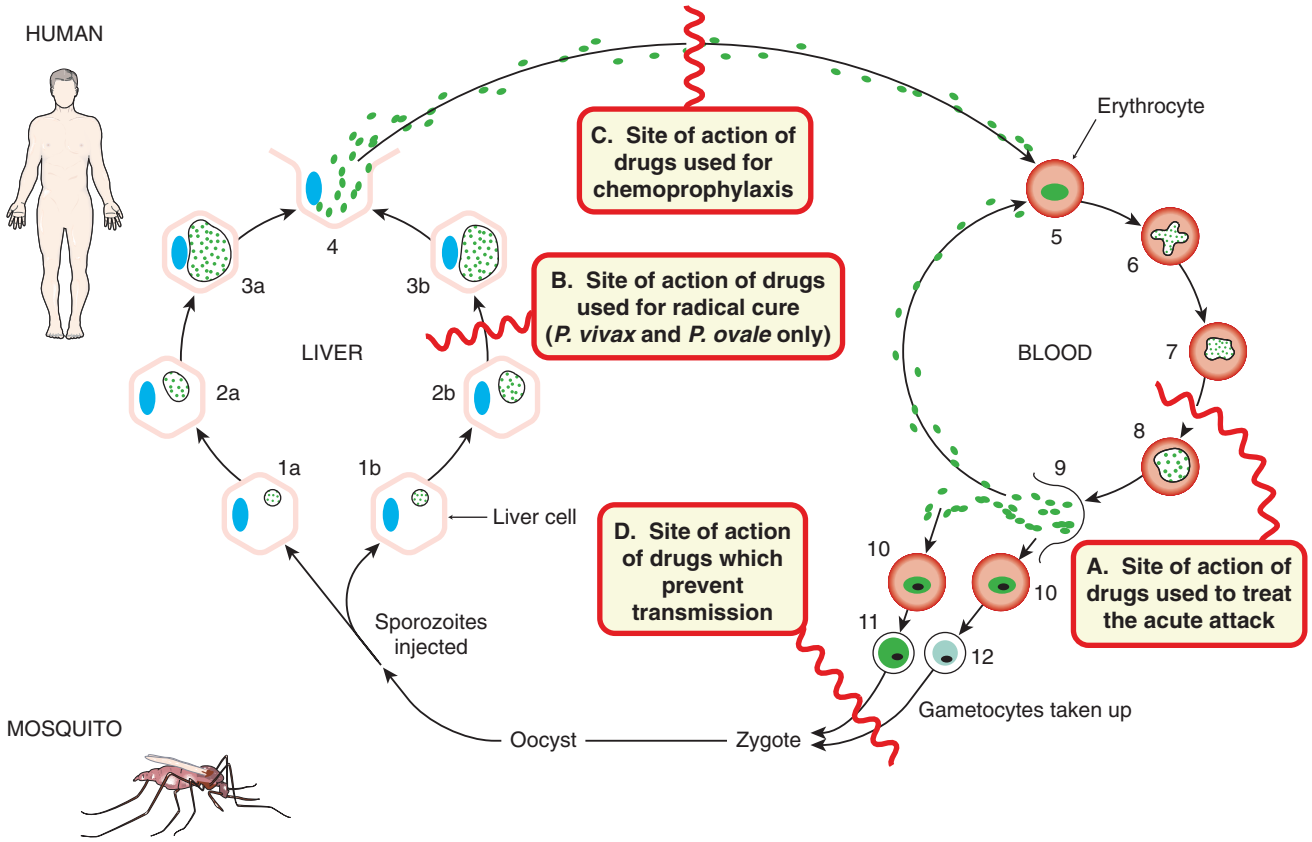

Fig. 53.1 **The life cycle of the malarial parasite and the site of action of antimalarial drugs.** The pre- or exoerythrocytic cycle in the liver and the erythrocytic cycle in the blood are shown: **1a** Entry of sporozoite into liver cell (the parasite is shown as a small circle containing dots, and the liver cell nucleus as a blue oval). **2a** and **3a** Development of the schizont in liver cell. **4** Rupture of liver cell with release of merozoites (some may enter liver cells to give resting forms of the parasite, hypnozoites). **5** Entry of merozoites into a red cell. **6** Trophozoite in red cell. **7** and **8** Development of schizont in red cell. **9** Rupture of red cell with release of merozoites, most of which parasitise other red cells. **10–12** Entry of merozoites into red cells and development of male and female gametocytes. **1b** Resting form of parasite in liver (hypnozoite). **2b** and **3b** Growth and multiplication of hypnozoites. Sites of drug action are as follow. **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.

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.

The sporozoites are thus inoculated into the bloodstream. Within 30 min, they disappear from the blood 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. At the end of this stage, the parasitised liver cells rupture, and a host of fresh *merozoites* are released. These bind to and enter the red cells of the blood and form motile intracellular parasites termed *trophozoites*. The development and multiplication of the plasmodia within these cells constitutes the *erythrocytic* stage. During maturation within the red cell, 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 (see below).

▼ Following mitotic replication of its nucleus, 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 forms

of the parasite, called *gametocytes*. These can complete their cycle only when taken up by the mosquito, when it sucks the blood of 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 tumour necrosis factor-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. 53.1 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. These parasitised red cells 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*—'benign' because it is less severe than falciparum malaria and is 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 and travellers to infected areas are advised to wear clothes that cover much of the skin and use insect repellents in living, and especially sleeping, areas, because mosquitoes tend to bite between dusk and dawn. 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 53.2), 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. 53.1).

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. Today monotherapy has been abandoned in favour of **artemisinin**-based combination therapy (ACT; see Table 53.3). 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 53.1. Newton & White (1999) and Baird (2005) give a more detailed coverage of the treatment of malaria around the world.

Drugs used to treat the acute attack

Blood schizonticidal agents (Fig. 53.1, 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:

- **artemesinin** and related compounds derived from the Chinese herb *qing hao*, 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.

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 53.2 Summary of drugs used for treatment and chemoprophylaxis of malaria[a]

Infections	Typical drug choices for acute attacks	Typical drug choices for chemoprophylaxis
Infection with chloroquine-resistant *Plasmodium falciparum* or with unknown or mixed organisms	Oral quinine plus: proguanil + atovoquone;[b] or artemether + lumefantrine[c]	**Short term (weeks):** atovoquone + proguanil or doxycycline **Long term (months/years):** chloroquine + proguanil or atovoquone + proguanil

[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 2008).
[b] *Malarone* is a proprietary combination of atovoquone and proguanil hydrochloride.
[c] *Riamet* is a proprietary combination of artemether and lumefantrine.

Table 53.3 Drug targets of antimalarial drugs

Parasite organelle	Target	Chemical class	Drugs
Cytosolic compartment	Inhibit or antagonise folic acid metabolism	Diaminopyridines Biguanides Sulfones Sulfonamides	Pyrimethamine Proguanil Dapsone Sulphadoxine
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 Aryl amino alcohols	Chloroquine, amodiaquine, mefloquine, quinine Lumefantrine
Membranes ?	Inhibition of Ca^{+}-dependent ATPase	Sesquiterpene lactones	Artemisinin derivatives

After Fidock et al., 2004.

Combinations of these agents are frequently used. Some antibiotics, such as the tetracycline **doxycycline** and **clindamycin** (see Ch. 50), 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. 53.1, 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 53.2) 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. 53.1, 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 53.2.

Drugs used to prevent transmission

Some drugs (e.g. primaquine, proguanil and pyrimethamine) can also destroy gametocytes (Fig. 53.1, 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 53.3 summarises what is known about the molecular targets of these drugs and Figure 53.2 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. 53.1, 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. The clinical use of chloroquine is summarised in Tables 53.2 and the *Antimalarial drugs* box.

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.

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

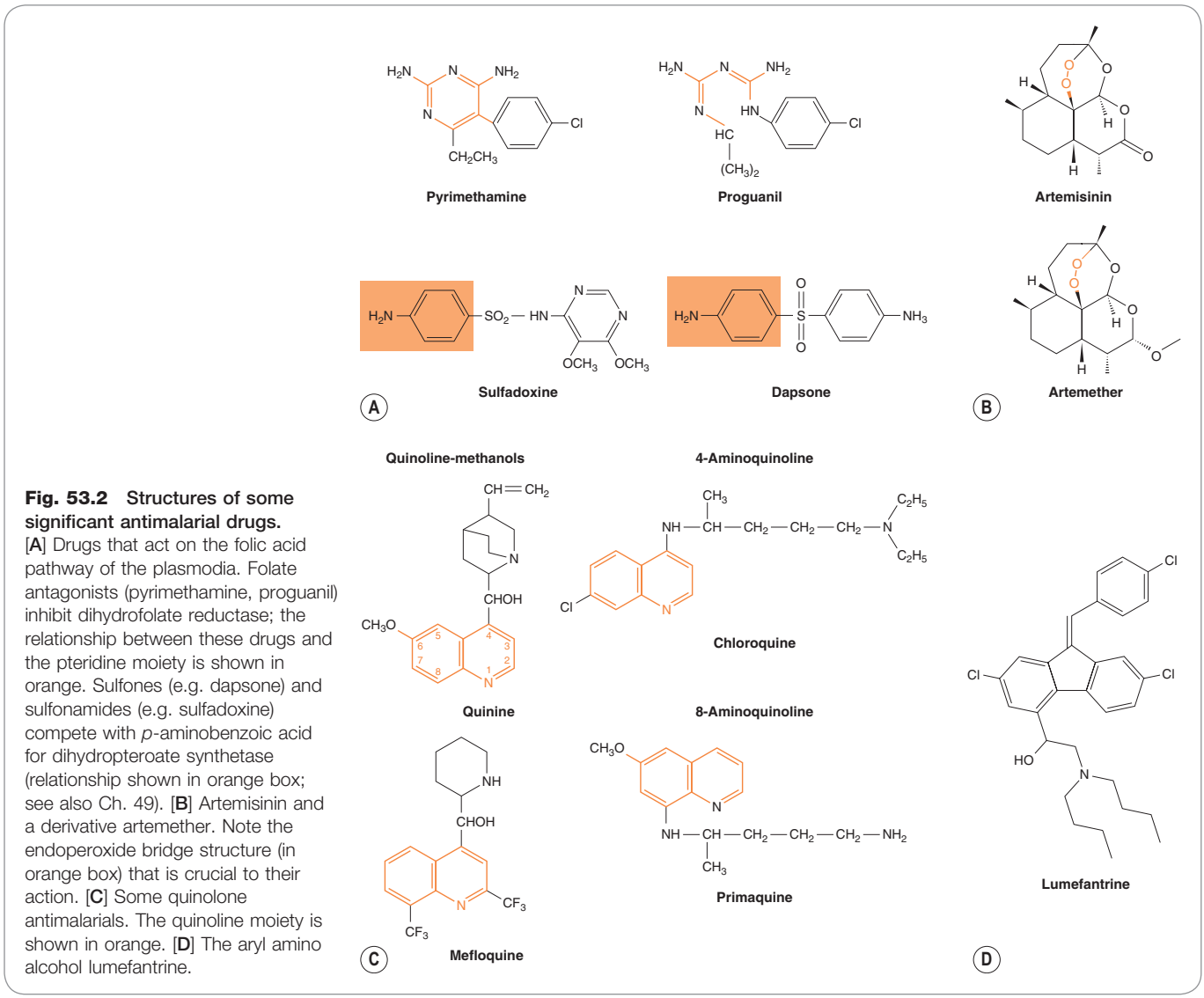

Fig. 53.2 Structures of some significant antimalarial drugs. [**A**] Drugs that act on the folic acid pathway of the plasmodia. Folate antagonists (pyrimethamine, proguanil) inhibit dihydrofolate reductase; the relationship between these drugs and the pteridine moiety is shown in orange. Sulfones (e.g. dapsone) and sulfonamides (e.g. sulfadoxine) compete with *p*-aminobenzoic acid for dihydropteroate synthetase (relationship shown in orange box; see also Ch. 49). [**B**] Artemisinin and a derivative artemether. Note the endoperoxide bridge structure (in orange box) that is crucial to their action. [**C**] Some quinolone antimalarials. The quinoline moiety is shown in orange. [**D**] The aryl amino alcohol lumefantrine.

the tissues and concentrated in parasitised red cells. The free base form of the drug is trapped in the acidic environment in the food vacuole of the malaria parasites where it disrupts the haemoglobin digestion pathway (Ch. 8). 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

The methanol quinolone, quinine, is derived from *cinchona* bark. It has been used for the treatment of 'fevers' since the 16th century, when Jesuit missionaries bought the bark to Europe from Peru. It is a blood schizonticidal drug effective against the erythrocytic forms of all four species of plasmodium (Fig. 53.1, 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*. 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. The clinical uses of quinine are given in Table 53.2 and in the *Antimalarial Drugs* box.

Some degree of resistance to quinine is developing 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. 53.2) is a blood schizonticidal compound active against *P. falciparum* and *P. vivax* (Fig. 53.1, 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 (see below) to eradicate the hypnozoites. Mefloquine acts in the same way as quinine, and is frequently combined with pyrimethamine.

Resistance 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. The clinical use of mefloquine is given in Table 53.2 and the *Antimalarial drugs* box.

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 CNS symptoms.

