The current status of antiparasite chemotherapy

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SUMMARY

Currently used antiparasitic drugs, including benzimidazoles, nitroimidazoles, avermectins, polyene ionophores, hydroxynaphthoquinones and sesquiterpene lactones, were identified through the empirical route to drug discovery. The modern rational approach to drug design is focused upon the structure and function of biochemical and molecular targets. The requisite pharmacological properties for new anti-parasite drugs should not be ignored in this process.

INTRODUCTION

There is wide variation in the availability and efficacy of drugs for the therapy and prophylaxis of parasitic diseases, both in humans and domestic animals. For many protozoal and helminth infections there is adequate therapy mainly as a result of drugs developed and introduced since the 1960s. The benzimidazoles (Fig. 1) have offered major advances in the therapy and prophylaxis of gastrointestinal helminth infections (Campbell, 1990; Lacey & Gill, 1994) as well as cestode infections, for example *Echinococcus* (Cook, 1990), and protozoa, for example *Giardia intestinalis* and microsporidia (Morgan, Reynoldson & Thompson, 1993; Lecuit, Oksenhendler & Sarfati, 1994). Since the 1960s, the 5-nitroimidazoles, for example metronidazole and tinidazole (Fig. 2), have been used extensively in the treatment of amoebiasis, giardiasis and trichomoniasis (Townson *et al*. 1994). A group of polyene antibiotics introduced in the 1970s, the ionophores monensin, lasalocid, narasin and salinomycin (Fig. 3), remain important prophylactic agents for avian coccidioisis (Haberkorn, 1996). The most successful anthelmintics include praziquantel (Fig. 4), which has significantly improved the treatment of schistosomiasis and other fluke infections (Brindley, 1994; Cioli, Pica-Mattoccia & Archer, 1995), and the avermectin and milbemycin antibiotics, in particular ivermectin (Fig. 5). The latter are used widely in the control of nematodes and ectoparasitic infections of domestic animals as well as for microfilarial infections in human onchocerciasis and lymphatic filariasis (Ottesen & Campbell, 1994). In the 1980s, the hydroxynaphthoquinones, parvaquone and buparvaquone (Fig. 6) became available for the treatment of theileriosis in cattle and other ungulates and, more recently, atovaquone has been on clinical trial for malaria (Hudson, 1993). Another group of compounds, the sesquiterpene lactones, derivatives of the plant product artemisinin, which was discovered in 1972 (Fig. 7), are now having a major impact on the treatment of malaria (Hien & White, 1993). In addition, several antibacterial and antifungal drugs are established alternatives for the treatment of some protozoal infections (Edlind, 1991; Urbina, this volume). Descriptions of many of these effective drugs currently prescribed for the therapy and prophylaxis of human parasitic diseases can be found in WHO (1995), Anon (1993) and Abdi *et al*. (1995) and for animal infections in Campbell & Rew (1986).

However, there remain major deficiencies in antiparasite chemotherapy. Drugs for human African trypanosomiasis, Chagas' disease, the leishmaniases and the adult stages of human filariasis are inadequate because of their variable efficacy, toxicity and requirements for long courses of parenteral administration, or a combination of these reasons. There are no clinically effective drugs for cryptosporidiosis and some forms of microsporidiosis. The value of drugs for the control of falciparum malaria, and for gastro-intestinal helminths in domestic animals, has been lost because of the development of widespread drug resistance. A problem of current major concern is that, in common with antibacterial and antifungal drugs, most antiparasite drugs exhibit poor activity in immunosuppressed patients. Moreover, for most parasitic infections the armamentarium is limited, there being few alternatives if resistance develops or drug availability becomes a problem. Given the economics of drug development and the limited resources currently available for the identification and development of new drugs for human and veterinary markets, it is important to clarify the priorities in our requirements. This review will focus on some key areas of antiparasite chemotherapy and consider certain broader aspects of drug design.