DRUGS AFFECTING FOLATE METABOLISM

Sulfonamides and sulfones, used as antibacterial drugs (see Ch. 50) inhibit the synthesis of folate 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** (see Fig. 53.3). Details of these drugs are given in Chapter 50. 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 (see Fig. 53.3) is similar in structure to the antibacterial drug **trimethoprim** (see Ch. 50). Proguanil has a slightly different structure (see Fig. 53.3) but its metabolite can assume a similar configuration. Both drugs have a greater affinity for the plasmodial enzyme than for the human enzyme. They have a slow action against the erythrocytic forms of the parasite (Fig. 53.1, site A), and proguanil is believed to have an additional effect on the initial hepatic stage (1a to 3a in Fig. 53.1) but not on the hypnozoites of *P. vivax* (Fig. 53.1, 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 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

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

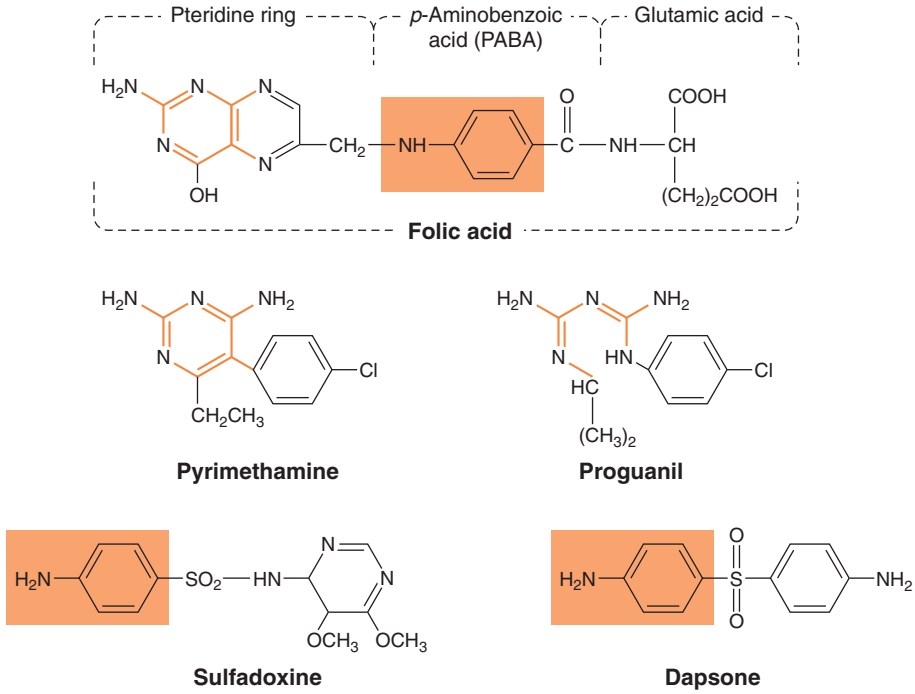

Fig. 53.3 Structures of some antimalarial drugs that act on the folic acid pathway of the plasmodia. Folate antagonists (pyrimethamine, proguanil) inhibit dihydrofolate reductase; the relationship between these drugs and the pteridine moiety is shown in orange. Sulfones (e.g. dapsone) and sulfonamides (e.g. sulfadoxine) compete with *p*-aminobenzoic acid for dihydropteroate synthetase (relationship shown in orange box).

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. 53.2). **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 the disease in 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.

ARTEMESININ AND RELATED COMPOUNDS

These sesquiterpene lactones are derived from the herb *qing hao*, a traditional Chinese remedy for malaria. 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). **Artesunate**, a water-soluble derivative, and the synthetic analogues **artemether** and **artether**, have higher activity and are better

[4]Having been used for thousands of years in China as a herbal extract for treating 'fevers', the active compound artemsinin 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 fourth century king of Halicarnassus; her sorrow on his death led her to mix his ashes with whatever she drank to make it bitter.

absorbed. The compounds are concentrated in parasitised red cells. The mechanism of action is probably through inhibition of a parasite Ca^{+}-dependent ATPase (Eckstein-Ludwig et al., 2003) and it is likely that the 'endoperoxide bridge' of this drug (see Fig. 53.2) 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 have been few unwanted effects reported to date. 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 it is developing in some countries.

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, the *qinghaosu* compounds 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, 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

Several new drugs are currently under test for antimalarial activity, with positive results in animals and in preliminary trials in humans. One of these, **pyronaridine**, has shown encouraging results. It is active against *P. falciparum* and *P. vivax*, and is also active in chloroquine-resistant *P. falciparum*. It is effective orally and has low toxicity. The mechanism of action is unknown. Other novel agents are reviewed by Lanteri et al. (2007).

In 2002, the results from the malaria genome sequencing project were published and it is highly likely that this information will eventually yield new candidate drugs with novel actions. A group of cysteine proteases used by the

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.

parasite to digest haem seems to be one attractive target that is currently receiving attention.

Given the extraordinary lifestyle of the malarial parasite with its many forms both inside and outside cells, the challenges facing vaccine developers are enormous. Nevertheless, there is cause for optimism, and a large-scale trial of the first candidate vaccine began in 2009. Discussion of this topic is beyond this chapter but further details may be found in Greenwood et al. (2008).

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.

The trophozoite lyses the colonic mucosal cells (hence 'histolytica') using *amoebapores* (peptides that form pores in cell membranes) and proteases or by inducing host cell apoptosis. The organism then invades the submucosa, where it secretes factors that 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 in many subjects a chronic intestinal infection may be present in the absence of dysentery. In some subjects, 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 extraintestinal symptom of the disease—amoebic liver abscesses.

The use of drugs to treat this condition (see *Drugs used in amoebiasis* box) 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 parasite 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 central nervous system (CNS) symptoms (dizziness, headache, sensory neuropathies). Metronidazole causes a disulfiram-like reaction to alcohol (see Ch. 48), 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**.

TRYPANOSOMIASIS AND TRYPANOCIDAL DRUGS

Trypanosomes belong to the group of pathogenic flagellate protozoa. The three main species that cause disease in humans are *Trypanosoma gambiense* and *Trypanosoma rhodesiense*, which cause sleeping sickness in Africa, and *Trypanosoma cruzi*, which causes Chagas' disease in South America. About 50–60 million people are thought by the WHO to be at risk of contracting sleeping sickness each year. The disease caused by *T. 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

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.

despite this, improved surveillance has resulted in a recent reduction in the total number of new cases reported. Related trypanosome infections also pose a major risk to livestock and thus have a secondary impact on human health and well-being.

▼ The vector 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. 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, or 'Chagas' disease' when parasites damage the heart, muscles and sometimes liver, spleen, bone and intestine. Left untreated, such infections are fatal.

The main drugs used for African sleeping sickness 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 are toxic. **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 from where it is 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. 50). 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

A relatively new drug, eflornithine inhibits the parasite *ornithine decarboxylase* enzyme. It shows good activity against *T. gambiense* and is used as a back-up for melarsoprol, although unfortunately it has limited activity against *T. 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. The recent publication of the complete genome sequence of some trypanasome species has led to optimism that new agents may be forthcoming in the medium term. The interested reader is referred to Gehrig & Efferth, 2008; Kennedy, 2008; and Myler, 2008 for recent accounts of these opportunities.

OTHER PROTOZOAL INFECTIONS AND DRUGS USED TO TREAT THEM

LEISHMANIASIS

Leishmania organisms are flagellate protozoa that cause disease (sometimes fatal). Some 350 million people in 90 countries are at risk, mainly in tropical and subtropical regions. The disease afflicts about 12 million people: there are about 2 million new cases each year of which some 60 000 die. With increasing international travel, leishmaniasis is being imported into new areas and opportunistic infections are now being reported (particularly in AIDS patients).

▼ The vector in this case is the sandfly, and the parasite exists in two forms, a flagellated form (*promastigote*) found in the gut of the infected insect, and a non-flagellated intracellular form (*amastigote*) that occurs in the infected mammalian host, where it is harboured by mononuclear phagocytes. Within this cell, the parasites thrive in modified phagolysosomes and protect themselves from the usual intracellular killing mechanisms by modifying the macrophage's microbiocidal systems, apparently by deploying a lipophosphoglycan on their surface (Handman & Bullen, 2002). 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.

The different species of Leishmania occur in different geographical zones and cause different clinical manifestations (see Table 53.1). Typical presentations include:

- a simple skin infection giving rise to an unpleasant chancre ('oriental sore', 'Chiclero's ulcer' and other names) that may heal spontaneously
- a mucocutaneous form ('espundia' and other names), in which there may be large ulcers of the mucous membranes
- a serious visceral form ('kala-azar' and other names), where the parasite spreads through the bloodstream and causes hepatomegaly, splenomegaly, anaemia and intermittent fever.

The main drugs used in visceral leishmaniasis are pentavalent antimony compounds such as **sodium stibogluconate**, pentamidine (see above) and amphotericin (see Ch. 52), which is sometimes used as a follow-up treatment. **Miltefosine**, an antitumour drug, which has been used with success to treat the disease, 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. Treatment may also be associated with increased incidence of herpes zoster. 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 (Verma & Dey, 2004).

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. Current drug usage and possible future approaches to the treatment of leishmaniasis are discussed by Mishra et al. (2007). The publication of complete leishmania genomes will initiate a major effort to discover novel parasite-specific pathways that could serve as useful drug targets (see Kumari et al., 2008).

There is no effective vaccine for leishmaniasis.

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. 50), 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

Toxoplasma organisms belong to the group of pathogenic Sporozoa. The cat is the definitive host of *Toxoplasma gondii* (i.e. it is the only host in which the sexual cycle can occur), and 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. 50) or combinations of **pyrimethamine** with **clindamycin**, **clarithromycin** or **azithromycin** (see Ch. 50) have shown promise.

PNEUMOCYSTIS

First recognised in 1909, *Pneumocystis carinii* (now known as *P. jirovecii*; see also Ch. 52) was presumed to belong to the protozoa, but recent studies have shown that it 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 a 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. 49) is the drug of choice in serious cases, with parenteral pentamidine (see above) 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 challenge, with each protozoa species posing its own distinct problems to the would-be designer of new antiprotozoal drugs. Where appropriate in this chapter, we have indicated possible future avenues for research and development, but the interested reader is referred to the reading list and Web sites listed below for further information.

It is abundantly clear that the diseases caused by the protozoa constitute a major global challenge, but the problems of provision and distribution of new drugs are daunting. Managing the costs of research and development in this area is complex. 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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Useful Web resources

http://malaria.who.int/ (*The WHO home page dealing with the global malaria programme contains links to all the major information on the site dealing with malaria – a terrific starting point for further investigation*)

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

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

54 Anthelminthic drugs

OVERVIEW

Among the most widespread of all chronic infections are those caused by various species of parasitic *helminths* (worms). It is estimated that over half the world's population is infected with gastrointestinal helminths. Inhabitants of tropical or subtropical low-income countries are most at risk; children often become infected with one or more species at birth and may remain so throughout their lives. In some cases (e.g. *threadworms*), these infections result mainly in discomfort and do not cause substantial ill health, but others, such as *schistosomiasis* (*bilharzia*) and *hookworm* disease, are associated with serious morbidity. Because of its prevalence, the treatment of helminthiasis is therefore of great practical therapeutic importance. Worm infections are also a major 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.

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.

Helminths have a complex life cycle, often involving several host species. Infection by helminths 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, other types 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 form that reproduces. 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. The usual intermediate hosts of the two most common tapeworms (*T. saginata* and *T. solium*) are cattle and pigs, respectively. 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 *Ankylostoma 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. Blood loss caused by intestinal hookworms 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 and produce inflammation of these organs, resulting 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. They may be ingested by mosquitoes or similar biting insects when they feed. After a period of development within this secondary host, the larvae pass to the mouth parts of the insect and are reinjected into humans. 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 infection, larvae 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 usually 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 often entering through the foot. *Toxocariasis* or visceral larva migrans is caused by larvae of cat and dog roundworms of the *Toxocara* genus.

ANTHELMINTHIC DRUGS

Mankind has attempted to treat helminth infections since antiquity. Extracts of herbs or plants such as male fern formed the basis of many early 'cures', but the 20th century saw the advent of a new group of drugs based on toxic metals such as arsenic (atoxyl) or antimony (tartar emetic), which were effective in trypanosome and schistosome infestations.

Current anthelminthic drugs act either by paralysing the parasite (e.g. by preventing muscular contraction), or by damaging the worm such that the immune system can eliminate it, or by altering its 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 be effective, a drug must be able to 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 anthelminthic drugs are therefore important.

Some individual anthelminthic drugs are described briefly below, and indications for their use are given in Table 54.1. Several of these drugs (i.e. **albendazole**, **ivermectin**, **levamisole**, **niclosamide**, **praziquantel** and **tiabendazole**) are available in the UK only on a 'named patient' basis.[1] For a more comprehensive coverage of antiparasitic drugs and their use in humans and animals, you are directed to the literature cited in the bibliography.

BENZIMIDAZOLES

One of the principal groups of anthelminthics used clinically are the substituted benzimidazoles. This group of broad-spectrum agents includes **mebendazole**, **tiabendazole** and **albendazole**. 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 is 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, like mebendazole, this may be increased by food, especially fats. It is metabolised extensively by first-pass metabolism to the 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 unsuitable for pregnant women or children less than 2 years old.

PRAZIQUANTEL

Praziquantel is a highly effective broad-spectrum anthelminthic 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 but also the immature forms and the cercariae—the form of the parasite that infects humans by penetrating the skin.

The drug apparently disrupts Ca^{2+} homeostasis in the parasite by binding to consensus protein kinase C-binding

[1]A relatively rare situation in which the physician seeks approval from a pharmaceutical company to use one of their drugs 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 licence (possibly for commercial reasons).