CURRENT SITUATION

Malaria

Malaria has re-emerged as a major public health problem over the past three decades mainly because of the development of worldwide resistance of

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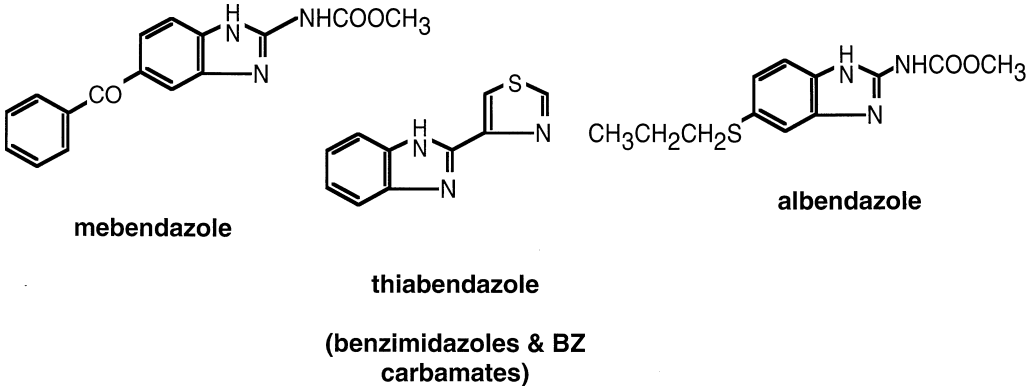

Fig. 1. Benzimidazoles with anthelmintic and antiprotozoal activity.

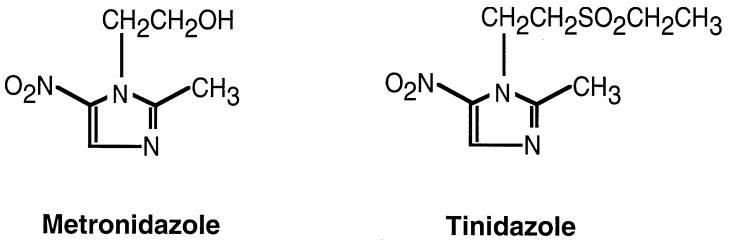

Fig. 2. 5-nitroimidazoles for treatment of trichomoniasis, amoebiasis and giardiasis.

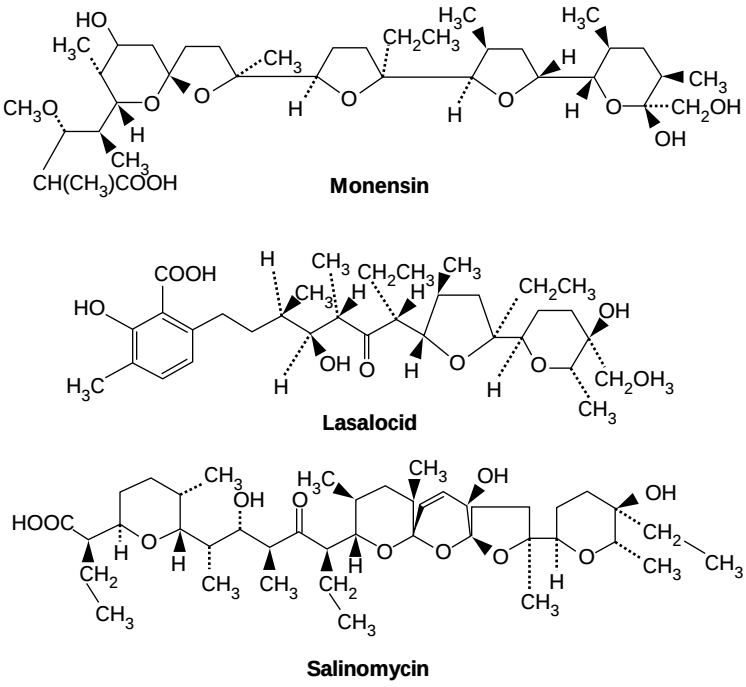

Fig. 3. Polyene antibiotics (ionophores) for avian and mammalian coccidiosis.

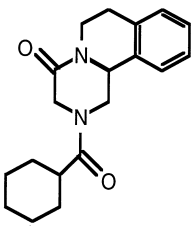

Fig. 4. Praziquantel (a pyrazinoisoquinoline) for schistosomiasis, fluke and cestode infections.