Table 54.1 Principal drugs used in helminth infections

Helminth(s)	Drug(s) used
Threadworm (pinworm)	
Enterobius vermicularis	Mebendazole, albendazole, piperazine
Strongyloides stercoralis (threadworm in the USA)	Albendazole, ivermectin
Common roundworm	
Ascaris lumbricoides	Levamisole, mebendazole, piperazine
Other roundworm (filariae)	
Wuchereria bancrofti, *Loa loa*	Diethylcarbamazine, ivermectin
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 (*Ankylostoma duodenale*, *Necator americanus*)	Mebendazole, albendazole
Whipworm (*Trichuris trichiura*)	Mebendazole, albendazole, diethylcarbamazine
Blood flukes (Schistosoma spp.)	
S. haematobium	Praziquantel
S. mansoni	Praziquantel
S. japonicum	Praziquantel
Cutaneous larva migrans	
Ankylostoma caninum	Albendazole, ivermectin, tiabendazole
Visceral larva migrans	
Toxocara canis	Albendazole, tiabendazole, diethylcarbamazine

(Sourced mainly from the British National Formulary 2008.)

sites in a β subunit of schistosome voltage-gated calcium channels (Greenberg, 2005). This induces an influx of the ion, 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 is considered to be a very safe drug with 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 (see below).

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. 37), 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. 29) 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 lightheadeness 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, arthralgias, 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.

When single-dose therapy is used, *unwanted effects* such as mild gastrointestinal disturbances are generally few and soon subside.

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,[2] and is the first choice of drug for the treatment of 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 the drug of choice for onchocerciasis, which causes river blindness and reduces the incidence of this 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 CNS toxicity seen in collie dogs (Ch. 8).

RESISTANCE TO ANTHELMINTHIC DRUGS

Resistance to anthelminthic 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 anthelminthic drugs. Parasites that develop such resistance pass this ability on to their offspring, leading to treatment failure. The widespread use of anthelminthic 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. 8) 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 thrive 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.

▼ In Chapter 6, we discussed the two main types of adaptive immune strategy, termed the *Th1* and the *Th2* responses, the latter being characterised by the development of an antibody-mediated response rather than the development of a cell-mediated immune response. It appears that many helminths can actually exploit this mechanism by steering the immune system away from a local Th1 response, 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).

Ironically, the ability of helminths to modify the host immune response in this way may confer some survival value on the hosts

[2]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.

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 mitigate some forms of malaria and other diseases, possibly conferring survival advantages in populations where these diseases are endemic. Indeed, the deliberate infestation of Crohn's disease patients with helminths has been evaluated as a strategy to induce remission of the disease (see Hunter & McKay, 2004; Reddy & Fried, 2007). On the negative side, however, they may also 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 have been few new anthelminthic drugs recently. On a more positive note, the sequencing of the transcriptomes of several helminths may make it possible to create a transgenic species that expresses mutations found in resistant parasitic worms, thus providing insights into the mechanisms underlying resistance. In addition, such databases may reveal new drug targets, as well as opening the way for other types of anthelmithic agent, such as those based on antisense DNA or small interfering RNA (see Boyle & Yoshino, 2003).

More progress has been made in the field of anthelminthic vaccines through the use of recombinant DNA technology. Protein antigens on the surface of the (highly infectious) larval stage have been cloned and used as immunogens. Considerable success has 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; Lightowlers et al., 2003). Qualified success has also been obtained with vaccines to other helminth species (see Capron et al., 2005; McManus & Loukas, 2008).

Efficacious helminth vaccines would revolutionise the treatment of these widespread infections, minimise the problem of drug resistance as well as reducing the environmental burden of residual pesticide residues, which sometimes occurs as a consequence of overenthusiastic anthelminth control campaigns. Looking further into the future, it may be possible to develop DNA vaccines against these organisms without having to produce any protein-based immunogen at all.

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55 Anticancer drugs

OVERVIEW

In this chapter, we deal with cancer and anticancer therapy, emphasising first the pathogenesis of cancer before proceeding to describe the drugs that can be used therapeutically. Finally, we consider the extent to which our new knowledge of cancer biology is leading to new treatments. The use of radioactive isotopes in cancer treatment is beyond the scope of this book.

INTRODUCTION

Cancer is a disease characterised by uncontrolled multiplication and spread of abnormal forms of the body's own cells. It is the second most common cause of death in the developed nations (cardiovascular disease has the dubious distinction of heading that table) and one in three people will be diagnosed with cancer during their lifetime. In the UK, over 365 000 new cases were reported and mortality in 2006 was in excess of 154 000 (Cancer Research UK). Cancer is responsible for approximately one-quarter of all deaths in the UK, with lung and bowel cancer comprising the largest category, closely followed by breast and prostate cancer. Statistics from most other countries in the developed world tell much the same story. At first sight, incidence figures for the past 100 years or so give the impression that the disease is increasing in developed countries, but cancer is largely a disease of later life, and with advances in public health and medical science, many more people now live to an age where they are more liable to contract cancer.

The terms *cancer*, *malignant neoplasm* (neoplasm simply means 'new growth') and *malignant tumour* are synonymous. Both benign and malignant tumours manifest uncontrolled proliferation, but the latter are distinguished by their capacity for *dedifferentiation*, their invasiveness and their ability to *metastasise* (spread to other parts of the body). In this chapter, we shall be concerned only with the therapy of malignant neoplasia or cancer. The appearance of these abnormal characteristics reflects altered patterns of gene expression in the cancer cells, resulting from inherited or acquired genetic mutations.

There are three main approaches to treating established cancer—*surgical excision*, *irradiation* and *drug therapy* (often called *chemotherapy*)—and the relative value of each of these approaches depends on the type of tumour and the stage of its development. Chemotherapy may be used on its own or as an adjunct to other forms of therapy.

Compared with that of bacterial diseases, cancer chemotherapy presents a difficult problem. In biochemical terms, microorganisms are both quantitatively and qualitatively different from human cells (see Ch. 49), but cancer cells and normal cells are so similar in most respects that it is more difficult to find general, exploitable, biochemical differences between them. In recent years, the focus of cancer chemotherapy has broadened to include, as well as conventional *cytotoxic drugs* (which act on all cells, and rely on a small margin of selectivity to be useful as anticancer agents), several drugs that affect either the hormonal regulation of tumour growth, or the defective cell cycle controls that underlie malignancy (see below and Ch. 5). Overall, this has been one of the most fruitful fields of drug development in recent years, in which genomics and biopharmaceuticals have played a major role. The flow of innovation seems set to continue.

THE PATHOGENESIS OF CANCER

To understand the action and drawbacks of current anticancer agents and to appreciate the therapeutic hurdles that must be surmounted by putative new drugs, it is important to consider in more detail the pathobiology of this disease.

Cancer cells manifest, to varying degrees, four characteristics that distinguish them from normal cells. These are:

- uncontrolled *proliferation*
- *dedifferentiation* and loss of function
- *invasiveness*
- *metastasis*.

THE GENESIS OF A CANCER CELL

A normal cell turns into a cancer cell because of one or more mutations in its DNA, which can be inherited or acquired, usually through exposure to viruses or *carcinogens* (e.g. tobacco products, asbestos). A good example is breast cancer; women who inherit a single defective copy of either of the tumour suppressor genes *BRCA1* and *BRCA2* (see below) have a significantly increased risk of developing breast cancer. However, carcinogenesis is a complex multistage process, usually involving more than one genetic change as well as other, *epigenetic* factors (hormonal, co-carcinogen and tumour promoter effects, etc.) that do not themselves produce cancer but which increase the likelihood that the genetic mutation(s) will eventually result in cancer.

There are two main categories of genetic change that are important:

1. The activation of *proto-oncogenes* to *oncogenes*. Proto-oncogenes are genes that normally control cell division, apoptosis and differentiation (see Ch. 5), but which can be converted to oncogenes that induce malignant change by viral or carcinogen action.
2. The inactivation of *tumour suppressor genes*. Normal cells contain genes that have the ability to suppress malignant change—termed tumour suppressor genes (antioncogenes)—and mutations of these genes are involved in many different cancers. The loss of function of tumour suppressor genes can be the critical event in carcinogenesis.

About 30 tumour suppressor genes and 100 dominant oncogenes have been identified. The changes that lead to malignancy are a result of point mutations, gene amplification or chromosomal translocation, often caused by viruses or chemical carcinogens.

THE SPECIAL CHARACTERISTICS OF CANCER CELLS

UNCONTROLLED PROLIFERATION

Many healthy cells, in the bone marrow and the epithelium of the gastrointestinal tract for example, have the property of continuous rapid division, and it is not generally true that cancer cells proliferate faster than normal cells. Some cancer cells multiply slowly (e.g. those in plasma cell tumours) and some much more rapidly (e.g. the cells of *Burkitt's lymphoma*). The significant issue is that cancer cells *have escaped from the mechanisms that normally regulate cell division and tissue growth*. It is this, rather than their rate of proliferation, that distinguishes them from normal cells.

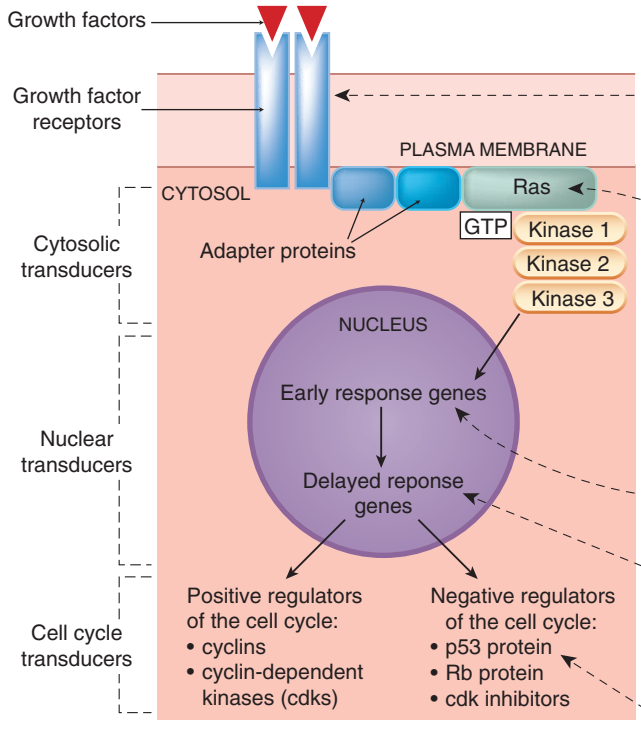
What are the changes that lead to the uncontrolled proliferation of tumour cells? Inactivation of tumour suppressor genes or transformation of proto-oncogenes into oncogenes can confer autonomy of growth on a cell and thus result in uncontrolled proliferation by producing changes in several cellular systems (see Fig. 55.1), including:

- *growth factors*, their receptors and signalling pathways
- the *cell cycle transducers*, for example cyclins, cyclin-dependent kinases (cdks) or the cdk inhibitors
- the *apoptotic machinery* that normally disposes of abnormal cells
- *telomerase expression*
- *local blood vessels*, resulting from tumour-directed angiogenesis.

Potentially all the genes coding for the above components could be regarded as oncogenes or tumour suppressor genes (see Fig. 55.2), although not all are equally prone to malignant transformation. It should be understood that malignant transformation of several components is needed for the development of cancer.

Resistance to apoptosis

Apoptosis is programmed cell death (Ch.5), and genetic mutations in the antiapoptotic genes are usually a



Proto-oncogene	Proto-oncogene products	Cancer	Anticancer drugs
Genes for growth factors e.g. for IGF	Growth factors e.g. IGF	Prostate, breast colorectal, etc.	Research in progress WTS
Gene for EGF receptors (e.g. c-*erbB*)	Her2* (a receptor tyrosine kinase)	Breast	Inhibited by **trastuzumab** (aka Herceptin)
Gene for PDGF (c-*sis*)	PDGF (a receptor tyrosine kinase)	Chronic myeloid leukaemia	Inhibited by **imatinib** (aka Glivec)
c-*ras*	Ras proteins	30% of all tumours	Ras inhibitors in clinical trial
abl	Abl tyrosine kinase (cytoplasmic)	Chronic myeloid leukaemia	Inhibited by **imatinib** (aka Glivec)
c-*src*	Cytoplasmic tyrosine kinase	Breast, pancreas, bone	Research in progress WTS
Genes for JAK, Lck		Leukaemias	
c-*jun*/c-*fos*	Transcription factors (Jun, Fos, Myc)	Colorectal	
c-*myc*		Lung, neural tissue	

Mutation of the delayed response nuclear proto-oncogenes... can alter expression of the regulators of the cell cycle, e.g. more than 50% of human tumours have mutations of the tumour supressor gene that codes for p53 protein

Fig. 55.1 Signal transduction pathways initiated by growth factors and their relationship to cancer development. A few examples of proto-oncogenes and the products they code for are given in the table, with examples of the cancers that are associated with their conversion to oncogenes. Many growth factor receptors are receptor tyrosine kinases, the cytosolic transducers including adapter proteins that bind to phosphorylated tyrosine residues in the receptors. Ras proteins are guanine nucleotide-binding proteins and have GTPase action; decreased GTPase action means that Ras remains activated. EGF, epidermal growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; WTS, watch this space. **Her2* is also termed *her2/neu*.

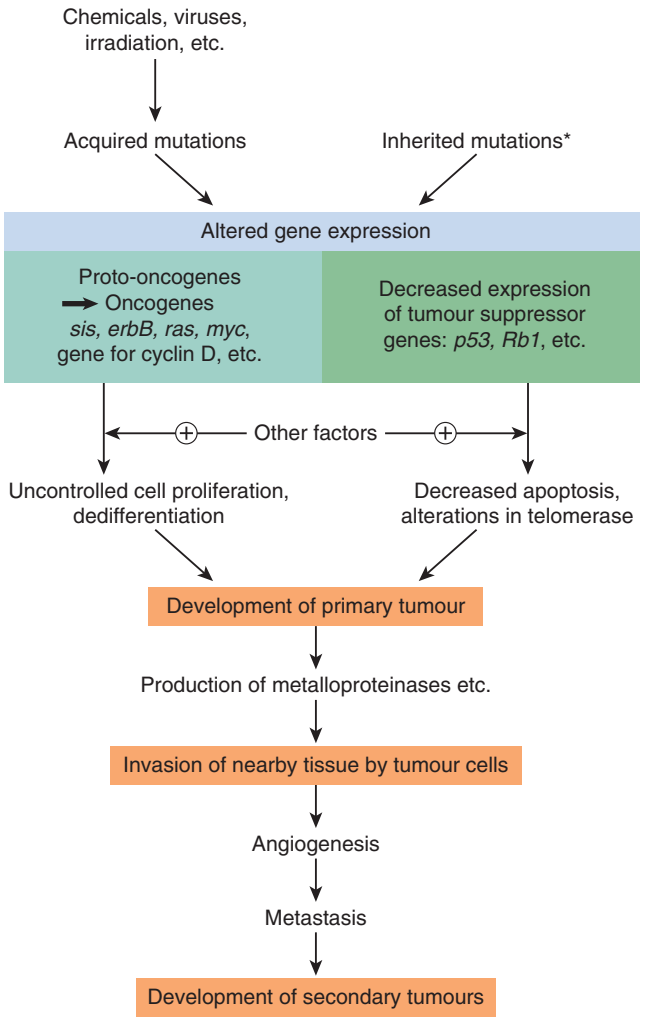


Fig. 55.2 Simplified outline of the genesis of cancer. The diagram summarises the information given in the text. The genesis of cancer is usually multifactorial, involving more than one genetic change. 'Other factors', as specified above, may involve the actions of promoters, co-carcinogens, hormones, etc. which, while not themselves carcinogenic, increase the likelihood that genetic mutation(s) will result in cancer.

prerequisite for cancer; indeed, resistance to apoptosis is a hallmark of the disease. It can be brought about by inactivation of proapoptotic factors or by activation of antiapoptotic factors.

Telomerase expression

Telomeres are specialised structures that cap the ends of chromosomes—like the small metal tubes on the end of shoelaces—protecting them from degradation, rearrangement and fusion with other chromosomes. Furthermore, DNA polymerase cannot easily duplicate the last few nucleotides at the ends of DNA, and telomeres prevent loss of the 'end' genes. With each round of cell division, a portion of the telomere is eroded, so that eventually it becomes non-functional. At this point, DNA replication ceases and the cell becomes senescent.

Rapidly dividing cells, such as stem cells and those of the bone marrow, the germline and the epithelium of the gastrointestinal tract, express *telomerase*, an enzyme that maintains and stabilises telomeres. While it is absent from most fully differentiated somatic cells, about 95% of late-stage malignant tumours do express the enzyme, and it is this that may confer 'immortality' on cancer cells.

The control of tumour-related blood vessels

The factors described above lead to the uncontrolled proliferation of individual cancer cells, but other factors, particularly blood supply, determine the actual growth of a solid tumour. Tumours 1–2 mm in diameter can obtain nutrients by diffusion, but any further expansion requires *angiogenesis*, the development of new blood vessels. Angiogenesis occurs in response to growth factors produced by the growing tumour (see Griffioen & Molema, 2000).

DEDIFFERENTIATION AND LOSS OF FUNCTION

The multiplication of normal cells in a tissue begins with division of the undifferentiated stem cells giving rise to *daughter cells*. These daughter cells eventually differentiate to become the mature cells of the relevant tissue, ready to perform their programmed functions. For example, when fibroblasts mature, they secrete and organise extracellular matrix; mature muscle cells are capable of contraction. One of the main characteristics of cancer cells is that they dedifferentiate to varying degrees. In general, poorly differentiated cancers multiply faster and carry a worse prognosis than well-differentiated cancers.

INVASIVENESS

Normal cells are not generally found outside their 'designated' tissue of origin. This is because, during differentiation and tissue or organ growth, normal cells develop certain spatial relationships with respect to each other. These relationships are maintained by various tissue-specific survival factors that prevent apoptosis (see Ch. 5). In this way, any cells that escape accidentally lose these survival signals and die.

Consequently, although the cells of the normal mucosal epithelium of the rectum proliferate continuously as the lining is shed, they remain as a lining epithelium. A cancer of the rectal mucosa, by comparison, invades other tissues forming the rectum and often the tissues of other pelvic organs. Cancer cells have not only lost, through mutation, the restraints that act on normal cells, but they also secrete enzymes (e.g. metalloproteinases; see Ch. 5) that break down the extracellular matrix, enabling them to move around.

METASTASIS

Metastases are secondary tumours ('secondaries') formed by cells that have been released from the initial or *primary tumour* and which have reached other sites through blood vessels or lymphatics, by transportation on other cells or as a result of being shed into body cavities. Metastases are the principal cause of mortality and morbidity in most cancers and constitute a major problem for cancer therapy.

As discussed above, dislodgment or aberrant migration of normal cells would lead to programmed cell death as a result of withdrawal of the necessary antiapoptotic factors. Cancer cells that metastasise have undergone a series of genetic changes that alter their responses to the regulatory factors that control the cellular architecture of normal tissues, enabling them to establish themselves

'extraterritorially'. Tumour-induced growth of new blood vessels locally (see above) favours metastasis.

Secondary tumours occur more frequently in some tissues than in others. For example, metastases of mammary cancers are often found in lung, bone and brain. The reason for this is that breast cancer cells express chemokine receptors such as CXR4 (see Ch. 17) on their surfaces, and chemokines that recognise these receptors are expressed at high level in these tissues but not in others (e.g. kidney), facilitating the selective accumulation of cells at these sites.

GENERAL PRINCIPLES OF CYTOTOXIC ANTICANCER DRUGS

In experiments with rapidly growing transplantable leukaemias in mice, it has been found that a given therapeutic dose of a cytotoxic drug[1] destroys a constant fraction of the malignant cells. Thus a dose that kills 99.99% of cells, if used to treat a tumour with 10^{11} cells, will still leave 10 million (10^7) viable malignant cells. As the same principle holds for fast-growing tumours in humans, schedules for chemotherapy are aimed at producing as near a total cell kill as possible because, in contrast to the situation that occurs in microorganisms, little reliance can be placed on the host's immunological defence mechanisms against the remaining cancer cells.

One of the major difficulties in treating cancer is that tumour growth is usually far advanced before cancer is diagnosed. Let us suppose that a tumour arises from a single cell and that the growth is exponential, as it may well be during the initial stages. 'Doubling' times vary, being, for example, approximately 24 h with Burkitt's lymphoma, 2 weeks in the case of some leukaemias, and 3 months with mammary cancers. Approximately 30 doublings would be required to produce a cell mass with a diameter of 2 cm, containing 10^9 cells. Such a tumour is within the limits of diagnostic procedures, although it could easily go unnoticed. A further 10 doublings would produce 10^{12} cells, a tumour mass that is likely to be lethal, and which would measure about 20 cm in diameter if it were one solid mass.

However, continuous exponential growth of this sort does not usually occur. In the case of most solid tumours (for example of lung, stomach, uterus and so on), as opposed to *leukaemias* (tumours of white blood cells), the growth rate falls as the neoplasm grows. This is partly because the tumour outgrows its blood supply, and partly because not all the cells proliferate continuously. The cells of a solid tumour can be considered as belonging to three compartments:

1. *Compartment A* consists of dividing cells, possibly being continuously in cell cycle.
2. *Compartment B* consists of resting cells (G_0 phase) which, although not dividing, are potentially able to do so.
3. *Compartment C* consists of cells that are no longer able to divide but which contribute to the tumour volume.

Essentially, only cells in *compartment A*, which may form as little as 5% of some solid tumours, are susceptible to the main current cytotoxic drugs, as is explained below. The cells in *compartment C* do not constitute a problem, but it is the existence of *compartment B* that makes cancer chemotherapy difficult, because these cells are not very sensitive to cytotoxic drugs and are liable to re-enter *compartment A* following chemotherapy.

Most current anticancer drugs, particularly cytotoxic agents, affect only one characteristic aspect of cancer cell biology—cell division—but have no specific inhibitory effect on invasiveness, the loss of differentiation or the tendency to metastasise. In many cases, the antiproliferative action results from an action during S phase of the cell cycle, and the resultant damage to DNA initiates apoptosis (see above). Furthermore, because their main target is cell division, they will affect all rapidly dividing normal tissues, and thus they are likely to produce, to a greater or lesser extent, the following general toxic effects:

- *bone marrow toxicity* (myelosuppression) with decreased leukocyte production and thus decreased resistance to infection
- *impaired wound healing*
- *loss of hair* (alopecia)
- damage to *gastrointestinal epithelium* (including oral mucous membranes)
- *depression of growth* in children
- *sterility*
- *teratogenicity*.

They can also, in certain circumstances, be themselves carcinogenic. Rapid cell destruction also entails extensive purine catabolism, and urates may precipitate in the renal tubules and cause kidney damage. Finally, in addition to

Cancer pathogenesis and cancer chemotherapy: general principles

- Cancer arises as a result of a series of genetic and epigenetic changes, the main genetic lesions being:
  - inactivation of tumour suppressor genes
  - the activation of oncogenes (mutation of the normal genes controlling cell division and other processes).
- Cancer cells have four characteristics that distinguish them from normal cells:
  - uncontrolled proliferation
  - loss of function because of lack of capacity to differentiate
  - invasiveness
  - the ability to metastasise.
- Cancer cells have uncontrolled proliferation often because of changes in:
  - growth factors and/or their receptors
  - intracellular signalling pathways, particularly those controlling the cell cycle and apoptosis
  - telomerase expression.
- This may be supported by tumour-related angiogenesis.
- Most anticancer drugs are antiproliferative—most damage DNA and thereby initiate apoptosis. They also affect rapidly dividing normal cells and are thus likely to depress bone marrow, impair healing and depress growth. Most cause nausea, vomiting, sterility, hair loss and teratogenicity.

[1]The term *cytotoxic* drug applies to any drug that can damage or kill cells. In practice, it is used more restrictively to refer to drugs that inhibit cell division and are therefore potentially useful in cancer chemotherapy.

specific toxic effects associated with individual drugs, virtually all cytotoxic drugs produce severe nausea and vomiting, which has been called the 'inbuilt deterrent' to patient compliance in completing a course of treatment with these agents.

ANTICANCER DRUGS

The main anticancer drugs can be divided into the following general categories:

- *Cytotoxic drugs*. The mechanism of action of these drugs is discussed more fully below and summarised in Table 55.1; they include:
  - *alkylating agents* and related compounds, which act by forming covalent bonds with DNA and thus impeding replication
  - *antimetabolites*, which block or subvert one or more of the metabolic pathways involved in DNA synthesis
  - *cytotoxic antibiotics*, i.e. substances of microbial origin that prevent mammalian cell division
  - *plant derivatives* (vinca alkaloids, taxanes, campothecins): most of these specifically affect microtubule function and hence the formation of the mitotic spindle.
- *Hormones*, of which the most important are steroids (e.g. glucocorticoids, oestrogens and androgens) as well as drugs that suppress hormone secretion or antagonise hormone action.
- *Monoclonal antibodies*: these are generally only of use in particular types of cancer.
- *Protein kinase inhibitors*: these drugs inhibit protein (usually tyrosine) kinases that transduce growth signals in rapidly dividing cells. They have a rather restricted use.
- *Miscellaneous agents* that do not easily fit into the above categories.

Table 55.1 An overview of anticancer drugs

Type	Group	Examples	Main mechanism
Alkylating and related agents	Nitrogen mustards	Cyclophosphamide, ifosfamide, chlorambucil, melphalan, estramustine,	Intrastrand cross-linking of DNA
	Nitrosoureas	Lomustine, carmustine,	
	Platinum compounds	Carboplatin, cisplatin, oxaliplatin	
	Other	Busulfan, treosulfan, thiotepa, dacarbazine, procarbazine, temozolimide	
Antimetabolites	Folate antagonists	Methotrexate, raltitrexed, pemetrexed	Blocking the synthesis of DNA and/or RNA
	Pyrimdine pathway	Fluorouracil, capecitabine, cytarabine, gemcitabine, tegafur	
	Purine pathway	Fludarabine, cladibrine, mercaptopurine, tioguanine, pentostatin, clofarabrine, nelarabine	
Cytotoxic antibiotics	Anthracyclines	Daunorubicin, doxorubicin, epirubicin, idarubicin, (mitoxantrine), (amascrine)	Multiple effects on DNA/RNA synthesis and topisomerase action
	Other	Bleomycin, dactinomycin, mitomycin	
Plant derivatives	Taxanes	Paclitaxel, docetaxel	Microtubule assembly; prevents spindle formation
	Vinca alkaloids	Vinblastine, vincristine, vindesine, vinorelbine	
	Campothecins	Irinotecan, topotecan, trabectedin	Inhibition of topoisomerase
	Other	Etoposide	
Hormones/antagonists	Hormones/analogues	Diethylstilbestrol, ethinyloestradiol, medroxyprogesterone, megesterol, norhisterone, goserelin, leuporelin, triptorelin, lanreotide, octreotide	Act as physiological antagonists, antagonists or hormone synthesis inhibitors to disrupt hormone-dependent tumour growth
	Antagonists	Tamoxifen, toremifine, fulvestrant, cyproterone, flutamide, bicalutamide	
	Aromatase inhibitors	Anastrozole, letrozole, exemastine	
Protein kinase inhibitors	Tyrosine kinase inhibitors	Dasatinib, erlotinib, imatinib, nilotinib, sunitinib	Inhibition of kinases involved in growth factor receptor transduction
	Pan kinase inhibitors	Sorafenib	
Monoclonal antibodies	Anti-EGF, EGF-2	Panitumumab, trastuzumab	Blocks cell proliferation
	Anti-CD20/CD52	Rituximab, alemtuzumab	Inhibition of lymphocyte proliferation
	Anti-VEGF	Bevacizumab	Prevents angiogenesis

Drugs in parentheses have similar pharmacological actions but are not necessarily chemically related.