Plasmodium falciparum to chloroquine, a drug which formed the basis for cheap and effective treatment and for prophylaxis of this disease, as well as to other established antimalarials, for example pyrimethamine (Wernsdorfer, 1994). The search for successors to chloroquine has not been easy and subsequent generations of drugs have not met the expectations left by this remarkable compound. It is a cheap, oral drug with high anti-plasmodial activity, low toxicity, good absorption and distribution, which has both

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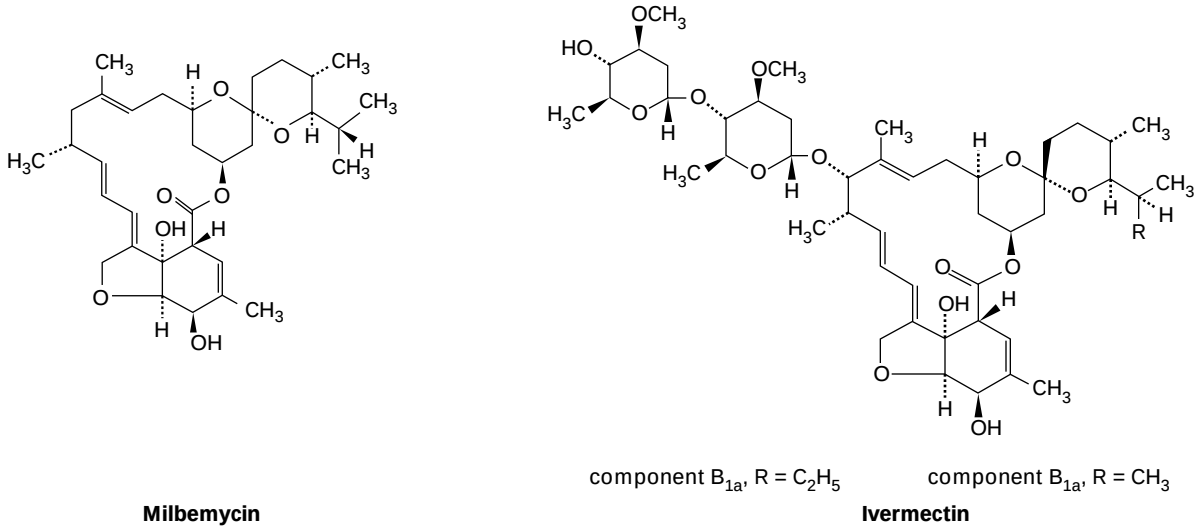

Fig. 5. Macrocyclic lactones for filarial and intestinal nematode infections.

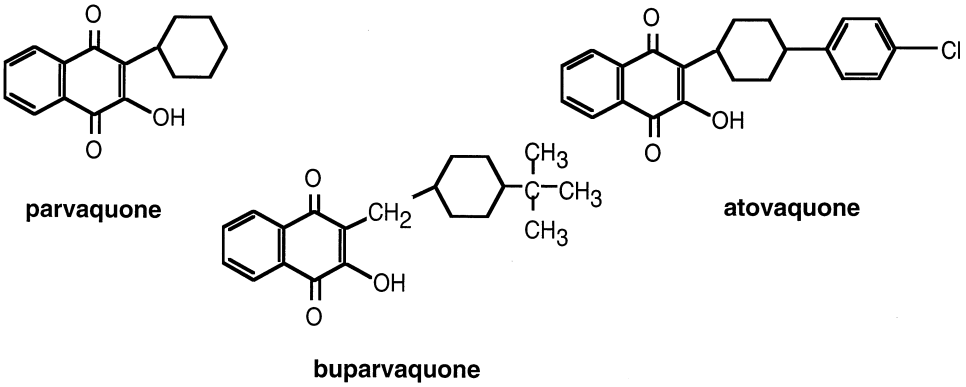

Fig. 6. Antiprotozoal hydroxynaphthoquinones.