The clinical use of anticancer drugs is the province of the specialist, who selects treatment regimens appropriate to the patient with the objective of curing, prolonging life or providing palliative therapy.[2] There are over 80 drugs available in the UK, which are often used in combination. Here we discuss mechanisms of action and the main unwanted effects of commonly used anticancer agents. A recent textbook (Airley, 2009) provides detailed information.

ALKYLATING AGENTS AND RELATED COMPOUNDS

Alkylating agents and related compounds contain chemical groups that can form covalent bonds with particular nucleophilic substances in the cell. With alkylating agents themselves, the main step is the formation of a *carbonium ion*—a carbon atom with only six electrons in its outer shell. Such ions are highly reactive and react instantaneously with an electron donor such as an amine, hydroxyl or sulfhydryl group. Most of the cytotoxic anticancer alkylating agents are *bifunctional*, i.e. they have two alkylating groups (Fig. 55.3).

The nitrogen at position 7 (N7) of guanine, being strongly nucleophilic, is probably the main molecular target for alkylation in DNA (Fig. 55.3), although N1 and N3 of adenine and N3 of cytosine may also be affected. A bifunctional agent, by reacting with two groups, can cause intra- or interchain cross-linking (Fig. 55.3). This interferes not only with transcription, but also with replication, which is probably the critical effect of anticancer alkylating agents. Other effects of alkylation at guanine N7 are excision of the guanine base with main chain scission, or pairing of the alkylated guanine with thymine instead of cytosine, and eventual substitution of the GC pair by an AT pair. Their main impact is seen during replication (S phase), when some zones of the DNA are unpaired and more susceptible to alkylation. This results in a block at G_2 (see Fig. 55.3) and subsequent apoptotic cell death.

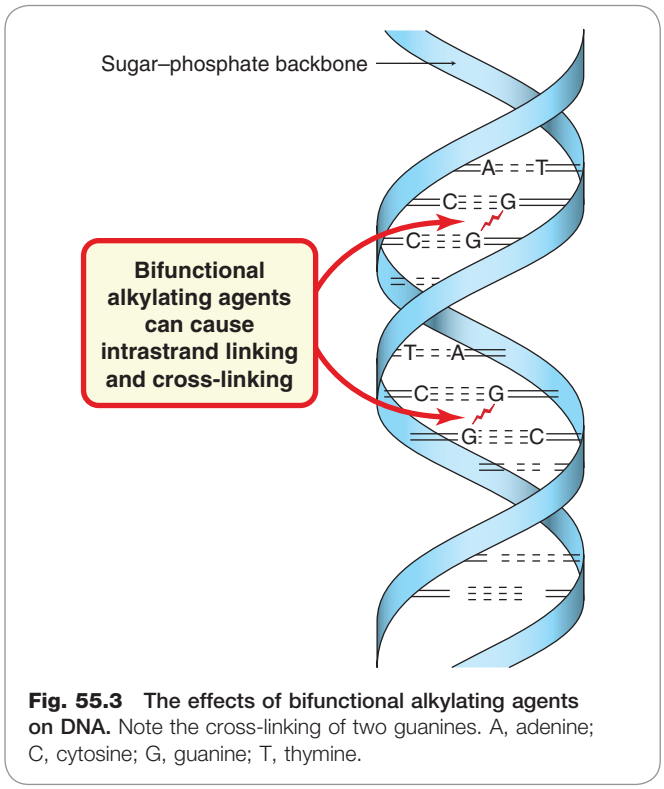


Fig. 55.3 The effects of bifunctional alkylating agents on DNA. Note the cross-linking of two guanines. A, adenine; C, cytosine; G, guanine; T, thymine.

All alkylating agents depress bone marrow function and cause gastrointestinal disturbances. With prolonged use, two further unwanted effects occur: depression of *gametogenesis* (particularly in men), leading to sterility, and an increased risk of acute *non-lymphocytic leukaemia* and other malignancies.

Alkylating agents are among the most commonly employed of all anticancer drugs. A large number are available for use in cancer chemotherapy (some dozen are approved in the UK at the time of writing). Only a few commonly used ones will be dealt with here.

Nitrogen mustards

Nitrogen mustards are related to the 'mustard gas' used during the First World War; their basic formula (R-*N*-*bis*-(2-chloroethyl)) is shown in Figure 55.4. In the body, each 2-chloroethyl side-chain undergoes an intramolecular cyclisation with the release of a Cl^-. The highly reactive *ethylene immonium* derivative so formed can interact with DNA (see Figs 55.3 and 55.4) and other molecules.

Cyclophosphamide is probably the most commonly used alkylating agent. It is inactive until metabolised in the liver by the P450 mixed function oxidases (see Ch. 9). It has a pronounced effect on lymphocytes and can also be used as an immunosuppressant (see Ch. 26). It is usually given orally or by intravenous injection but may also be given intramuscularly. Important toxic effects are nausea and vomiting, bone marrow depression and haemorrhagic cystitis. This last effect (which also occurs with the related drug **ifosfamide**) is caused by the metabolite *acrolein* and can be ameliorated by increasing fluid intake and administering compounds that are sulfhydryl donors, such as ***N*-acetylcysteine** or **mesna** (sodium-2-mercaptoethane sulfonate). These agents interact specifically with acrolein, forming a non-toxic compound. See also Chapters 9 and 57. Other nitrogen mustards used include **melphalan** and **chlorambucil**.

Estramustine is a combination of chlormethine (mustine) with an oestrogen. It has both cytotoxic and hormonal action, and is generally used for the treatment of prostate cancer.

Nitrosoureas

Examples include **lomustine** and **carmustine**. As they are lipid soluble and cross the blood–brain barrier, they may be used against tumours of the brain and meninges. However, most nitrosoureas have a severe cumulative depressive effect on the bone marrow that starts 3–6 weeks after initiation of treatment.

Other alkylating agents

Busulfan has a selective effect on the bone marrow, depressing the formation of granulocytes and platelets in low dosage and of red cells in higher dosage. It has little or no effect on lymphoid tissue or the gastrointestinal tract. It is used in chronic granulocytic leukaemia.

[2]You will have gathered that many anticancer drugs are toxic. 'To be an oncologist,' one practitioner quipped, 'one has to hate cancer more than one loves life.'

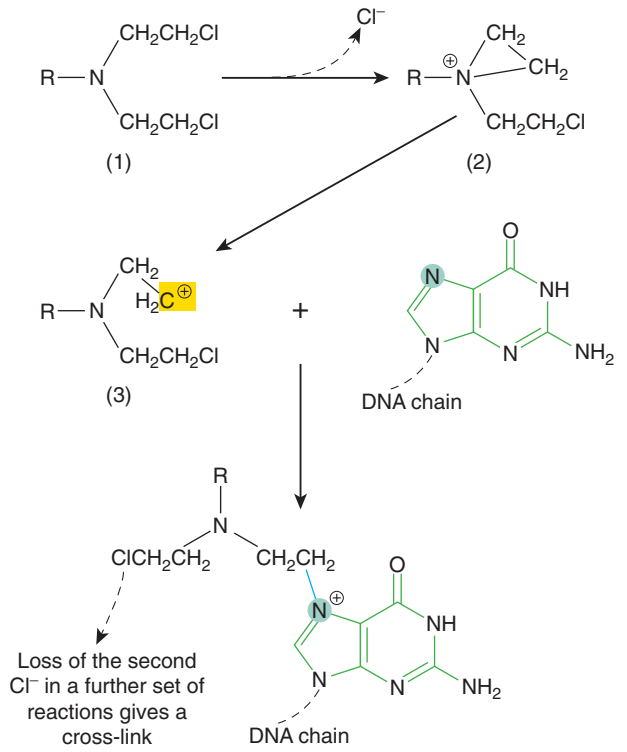


Fig. 55.4 An example of alkylation and cross-linking of DNA by a nitrogen mustard. A bis(chloroethyl)amine (1) undergoes intramolecular cyclisation, forming an unstable ethylene immonium cation (2) and releasing Cl^-, the tertiary amine being transformed to a quaternary ammonium compound. The strained ring of the ethylene immonium intermediate opens to form a reactive carbonium ion (in yellow box) (3), which reacts immediately with N7 of guanine (in green circle) to give 7-alkylguanine (bond shown in blue), the N7 being converted to a quaternary ammonium nitrogen. These reactions can then be repeated with the other $–CH_2CH_2Cl$ to give a cross-link.

Anticancer drugs: alkylating agents and related compounds

- Alkylating agents have groups that form covalent bonds with cell substituents; a carbonium ion is the reactive intermediate. Most have two alkylating groups and can cross-link two nucleophilic sites such as the N7 of guanine in DNA. Cross-linking can cause defective replication through pairing of alkylguanine and thymine, leading to substitution of AT for GC, or it can cause excision of guanine and chain breakage.
- Their principal effect occurs during DNA synthesis and the resulting damage triggers apoptosis.
- Unwanted effects include myelosuppression, sterility and risk of non-lymphocytic leukaemia.
- The main alkylating agents are:
  - nitrogen mustards, for example **cyclophosphamide**, which is activated to give aldophosphamide, then converted to phosphoramide mustard (the cytotoxic molecule) and acrolein (which causes bladder damage that can be ameliorated by mesna). Cyclophosphamide myelosuppression affects particularly the lymphocytes
  - nitrosoureas, for example **lomustine**, may act on non-dividing cells, can cross the blood–brain barrier and cause delayed, cumulative myelotoxicity.
- Platinum compounds (e.g. **cisplatin**) cause intrastrand linking in DNA. Cisplatin has low myelotoxicity but causes severe nausea and vomiting, and can be nephrotoxic. It has revolutionised the treatment of germ cell tumours.

Dacarbazine, a prodrug, is activated in the liver, and the resulting compound is subsequently cleaved in the target cell to release an alkylating derivative. Unwanted effects include myelotoxicity and severe nausea and vomiting. **Temozolomide** is a related compound with a restricted usage (malignant glioma).

Procarbazine inhibits DNA and RNA synthesis and interferes with mitosis at interphase. Its effects may be mediated by the production of active metabolites. It is given orally, and its main use is in Hodgkin's disease. It causes **disulfiram**-like actions with alcohol (see Ch. 56), exacerbates the effects of central nervous system depressants and, because it is a weak monoamine oxidase inhibitor, can produce hypertension if given with certain sympathomimetic agents (see Ch. 46). It causes the usual unwanted effects, and can be leukaemogenic, carcinogenic and teratogenic. Allergic skin reactions may necessitate cessation of treatment.

Other alkylating agents in clinical use include **thiotepa** and **treosulfan**.

Platinum compounds

Cisplatin is a water-soluble planar coordination complex containing a central platinum atom surrounded by two chlorine atoms and two ammonia groups. Its action is analogous to that of the alkylating agents. When it enters the cell, Cl^- dissociates, leaving a reactive complex that reacts with water and then interacts with DNA. It causes intrastrand cross-linking, probably between N7 and O6 of adjacent guanine molecules, which results in local denaturation of DNA.

Cisplatin has revolutionised the treatment of solid tumours of the testes and ovary. Therapeutically, it is given by slow intravenous injection or infusion. It is seriously nephrotoxic, and strict regimens of hydration and diuresis must be instituted. It has low myelotoxicity but causes very severe nausea and vomiting. The $5\text{-}HT_3$ receptor antagonists (e.g. **ondansetron**; see Chs 15, 29 and 38) are very effective in preventing this and have transformed cisplatin-based chemotherapy. Tinnitus and hearing loss in the high-frequency range may occur, as may peripheral neuropathies, hyperuricaemia and anaphylactic reactions.

Carboplatin is a derivative of cisplatin. Because it causes less nephrotoxicity, neurotoxicity, ototoxicity, nausea and vomiting than cisplatin (although it is more myelotoxic), it is sometimes given on an outpatient basis. **Oxaliplatin** is another platinum-containing compound with a restricted application.

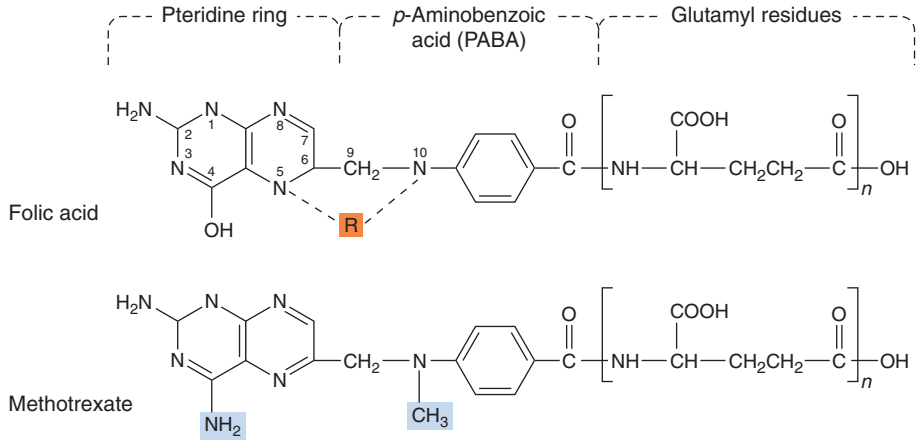


Fig. 55.5 Structure of folic acid and methotrexate. Both compounds are shown as polyglutamates. In tetrahydrofolate, one-carbon groups (R, in orange box) are transported on N5 or N10 or both (shown dotted). The points at which methotrexate differs from endogenous folic acid are shown in the blue boxes.

ANTIMETABOLITES

Folate antagonists

The main folate antagonist is **methotrexate**, one of the most widely used antimetabolites in cancer chemotherapy. Folates are essential for the synthesis of purine nucleotides and thymidylate, which in turn are essential for DNA synthesis and cell division. (This topic is also dealt with in Chs 25, 49 and 53.) The main action of the folate antagonists is to interfere with thymidylate synthesis.

In structure, folates consist of three elements: a *pteridine ring*, p-*aminobenzoic acid* and *glutamic acid* (Fig. 55.5). Folates are actively taken up into cells, where they are converted to polyglutamates. In order to act as coenzymes, folates must be reduced to tetrahydrofolate (FH_4). This two-step reaction is catalysed by *dihydrofolate reductase*, which converts the substrate first to dihydrofolate (FH_2), then to FH_4 (Fig. 55.6). FH_4 functions as an essential co-factor carrying the methyl groups necessary for the transformation of 2′-deoxyuridylate (DUMP) to the 2′-deoxythymidylate (DTMP) required for the synthesis of DNA and purines. During the formation of DTMP from DUMP, FH_4 is converted back to FH_2, enabling the cycle to repeat. Methotrexate has a higher affinity than FH_2 for dihydrofolate reductase and thus inhibits the enzyme (Fig. 55.6), depleting intracellular FH_4. The binding of methotrexate to dihydrofolate reductase involves an additional bond not present when FH_2 binds. The reaction most sensitive to FH_4 depletion is DTMP formation.

Methotrexate is usually given orally but can also be given intramuscularly, intravenously or intrathecally. The drug has low lipid solubility and thus does not readily cross the blood–brain barrier. It is, however, actively taken up into cells by the folate transport system and is metabolised to polyglutamate derivatives, which are retained in the cell for weeks (or even months in some cases) in the absence of extracellular drug. Resistance to methotrexate may develop in tumour cells by a variety of mechanisms (see below). Methotrexate is also used as an immunosuppressant drug to treat rheumatoid arthritis and other autoimmune conditions (see Ch. 26).

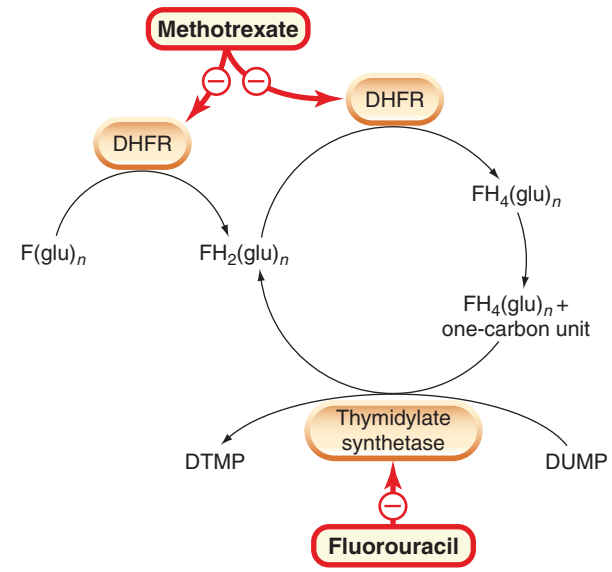


Fig. 55.6 Simplified diagram of action of methotrexate and fluorouracil on thymidylate synthesis. Tetrahydrofolate polyglutamate $FH_4(glu)_n$ functions as a carrier of a one-carbon unit, providing the methyl group necessary for the conversion of 2′-deoxyuridylate (DUMP) to 2′-deoxythymidylate (DTMP) by thymidylate synthetase. This one-carbon transfer results in the oxidation of $FH_4(glu)_n$ to $FH_2(glu)_n$. Fluorouracil is converted to FDUMP, which inhibits thymidylate synthetase. DHFR, dihydrofolate reductase.

Unwanted effects include depression of the bone marrow and damage to the epithelium of the gastrointestinal tract. Pneumonitis can occur. In addition, high-dose regimens—doses 10 times greater than the standard doses, sometimes used in patients with methotrexate resistance—can lead to nephrotoxicity, caused by precipitation of the drug or a metabolite in the renal tubules. High-dose regimens must be followed by 'rescue' with *folinic acid* (a form of FH_4).

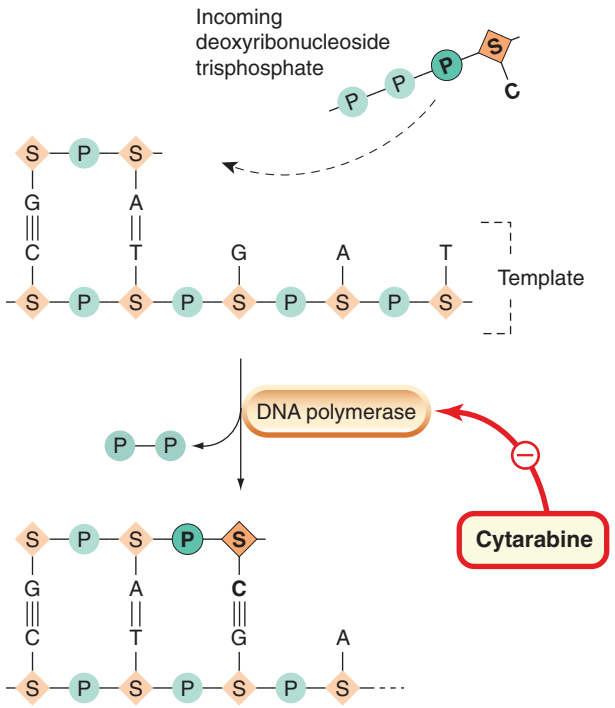


Fig. 55.7 The mechanism of action of cytarabine (cytosine arabinoside). For details of DNA polymerase action, see Figure 49.8. Cytarabine is an analogue of cytosine.

Chemically related to folate, **raltitrexed** also inhibits thymidylate synthetase and **pemetrexed**, thymidylate transferase.

Pyrimidine analogues

Fluorouracil, an analogue of uracil, also interferes with DTMP synthesis (Fig. 55.6). It is converted into a 'fraudulent' nucleotide, *fluorodeoxyuridine monophosphate* (FDUMP). This interacts with thymidylate synthetase but cannot be converted into DTMP. The result is inhibition of DNA but not RNA or protein synthesis.

Fluorouracil is usually given parenterally. The main *unwanted effects* are gastrointestinal epithelial damage and myelotoxicity. Cerebellar disturbances can also occur. Another drug, **capecitabine**, is metabolised to fluorouracil as is **tegafur**.

Cytarabine (cytosine arabinoside) is an analogue of the naturally occurring nucleoside 2'-deoxycytidine. The drug enters the target cell and undergoes the same phosphorylation reactions as the endogenous nucleoside to give *cytosine arabinoside trisphosphate*, which inhibits DNA polymerase (see Fig. 55.7). The main *unwanted effects* are on the bone marrow and the gastrointestinal tract. It also causes nausea and vomiting.

Gemcitabine, an analogue of cytarabine, has fewer *unwanted actions*, mainly an influenza-like syndrome and mild myelotoxicity. It is often given in combination with other drugs such as cisplatin.

Purine analogues

The main anticancer purine analogues include **fludarabine**, **pentostatin**, **cladribine**, **clofarabrine**, **nelarabrine**, **mercaptopurine** and **tioguanine**.

Anticancer drugs: antimetabolites

- Antimetabolites block or subvert pathways of DNA synthesis.
- *Folate antagonists*. **Methotrexate** inhibits dihydrofolate reductase, preventing generation of tetrahydrofolate interfering with thymidylate synthesis. Methotrexate is taken up into cells by the folate carrier and, like folate, is converted to the polyglutamate form. Normal cells affected by high doses can be 'rescued' by folinic acid. Unwanted effects are myelosuppression and possible nephrotoxicity.
- *Pyrimidine analogues*. **Fluorouracil** is converted to a 'fraudulent' nucleotide and inhibits thymidylate synthesis. **Cytarabine** in its trisphosphate form inhibits DNA polymerase. They are potent myelosuppressives.
- *Purine analogues*. **Mercaptopurine** is converted into fraudulent nucleotide. **Fludarabine** in its trisphosphate form inhibits DNA polymerase and is myelosuppressive. **Pentostatin** inhibits adenosine deaminase—a critical pathway in purine metabolism.

Fludarabine is metabolised to the trisphosphate and inhibits DNA synthesis by actions similar to those of cytarabine. It is myelosuppressive. Pentostatin has a different mechanism of action. It inhibits *adenosine deaminase*, the enzyme that transforms adenosine to inosine. This action interferes with critical pathways in purine metabolism and can have significant effects on cell proliferation. Cladribine, mercaptopurine and tioguanine are used mainly in the treatment of leukaemia.

CYTOTOXIC ANTIBIOTICS

This is a widely used group of drugs that mainly produce their effects through direct action on DNA. As a rule, they should not be given together with radiotherapy, as the cumulative burden of toxicity is very high.

Doxorubicin and the anthracyclines

The main anticancer anthracycline antibiotic is **doxorubicin**. Other related compounds include **idarubicin**, **daunorubicin**, **epirubicin** and **mitoxantrone** (**mitozantrone**). **Amascrine** has a similar action to this group.

Doxorubicin has several cytotoxic actions. It binds to DNA and inhibits both DNA and RNA synthesis, but its main cytotoxic action appears to be mediated through an effect on topoisomerase II (a DNA gyrase; see Ch. 49), the activity of which is markedly increased in proliferating cells. The significance of the enzyme lies in the fact that, during replication of the DNA helix, reversible swivelling needs to take place around the replication fork in order to prevent the daughter DNA molecule becoming inextricably entangled during mitotic segregation. The 'swivel' is produced by topoisomerase II, which nicks both DNA strands and subsequently reseals the breaks. Doxorubicin intercalates in the DNA, and its effect is, in essence, to stabilise the DNA–topoisomerase II complex after the strands have been nicked, thus halting the process at this point.

Doxorubicin is given by intravenous infusion. Extravasation at the injection site can cause local necrosis. In addition to the general unwanted effects, the drug can cause cumulative, dose-related cardiac damage, leading to dysrhythmias and heart failure. This action may be the result of generation of free radicals. Marked hair loss frequently occurs.

Dactinomycin

Dactinomycin intercalates in the minor groove of DNA between adjacent guanosine–cytosine pairs, interfering with the movement of RNA polymerase along the gene and thus preventing transcription. There is also evidence that it has a similar action to that of the anthracyclines on topoisomerase II. It produces most of the toxic effects outlined above, except cardiotoxicity. It is mainly used for treating paediatric cancers.

Bleomycins

The bleomycins are a group of metal-chelating glycopeptide antibiotics that degrade preformed DNA, causing chain fragmentation and release of free bases. This action is thought to involve chelation of ferrous iron and interaction with oxygen, resulting in the oxidation of the iron and generation of superoxide and/or hydroxyl radicals. Bleomycin is most effective in the G_2 phase of the cell cycle and mitosis, but it is also active against non-dividing cells (i.e. cells in the G_0 phase; Fig. 5.4). It is often used to treat germline cancer. In contrast to most anticancer drugs, bleomycin causes little myelosuppression: its most serious toxic effect is pulmonary fibrosis, which occurs in 10% of patients treated and is reported to be fatal in 1%. Allergic reactions can also occur. About half the patients manifest mucocutaneous reactions (the palms are frequently affected), and many develop hyperpyrexia.

Mitomycin

Following enzymic activation, **mitomycin** functions as a bifunctional alkylating agent, binding preferentially at O6 of the guanine nucleus. It cross-links DNA and may also degrade DNA through the generation of free radicals. It causes marked delayed myelosuppression and can also cause kidney damage and fibrosis of lung tissue.

Anticancer drugs: cytotoxic antibiotics

- **Doxorubicin** inhibits DNA and RNA synthesis; the DNA effect is mainly through interference with topoisomerase II action. Unwanted effects include nausea, vomiting, myelosuppression and hair loss. It is cardiotoxic in high doses.
- **Bleomycin** causes fragmentation of DNA chains. It acts on non-dividing cells. Unwanted effects include fever, allergies, mucocutaneous reactions and pulmonary fibrosis. There is virtually no myelosuppression.
- **Dactinomycin** intercalates in DNA, interfering with RNA polymerase and inhibiting transcription. It also interferes with the action of topoisomerase II. Unwanted effects include nausea, vomiting and myelosuppression.
- **Mitomycin** is activated to give an alkylating metabolite.

PLANT DERIVATIVES

Several naturally occurring plant products exert potent cytotoxic effects and have earned a place in the arsenal of anticancer drugs on that basis.

Vinca alkaloids

The vinca alkaloids are derived from the *Madagascar periwinkle (Catharanthus roseus)*. The principal members of the group are **vincristine**, **vinblastine** and **vindesine**. **Vinorelbine** is a semisynthetic vinca alkaloid with similar properties that is mainly used in breast cancer. The drugs bind to tubulin and inhibit its polymerisation into microtubules, preventing spindle formation in dividing cells and causing arrest at metaphase. Their effects become manifest only during mitosis. They also inhibit other cellular activities that involve the microtubules, such as leukocyte phagocytosis and chemotaxis, as well as axonal transport in neurons.

The vinca alkaloids are relatively non-toxic. Vincristine has very mild myelosuppressive activity but causes paraesthesias (sensory changes), abdominal pain and muscle weakness fairly frequently. Vinblastine is less neurotoxic but causes leukopenia, while vindesine has both moderate myelotoxicity and neurotoxicity. All members of the group can cause reversible alopecia.

Paclitaxel and docetaxel

These *taxanes* are derived from a naturally occurring compound found in the bark of the yew tree (*Taxus* spp.). They act on microtubules, stabilising them (in effect 'freezing' them) in the polymerised state, achieving a similar effect to that of the vinca alkaloids. Paclitaxel is given by intravenous infusion and docetaxel by mouth. Both have a place in the treatment of breast cancer, and paclitaxel, given with carboplatin, is the treatment of choice for ovarian cancer.