prophylactic and therapeutic activity for the malaria caused by all four species of *Plasmodium* that infect humans. The first major programme to search for new antimalarials in the post-chloroquine world was established at the Walter Reed Army Institute for Research (WRAIR); between 1965 and 1986 WRAIR tested over 300000 compounds and mefloquine (a 4-quinolinemethanol) and halofantrine (a 9-phenanthrenemethanol) emerged as candidate drugs (Peters, 1987). However, since their introduction in the 1980s there have been reports of decreasing sensitivity and resistance of *P. falciparum* to both these drugs and to the structurally related quinine (Wernsdorfer, 1994). Cure rates of 15 mg/kg (total dose) mefloquine had dropped from 98–100 % in the 1980s to 40 % by 1995 (Mockenhaupt, 1995). Fortunately new antimalarials, mostly from different chemical classes and with little cross-resistance to established drugs, have also been discovered in this period. The most important of these for the therapy of *P. falciparum* malaria was the identification of the sesquiterpene lactone peroxide artemisinin (qinghaosu) (Fig. 7), as the antimalarial component of *Artemisia annua*, a plant used in traditional Chinese medicine over two millennia. Since this discovery by Chinese scientists in 1972, artemisinin and its derivatives have been used to treat over three million cases of falciparum malaria in South East Asia (White, 1994). Derivatives of artemisinin, artemether, artesunate and dihydroartesmisinin, retain the potent activity of the parent compound against erythrocytic stages with the rapid clearance of fever, but also have greater solubility and consequently have been developed for easier oral, injectable and suppository administration. The effectiveness of artemisinin and derivatives against multidrug resistant *P. falciparum* malaria has now been shown in sub-Saharan Africa (Murphy *et al.* 1996) and combinations of artemether or artesunate with mefloquine are proving to be highly effective in the treatment of multidrug resistant malaria in South East Asia (Looareesuwan, 1994). Artemether and artesunate formulations are now also being produced by pharmaceutical companies outside China and a further derivative, arteether, is in development (Olliaro & Trigg, 1995). The identification of the endoperoxide moiety of artesmisinin as being of prime importance in the anti-plasmodial activity of this drug, by the generation of protein alkylating radicals, has provided a stimulus for the synthesis of

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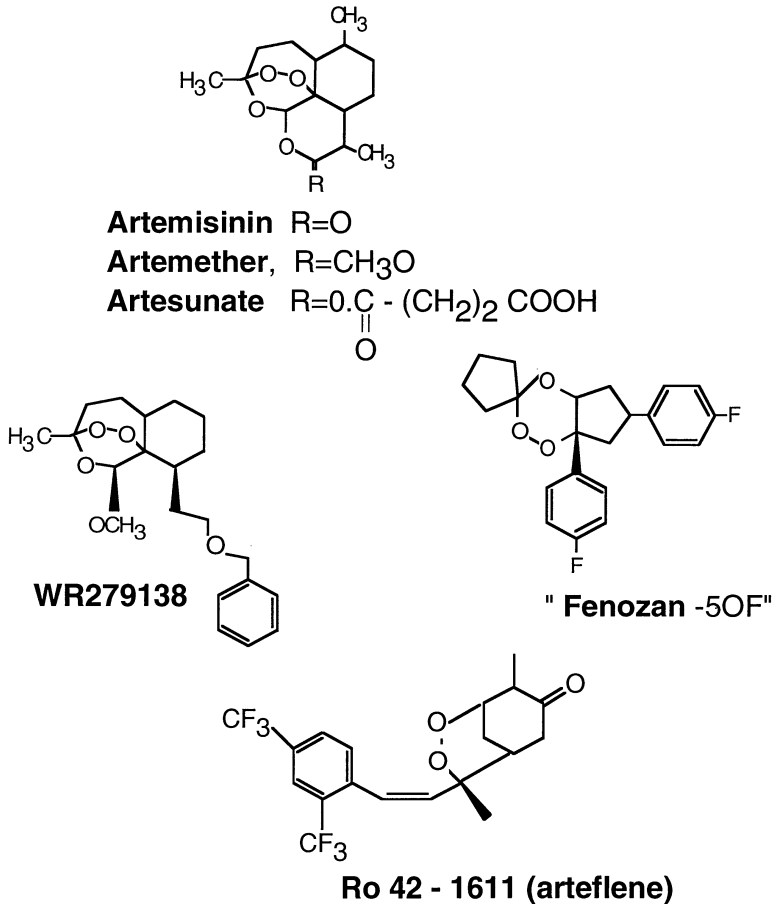

Fig. 7. Sesquiterpene peroxides with antiprotozoal activity.

novel trioxane and tetroxane compounds, some of which have shown promising activity in experimental antimalarial models (Meshnick *et al*. 1996). The dioxane, Ro 42-1611 (arteflene), a derivative of Yingzhaosu, a product of another plant, *Atrobotrys unicinatus*, reached phase II clinical trials (Somo-Moyou *et al*. 1994) before being discontinued because of high recrudescence rates. Two other compounds have emerged from China since the 1970s and have been used to treat *P. falciparum* malaria in that country, the azacrine, pyronaridine, and the fluoromethanol, benfumetol. Recent trials with pyronaridine in West Africa and Thailand have confirmed its activity against multidrug resistant *P. falciparum* (Ringwld, Bickii & Basco, 1996). A combination of benflumetol with artemether is in development (Olliaro & Trigg, 1995). The hydroxynaphthoquinone, atovaquone (Fig. 6), has also shown high activity against the erythocytic stage of *P. falciparum*, including chloroquine-resistant strains (Hudson, 1993). However, in clinical trials 30% recrudescence rates were reported when atovaquone was used as a monotherapy. Phase III clinical trials of a combination of atovaquone and the biguanide, proguanil (Blanchard *et al*. 1994) are in progress; these two compounds have synergistic anti-plasmodial activity (Canfield, Pudney & Gutteridge, 1995).

The antifolate pyrimethamine-sulphonamide combination has also played an important role in malaria therapy, and Fansidar® has become especially important in Africa following the spread of chloroquine resistance in that continent. However, the use of antifolates is also compromised by resistance; mutations in the dihydrofolate reductase gene which confer resistance to pyrimethamine and cycloguanil (the active metabolite of the pro-drug proguanil) are widespread (Wemsdorfer, 1994). There is a search for new antifolates but, so far, the only drugs that have been identified, or are on trial, have a similar structure. A chlorproguanil-dapsone combination has shown potential in the treatment of falciparum malaria, and a proguanil analogue, PS-15, has given promising results in experimental studies (Rieckmann, Yeo & Edstein, 1996). These developments also have important implications for antimalarial prophylaxis where the pyrimethamine-sulphonamide/sulphone combinations and the biguanide proguanil have been used for over 40 years. Primaquine remains the most widely used treatment for exoerythrocytic malaria caused by *P. vivax*, despite toxicity problems; the potential for resistance to this antimalarial is less clear (Collins & Jeffery, 1996). An alternative 8-aminoquinoline, WR 238,605, which is more active and less toxic than primaquine and has appreciably improved blood

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schizonticidal activity has been identified. This compound has reached phase I clinical trials (Olliaro & Trigg, 1995).

Trypanosomiasis and leishmaniasis

Trypanosomiasis and leishmaniasis were amongst the first diseases to benefit from new approaches to chemotherapy in the first two decades of this century. Ehrlich, Bramachari & Mesnil and Nicolle led the way in studies of drugs based upon arsenicals, antimonials and acidic azo dyes. It is disappointing how few advances have been made in the chemotherapy of these diseases since that period. The treatment of human African trypanosomiasis (HAT) is still reliant upon drugs that were first described over 50 years ago: the diamidine, pentamidine, and the sulphonated napththylamine, suramin, are recommended for the early haemolymphatic stages of the disease and the trivalent arsenical, melarsoprol, for the later CNS stages of the infection. Melarsoprol requires parenteral administration and is toxic, causing a reactive encephalopathy in up to 10% patients treated with a mortality of up to 5% (Pépin & Milord, 1994). Alternative treatments have included two other antitrypanosomal drugs, nifurtimox and berenil. Recently a re-examination of the clinical pharmacokinetics of melarsoprol has led to a proposal for a new dose regimen that could reduce toxicity (Burri, Blum & Brun, 1994). Despite extensive use of melarsoprol over four decades, rates of relapse following treatment are constant, suggesting that acquired resistance is not a problem (Pépin & Milord, 1994). The only new drug to be developed for the treatment of HAT since 1949 has been eflornithine [Ornidyl®]. This is a selective and irreversible inhibitor of ornithine decarboxylase, a key enzyme in polyamine biosynthesis in *Trypanosoma brucei*. Since the early 1980s, when this drug was first shown to have activity against CNS infections of *T. brucei* in rodents, eflornithine has proved to be an effective treatment for late stage infections caused by *T. b. gambiense* in West and Central Africa, including many cases resistant to arsenicals (Pépin & Milord, 1994), and was registered for use in the early 1990s (Kuzoe, 1993). However, eflornithine has many drawbacks: treatment requires the parenteral administration of high doses, there are toxic side-effects in up to 40% patients, the cost of treatment is over $250/patient, and, most importantly, the drug is ineffective as a monotherapy against *T. b. rhodesiense* (Iten *et al*. 1995). A combination of eflornithine and suramin is on trial for the treatment of late stage *T. b. rhodesiense* sleeping sickness (Taelman *et al*. 1996).