Unwanted effects, which can be serious, include bone marrow suppression and cumulative neurotoxicity. Resistant fluid retention (particularly oedema of the legs) can occur with docetaxel. Hypersensitivity to both compounds is liable to occur and requires pretreatment with corticosteroids and antihistamines.

Camptothecins

The camptothecins **irinotecan** and **topotecan**, isolated from the stem of the tree *Camptotheca acuminata*, bind to and inhibit topoisomerase I, high levels of which occur throughout the cell cycle. Diarrhoea and reversible bone marrow depression occur but, in general, these alkaloids have fewer unwanted effects than most other anticancer agents.

Etoposide

Etoposide is derived from mandrake root (*Podophyllum peltatum*). Its mode of action is not clearly known, but it may act by inhibiting mitochondrial function and nucleoside transport, as well as having an effect on topoisomerase II similar to doxorubicin (see above). *Unwanted effects* include nausea and vomiting, myelosuppression and hair loss.

HORMONES

Tumours arising in hormone-sensitive tissues (e.g. breast, uterus, prostate gland) may be *hormone dependent*, an effect related to the presence of hormone receptors in the malignant cells. Their growth can be inhibited by hormones with opposing actions, by hormone antagonists or by agents

Anticancer drugs: plant derivatives

- **Vincristine** inhibits mitosis at metaphase by binding to tubulin. It is relatively non-toxic but can cause unwanted neuromuscular effects.
- **Etoposide** inhibits DNA synthesis by an action on topoisomerase II and also inhibits mitochondrial function. Common unwanted effects include vomiting, myelosuppression and alopecia.
- **Paclitaxel** stabilises microtubules, inhibiting mitosis; it is relatively toxic and hypersensitivity reactions occur.
- **Irinotecan** inhibits topoisomerase I; it has relatively few toxic effects.

that inhibit the endogenous hormone synthesis. Hormones or their analogues that have inhibitory actions on target tissues can be used in treatment of tumours of those tissues. Such procedures alone rarely effect a cure but do retard tumour growth and mitigate the symptoms of the cancer, and thus play an important part in the clinical management of sex hormone-dependent tumours.

Glucocorticoids

Glucocorticoids such as **prednisolone** and **dexamethasone** have marked inhibitory effects on lymphocyte proliferation (see Ch. 26) and are used in the treatment of leukaemias and lymphomas. Their ability to lower raised intracranial pressure, and to mitigate some of the side effects of anticancer drugs, such as nausea and vomiting, makes them useful as supportive therapy when treating other cancers, as well as in palliative care.

Oestrogens

Diethylstilbestrol and **ethinyloestradiol** are two oestrogens used clinically as physiological antagonists in the palliative treatment of androgen-dependent prostatic tumours. The latter compound has fewer side effects. These tumours are also treated with gonadotrophin-releasing hormone analogues (see below).

Oestrogens can also be used to recruit resting mammary cancer cells (i.e. cells in compartment B; see above) into the proliferating pool of cells (i.e. into compartment A), thus facilitating killing by other, cytotoxic drugs.

Progestogens

Progestogens such as **megestrol**, **norehisterone** and **medroxyprogesterone** have been useful in endometrial neoplasms and in renal tumours.

Gonadotrophin-releasing hormone analogues

As explained in Chapter 34, analogues of the gonadotrophin-releasing hormones, such as **goserelin**, **buserelin**, **leuprorelin** and **triptorelin**, can, under certain circumstances, inhibit gonadotrophin release. These agents are therefore used to treat advanced breast cancer in premenopausal women and prostate cancer. The effect of the transient surge of testosterone secretion that can occur in patients treated in this way for prostate cancer must be prevented by an antiandrogen such as **cyproterone**.

Somatostatin analogues

Analogues of somatostatin such as **octreotide** and **lanreotide** (see Ch. 32) are used to relieve the symptoms of neuroendocrine tumours, including hormone-secreting tumours of the gastrointestinal tract such as VIPomas, glucagonomas, carcinoid tumours and gastrinomas. These tumours express somatostatin receptors, activation of which inhibits cell proliferation as well as hormone secretion.

HORMONE ANTAGONISTS

In addition to the hormones themselves, hormone antagonists can also be effective in the treatment of several types of hormone-sensitive tumours.

Antioestrogens

An antioestrogen, **tamoxifen**, is remarkably effective in some cases of hormone-dependent breast cancer and may have a role in preventing these cancers. In breast tissue, tamoxifen competes with endogenous oestrogens for the oestrogen receptors and therefore inhibits the transcription of oestrogen-responsive genes. Tamoxifen is also reported to have cardioprotective effects, partly by virtue of its ability to protect low-density-lipoproteins against oxidative damage.

Unwanted effects are similar to those experienced by women following the menopause. Potentially more serious are hyperplastic events in the endometrium, which may progress to malignant changes, and the risk of thromboembolism.

Other oestrogen receptor antagonists include **toremifene** and **fulvestrant**. Aromatase inhibitors such as **anastrozole**, **letrozole** and **exemestane**, which suppress the synthesis of oestrogen from androgens, are also effective in the treatment of breast cancer. **Aminoglutethimide**, which blocks the generation of all steroids, has been largely replaced by the aromatase inhibitors.

Antiandrogens

The androgen antagonists, **flutamide**, **cyproterone** and **bicalutamide**, may be used either alone or in combination with other agents to treat tumours of the prostate. They are also used to control the testosterone surge ('flare') that is seen when treating patients with gonadorelin analogues (see above).

Adrenal hormone synthesis inhibitors

Several agents that inhibit synthesis of adrenal hormones have effects in postmenopausal breast cancer. The drugs used are **trilostane** and (rarely today) aminoglutethimide, which inhibit the early stages of sex hormone synthesis. Replacement of corticosteroids is necessary with these agents.

MONOCLONAL ANTIBODIES

Monoclonal antibodies are immunoglobulins, of one molecular type,[3] produced by hybridoma cells in culture, that react with defined target proteins expressed on cancer cells. Some are *humanised*, meaning that they are hybrids

[3]As opposed to the 'polyclonal' antibodies produced by the body in response to a foreign antigen, which comprise a complex (and variable) molecular species.

Anticancer agents: hormones

Hormones or their antagonists are used in hormone-sensitive tumours:

- **Glucocorticoids** for leukaemias and lymphomas.
- **Tamoxifen** for breast tumours.
- **Gonadotrophin-releasing hormone analogues** for prostate and breast tumours.
- **Antiandrogens** for prostate cancer.
- **Inhibitors of sex hormone synthesis** for postmenopausal breast cancer.

or *chimeras* of human antibodies with a murine or primate backbone[4] (and hence are less likely to be immunogenic in their own right; see Ch. 59 for more details). In some cases, binding of the antibody to its target activates the host's immune mechanisms and the cancer cell is killed by complement-mediated lysis or by killer T cells (see Ch. 6). Other monoclonal antibodies attach to and inactivate growth factor receptors on cancer cells, thus inhibiting the survival pathway and promoting apoptosis (Fig. 5.5).

Monoclonal antibodies are relatively recent additions to the anticancer armamentarium. Unlike most of the cytotoxic drugs described above, they offer the prospect of highly targeted therapy without many of the side effects of conventional chemotherapy. This advantage is offset in most instances as they are often given in combination with more traditional drugs. Several monoclonals are in current clinical use. Their high cost is a significant problem.

Rituximab

Rituximab is a monoclonal antibody that is licensed (in combination with other chemotherapeutic agents) for treatment of certain types of *lymphoma*. It lyses B lymphocytes by binding to the calcium channel-forming CD20 protein and activating complement. It also sensitises resistant cells (see below) to other chemotherapeutic drugs. It is effective in 40–50% of cases when combined with standard chemotherapy.

The drug is given by infusion, and its plasma half-life is approximately 3 days when first given, increasing with each administration to about 8 days by the fourth administration.

Unwanted effects include hypotension, chills and fever during the initial infusions and subsequent hypersensitivity reactions. A cytokine release reaction can occur and has been fatal. The drug may exacerbate cardiovascular disorders.

Alemtuzumab is another monoclonal antibody that lyses B lymphocytes, and is used in the treatment of resistant chronic lymphocytic leukaemia. It may also cause a similar cytokine release reaction to that with rituximab.

Trastuzumab

Trastuzumab (Herceptin) is a humanised murine monoclonal antibody that binds to an oncogenic protein termed *HER2* (the human epidermal growth factor receptor 2), a member of the wider family of receptors with integral tyrosine kinase activity (Fig. 55.1). There is some evidence that, in addition to inducing host immune responses, trastuzumab induces cell cycle inhibitors p21 and p27 (Fig. 5.2). Tumour cells, in about 25% of breast cancer patients, overexpress this receptor and the cancer proliferates rapidly. Early results show that trastuzumab given with standard chemotherapy has resulted in a 79% 1-year survival rate in treatment-naive patients with this aggressive form of breast cancer. The drug is often given with a taxane such as docetaxel.

Two mechanistically related compounds are **panitumumab** and **cetuximab**, which bind to epidermal growth factor (EGF) receptors (also overexpressed in a high proportion of tumours). They are used for the treatment of colorectal cancer usually in combination with other agents.

Unwanted effects are similar to those with rituximab.

Bevacizumab

Bevacizumab is a humanised monoclonal antibody that is also used for the treatment of colorectal cancer but would be expected to be useful for treating other cancers too. It neutralises *VEGF* (vascular endothelial growth factor), thereby preventing the angiogenesis that is crucial to tumour survival. It is administered by intravenous infusion and is generally combined with other agents. It is also given by direct injection into the eye to retard the progress of *acute macular degeneration* (AMD), a common cause of blindness associated with increased retinal vascularisation.

PROTEIN KINASE INHIBITORS

Imatinib

Hailed as a conceptual breakthrough in targeted chemotherapy, **imatinib** is a small-molecule inhibitor of signalling pathway kinases. It inhibits an oncogenic cytoplasmic kinase (Bcr/Abl, see Fig. 55.1 and Fig. 55.8) considered to be a unique factor in the pathogenesis of chronic myeloid leukaemia (CML), and also inhibits platelet-derived growth factor (a receptor tyrosine kinase; Fig. 55.1). It has greatly improved the hitherto poor prognosis of patients with CML, and is also used for the treatment of some gastrointestinal tumours not susceptible to surgery.

The drug is given orally. The half-life is long, about 18 h, and the main site of metabolism is in the liver, where approximately 75% of the drug is converted to a metabolite that is also biologically active. The bulk (81%) of the metabolised drug is excreted in the faeces.

Unwanted effects include gastrointestinal symptoms (pain, diarrhoea, nausea), fatigue, headaches and sometimes rashes. Resistance to imatinib, resulting from mutation of the kinase gene, is a growing problem. It results in little or no cross-resistance to other kinase inhibitors (see below).

Other mechanistically similar drugs which inhibit the bcr-abl kinase include **dasatinib** and **nilotinib** while **erlotinib** targets the EGFR kinase and **sunitinib** another tyrosine kinase. **Sorafenib** inhibits all these kinases. Several kinase inhibitors are currently in development, and are expected to make a significant contribution to cancer therapy in the foreseeable future.

[4]The nomenclature can be confusing: by convention the suffix '-mab' denotes a 'monoclonal antibody'; '-momab' a mouse; '-ximab' a chimeric; '-zumab' a humanised; and '-umab' a human antibody.

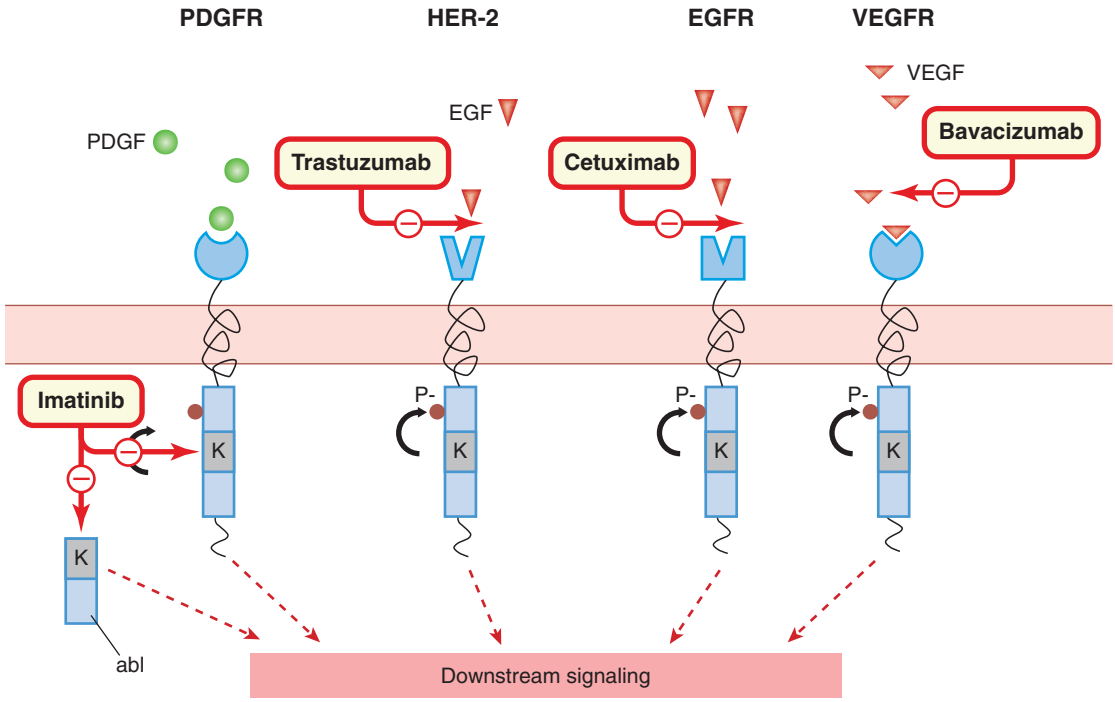


Fig. 55.8 The mechanism of action of anticancer monoclonal antibodies and protein kinase inhibitors. Many tumours overexpress growth factor receptors such as EGFR, the proto-oncogene HER2 or VEGFR. Therapeutic monoclonals can prevent this by interacting directly with the receptor itself (e.g. trastuzumab, cetuximab) or with the ligand (e.g. bevacizumab). An alternate way of reducing this drive on cell proliferation is by inhibiting the downstream signalling cascade. The receptor tyrosine kinases are good targets as are some oncongenic kinases such as bcr/abl.