In Africa, South America and Asia, trypanosomiasis is a threat to livestock; *T. brucei*, *T. congolense* and *T. vivax* cause disease in cattle, sheep and goats and *T. evansi* in camels, horses and buffalo. Diminazene (berenil, a diamidine) and homidium and isometamidium (phenanthridiniums) have been the long-standing drugs for the treatment and prophylaxis of the former group of trypanosomes and quinapyramine and suramin for *T. evansi*. Resistance and cross-resistance to these drugs has been reported for over 30 years (Peregrine, 1994). The only recent addition has been the introduction of another arsenical, melarsamine (Cymelarsen®), during the past decade for the treatment, but not the prophylaxis, of *T. evansi* infections (Otsyula *et al*. 1992). No other drugs are in pharmaceutical development for African trypanosomiasis.

The situation for the treatment of South American trypanosomiasis (Chagas' disease) is no more promising. Current treatment is dependent upon two drugs, the nitrofuran nifurtimox (no longer manufactured), and the 2-nitroimidazole, benznidazole. Both drugs have the advantage of being orally available but both have serious toxic side-effects, including neuropathy and dermopathy, in up to 40% of cases treated. Although both drugs have significant activity against the acute phase of Chagas' disease, a marked variation in the drug sensitivity of different strains of *Trypanosoma cruzi* has been observed. In addition, nifurtimox and benznidazole have no or limited efficacy against the chronic phase of the disease (De Castro, 1993). Clinical trials have been undertaken with the pyrazolopyrimidine allopurinol, a drug which is taken up by the *T. cruzi* purine salvage pathway and interferes with nucleic acid synthesis, and the antifungals, ketoconazole and itraconazole, inhibitors of sterol biosynthesis. These drugs were able to suppress but not eliminate the infection (Brener *et al*. 1993). The antiprotozoal action of sterol biosynthesis inhibitors and the promise of a more recently developed bis-triazole D0870 for the treatment of Chagas' disease are discussed elsewhere in this volume (Urbina, this volume). Transmission of *T. cruzi* by blood transfusion is a major problem in urban areas. Gentian violet, introduced to sterilize blood *T. cruzi* trypomastigotes in 1952, remains in use in South American blood banks; alternatives to this toxic mutagenic compound are required (De Castro, 1993).

Treatment for both visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) has been improved by the introduction of alternative drug regimes and formulations over the past 15 years. A re-examination of the pharmacokinetics of the standard pentavalent antimonials led to the use of higher doses in the 1980s; a necessity for the treatment of VL in India and Kenya following dramatic decreases in cure rates at previously recommended doses (Olliaro & Bryceson, 1993). Antimonials have never been as effective against CL and mucocutaneous leishmaniasis, possibly because of their pharmacokinetics and variation in species sensitivity (Navin *et al*. 1992). Two antibiotics have re-emerged as

important antileishmanials due to the development of improved formulations. The use of the polyene antibiotic, amphotericin B, as an antifungal and as an antileishmanial was, until recently, limited by its toxicity. Lipid amphotericin B formulations with lower toxicity than the parent compound, originally developed for the treatment of systemic mycoses, has been successfully exploited for VL with the additional advantage of targeting to the site of infection. Three amphotericin B lipid formulations are now marketed and all have been on clinical trial for leishmaniasis. The unilamellar liposome formulation, AmBisome®, has proved to be highly effective against VL in immunocompetent adults and children in Europe, Sudan, Kenya and India (Davidson *et al*. 1996). A second antibiotic, the aminoglycoside paromomycin (aminosidine), has been formulated in an ointment containing a transdermal enhancing agent (methyl benzethonium chloride), for the treatment of CL (El-On *et al*. 1992) and a parenteral formulation has proved to be an effective treatment of VL (Olliaro & Bryceson, 1993). Allopurinol has also been on clinical trial for VL and CL, as have ketoconazole and itraconazole against CL, but the results have been equivocal. There appears to be variation in species sensitivity to these drugs; ketoconazole is more active against *L. mexicana* than *L. braziliensis* (Navin *et al*., 1992), as are many other antileishmanials. Trials with the 8-aminoquinoline WR6026 for VL started in Kenya several years ago (Sherwood *et al*. 1994) but have now been transferred to Brazil. As *Leishmania* are obligate parasites of resting macrophages, immunotherapeutic approaches to activate the host cells have been pursued. Interferon-γ, in combination with antimonials, has been useful in cases of VL unresponsive to antimonials alone (Sundar, Rosenkaimer & Murray, 1994). The treatment of canine leishmaniasis remains problematic; antimonials, amphotericin B and paromomycin have limited efficacy.