Anticancer drugs: monoclonal antibodies and protein kinase inhibitors

- Many tumours overexpress growth factor receptors that therefore stimulate cell proliferation and tumour growth. This can be inhibited by:
  - monoclonal antibodies which bind to the extracellular domain of EGF (e.g. **panitumumab**) the oncogenic receptor HER2 (e.g. **trastuzumab**) or which neutralise the growth factors themselves (e.g. VEGF; **bevacizumab**)
  - protein kinase inhibitors which prevent downstream signalling triggered by growth factors by inhibiting specific oncogenic kinases (e.g. **imatinib**; bcr/abl) or by inhibiting specific receptor tyrosine kinases (e.g. EGF receptor; **erlotinib**) or several receptor-associated kinases (e.g. **sorefenib**).
- Some monoclonals act directly on lymphocyte cell surface proteins to cause lysis (e.g. **rituximab**), thereby preventing proliferation.

MISCELLANEOUS AGENTS

Crisantaspase

▼ Crisantaspase is a preparation of the enzyme *asparaginase*, given intramuscularly or intravenously. It converts asparagine to aspartic acid and ammonia, and is active against tumour cells, such as those of acute lymphoblastic leukaemia, that have lost the capacity to synthesise asparagine and therefore require an exogenous source. As most normal body cells are able to synthesise asparagine, the drug has a fairly selective action and has very little suppressive effect on the bone marrow, the mucosa of the gastrointestinal tract or hair follicles. It may cause nausea and vomiting, central nervous system depression, anaphylactic reactions and liver damage.

Hydroxycarbamide

▼ Hydroxycarbamide (hydroxyurea) is a urea analogue that inhibits ribonucleotide reductase, thus interfering with the conversion of ribonucleotides to deoxyribonucleotides. It is mainly used to treat polycythaemia rubra vera (a myeloproliferative disorder of the red cell lineage) and (in the past) chronic myelogenous leukaemia. Its use (in somewhat lower dose) in sickle cell anaemia is described in Chapter 25. It has the familiar spectrum of unwanted effects, bone marrow depression being significant.

Bortezomib

▼ Bortezomib is a boron-containing tripeptide that inhibits cellular proteasome function. For some reason, rapidly dividing cells are more sensitive than normal cells to this drug, making it a useful anticancer agent. It is generally used for the treatment of myeloma (a malignant bone marrow tumour).

Thalidomide

▼ Investigations of the notorious teratogenic effect of thalidomide showed that it has multiple effects on gene transcription, angiogenesis and proteasome function, leading to trials of its efficacy as an anticancer drug. In the event, it proved efficacious in myeloma, for which it is now widely used. The main adverse effect of thalidomide, apart from teratogenesis (irrelevant in myeloma treatment), is peripheral neuropathy, leading to irreversible weakness and sensory loss. It also increases the incidence of thrombosis and stroke.

A thalidomide derivative **lenalidomide** is thought to have fewer adverse effects, but unlike thalidomide, can cause bone marrow depression and neutropenia.

Biological response modifiers

▼ Agents that enhance the host's response are referred to as *biological response modifiers*. Some, for example **interferon-α** (and its pegylated derivative), are used in treating some solid tumours and lymphomas, and **aldesleukin** (recombinant interleukin-2) is used in some cases of renal tumours. **Tretinoin** (a form of vitamin A) is a powerful inducer of differentiation in leukaemic cells and is used as an adjunct to chemotherapy to induce remission.

RESISTANCE TO ANTICANCER DRUGS

The resistance that neoplastic cells manifest to cytotoxic drugs is said to be *primary* (present when the drug is first given) or *acquired* (developing during treatment with the drug). Acquired resistance may result from either *adaptation* of the tumour cells or *mutation*, with the emergence of cells that are less susceptible or resistant to the drug and consequently have a selective advantage over the sensitive cells. The following are examples of various mechanisms of resistance. See Mimeault et al. (2008) for an up-to-date appraisal of this issue.

- *Decreased accumulation of cytotoxic drugs* in cells as a result of the increased expression of cell surface, energy-dependent drug transport proteins. These are responsible for multidrug resistance to many structurally dissimilar anticancer drugs (e.g. doxorubicin, vinblastine and dactinomycin; see Gottesman et al., 2002). An important member of this group is *P-glycoprotein* (P-gp/MDR1; see Ch. 8). The physiological role of P-glycoprotein is thought to be the protection of cells against environmental toxins. It functions as a hydrophobic 'vacuum cleaner', picking up foreign chemicals, such as drugs, as they enter the cell membrane and expelling them. Non-cytotoxic agents that reverse multidrug resistance are being investigated as potential adjuncts to treatment.
- *A decrease in the amount of drug taken up by the cell* (e.g. in the case of methotrexate).
- *Insufficient activation of the drug*. Some drugs require metabolic activation to manifest their antitumour activity. If this fails, they may no longer be effective. Examples include conversion of fluorouracil to FDUMP, phosphorylation of cytarabine and conversion of mercaptopurine to a fraudulent nucleotide.
- *Increase in inactivation* (e.g. cytarabine and mercaptopurine).
- *Increased concentration of target enzyme* (methotrexate).
- *Decreased requirement for substrate* (crisantaspase).
- *Increased utilisation of alternative metabolic pathways* (antimetabolites).
- *Rapid repair of drug-induced lesions* (alkylating agents).
- *Altered activity of target*, for example modified topoisomerase II (doxorubicin).
- *Mutations in various genes*, giving rise to resistant target molecules. For example, the p53 gene and overexpression of the *Bcl-2* gene family (several cytotoxic drugs).

TREATMENT SCHEDULES

Treatment with combinations of several anticancer agents increases the cytotoxicity against cancer cells without necessarily increasing the general toxicity. For example, methotrexate, with mainly myelosuppressive toxicity, may be used in a regimen with vincristine, which has mainly neurotoxicity. The few drugs we possess with low myelotoxicity, such as cisplatin and bleomycin, are good candidates for combination regimens. Treatment with combinations of drugs also decreases the possibility of the development of resistance to individual agents. Drugs are often given in large doses intermittently in several courses, with intervals of 2–3 weeks between courses, rather than in small doses continuously, because this permits the bone marrow to regenerate during the intervals. Furthermore, it has been shown that the same total dose of an agent is more effective when given in one or two large doses than in multiple small doses.

Drug action during the cell cycle

▼ Cells that are constantly replicating constitute the 'growth fraction' of the tumour. Some anticancer drugs act at particular phases on the cell cycle, as shown below, and in principle this information could be of value in selecting individual agents or combinations for clinical use. However, not all authorities agree that treatment schedules based on these principles are better than purely empirical schedules.

- *Phase-specific agents*. Many cytotoxic drugs act at different points in the cycle. For example, the vinca alkaloids act in mitosis, whereas cytarabine, hydroxycarbamide, fluorouracil, methotrexate and mercaptopurine act in S phase. Some of these compounds also have some action during G_1 phase and thus may slow the entry of a cell into S phase, where it would be more susceptible to the drug.
- *Cycle-specific agents*. These act at all stages of the cell cycle but do not have much effect on cells out of cycle (e.g. alkylating agents, dactinomycin, doxorubicin and cisplatin).
- *Cycle non-specific agents*. These act on cells whether in cycle or not (e.g. bleomycin and nitrosoureas).

CONTROL OF EMESIS AND MYELOSUPPRESSION

EMESIS

The nausea and vomiting induced by many cancer chemotherapy agents constitute an inbuilt deterrent to patient compliance (see also Ch. 29). It is a particular problem with cisplatin but also complicates therapy with many other compounds, such as the alkylating agents. 5-hydroxytryptamine $(HT)_3$ receptor antagonists such as **ondansetron** or **granisetron** (see Chs 15 and 29) are effective against cytotoxic drug-induced vomiting and have revolutionised cisplatin chemotherapy. Of the other antiemetic agents available, **metoclopramide**, given intravenously in high dose, has proved useful and is often combined with dexamethasone (Ch. 32) or **lorazepam** (Ch. 43), both of which further mitigate the unwanted effects of chemotherapy. As metoclopramide commonly causes extrapyramidal side effects in children and young adults, **diphenhydramine** (Ch. 26) can be used instead.

MYELOSUPPRESSION

Myelosuppression limits the use of many anticancer agents. Regimens contrived to surmount the problem have included removal of some of the patient's own bone marrow prior to treatment, purging it of cancer cells (using specific monoclonal antibodies; see below) and replacing it after cytotoxic therapy is finished. A protocol in which aliquots of stem cells, harvested from the blood following administration of the growth factor **molgramostim**, are expanded in vitro using further haemopoietic growth

factors (Ch. 25) is now frequently used. The use of such growth factors after replacement of the marrow has been successful in some cases. A further possibility is the introduction, into the extracted bone marrow, of the mutated gene that confers multidrug resistance, so that when replaced, the marrow cells (but not the cancer cells) will be resistant to the cytotoxic action of the anticancer drugs.

FUTURE DEVELOPMENTS

As the reader will have judged by now, our current approach to cancer chemotherapy embraces an eclectic mixture of drugs and techniques, all designed to target selectively cancer cells. Real therapeutic progress has been achieved, although 'cancer' as a disease (actually many different diseases with a similar outcome) has not been defeated and remains a massive challenge for future generations of researchers. In this therapeutic area, probably more than in any other, the debate about the risk–benefit of treatment and the patient quality of life issues has taken centre stage and remains a major area of concern. These sensitive issues have been explored by Duric & Stockler (2001) and Klastersky & Paesmans (2001).

The quest for less toxic forms of therapy is, of course, central to anticancer initiatives, and many new drugs or novel combination regimens are in clinical trial or at earlier stages of development (see, for example, Kurtz et al., 2003). What follows is a selection of new and different approaches to the treatment of cancer that may bear fruit over the next decade.

Angiogenesis and metalloproteinase inhibitors

Tumour cells produce metalloproteinases and angiogenic factors that facilitate tumour growth, invasion of normal tissue and metastases. Targeting the mechanisms involved could provide us with drugs that block metastases. Several existing drugs already target this process (e.g. bevacizumab) and it is likely that this area will see further development (see Griffioen & Molema, 2000; Thijssen et al., 2007).

Cyclo-oxygenase inhibitors

There is strong epidemiological and experimental evidence suggesting that chronic use of cyclo-oxygenase (COX) inhibitors (see Ch. 26) protects against cancer of the gastrointestinal tract and possibly other sites as well. The COX-2 isoform is overexpressed in about 85% of cancers, and prostanoids originating from this source may activate signalling pathways that enable cells to escape from apoptotic death. The COX-2 inhibitor **celecoxib** reduces mammary and gastrointestinal cancer incidence in animal models and causes regression of existing tumours. It is in trial in humans as an inhibitor of a familial type of colon tumour. Overall, COX-2 is now considered to be a potentially important target for anticancer drug development although, ironically, some argue that the mechanism of action is unrelated to COX inhibition. The literature is daunting and often controversial; see Karamouzis & Papavassiliou (2004) for recent comment.

Antisense oligonucleotides

Genetic approaches are seen by many experts as the hope for the future. *Antisense oligonucleotides* (see Ch. 59) are synthetic sequences of single-stranded DNA complementary to specific coding regions of mRNA, which can inhibit gene expression. An antisense drug, **augmerosen**, downregulates the antiapoptotic factor Bcl-2. In an early clinical trial, it sensitised malignant melanoma to standard anticancer drugs.

Gene therapy

The introduction of engineered genes, antisense oligonucleotides or siRNA by *gene therapy* (see Ch. 59) offers, in principle, enormous advantages over conventional approaches in terms of selective toxicity to cancer cells. There are many technical problems yet to be solved with the delivery of the genes, (e.g. p53 or growth factor antisense DNA) into the target cells. There have been clinical trials, some of which showed modest success (see, for example, Wolf & Dwayne Jenkins, 2002, on ovarian cancer trials), but progress has been disappointingly slow.

Reversal of multidrug resistance

Several non-cytotoxic drugs (e.g. **verapamil**) that inhibit P-glycoprotein can reverse multidrug resistance. Other drugs with this action are being investigated. In addition, the use of antibodies, immunotoxins, antisense oligonucleotides (see above) or liposome-encapsulated agents may be useful in the elimination of cells with multidrug resistance (reviewed by Gottesman & Pastan, 1993).

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Wolf, J.K., Dwayne Jenkins, A., 2002. Gene therapy for ovarian cancer (review). Int. J. Oncol. 21, 461–468. (*Very readable review of ovarian cancer and basic concepts in gene therapy, coupled with a round-up of data on compounds in clinical trial*)

Useful Web resources

http://www.cancer.org/ (*The US equivalent of the Web site below. The best sections for you are those marked* Health Information Seekers *and* Professionals)

http://www.cancerresearchuk.org (*The Web site of Cancer Research UK, the largest cancer charity in the UK. Contains valuable data on the epidemiology and treatment of cancer, including links to clinical trials. An excellent resource*)