Other coccidian parasites and microsporidia

In immunocompetent humans only 10–20 % of acute infections by *Toxoplasma gondii* are symptomatic and these are not normally treated. However, in congenitally infected neonates and immunocompromised humans, infection involves many organs. *T. gondii* is the most frequent CNS infection in AIDS, being seen in 33 % of all positive HIV-infected patients in the USA. The standard treatment for toxoplasmosis in immunocompetent patients has been the antifolate combination of pyrimethamine–sulphadiazine (with leucovorin rescue to counteract bone marrow toxicity) for nearly 4 decades; this treatment produces clinical improvement in 80–90 % of patients. However, there are two major problems in the treatment of human toxoplasmosis. First, none of the drugs currently used is effective against the *T. gondii* tissue cyst stages. Consequently, relapse occurs in immunocompromised patients and maintenance therapy is required, which can be life-long for AIDS patients. Second, the toxic side-effects of diaminopyrimidine-sulphonamide combinations, due in part to the reactive hydroxyamine metabolites of sulphonamides (Van der Ven *et al*. 1995), preclude their use in up to 40 % of patients. Recommended alternatives include combinations of clindamycin-pyrimethamine, trimethoprim-sulphamethoxazole and dapsone-pyrimethamine (Richards, Kovacs & Luft, 1995). New folate pathway inhibitors, diaminopyrimidines and diaminoquinazolines, have been tested experimentally; one of these, epiroprim, has better pharmacokinetics and higher activity than trimethoprim (Martinez, Allegra & Kovacs, 1996). The azalide and macrolide antibiotics, azithromycin, clarithromycin and roxithromycin, have shown good experimental activity but there is limited activity against the cyst stage. The macrolide, spiramycin, is the recommended drug to ameliorate foetal infection resulting from acute maternal infection during pregnancy. Atovaquone has anti-cyst activity in experimental models (Ferguson *et al*. 1994) but in a clinical trial for toxoplasmosis in AIDS patients, relapse was observed in 52 % of cases (Kovacs, 1992). Feline toxoplasmosis is treatable with the ionophore, monensin, and the triazinetrione, toltazuril (Haberkom, 1996). Dogs infected with the closely related parasite, *Neospora caninum*, have been treated with trimethoprim and pyrimethamine, but an effective treatment is needed for cattle because this parasite can cause abortion.

Infection by another coccidian parasite, *Cryptosporidium parvum*, causes chronic diarrhoea in immunosuppressed humans. Cryptosporidiosis has been reported in 10–50 % of AIDS patients in various studies and is considered to be a major cause of morbidity and mortality in these patients. The organism has a complex life cycle, including autoinfection and dissemination to the biliary tract. Many drugs have been tried in the treatment of cryptosporidiosis from the pyrimethamine–sulphonamide combination, through the macrolides (clarithromycin, spiramycin and azithromycin), various anticoccidials (amprolium, diclazuril and its analogue letrazuril), and albendazole, to the somatostatin analogues octreotide and vapreotide (Hoepelman, 1996). At present there is no recommended chemotherapeutic or chemoprophylactic drug. Only the aminoglycoside paromomycin, which is poorly absorbed, has produced dose-related effects and both clinical and parasitological improvement were observed at high doses of 1–2 g/day (Bissuel *et al*. 1994; Clinton White *et al*. 1994). Maintenance therapy is required to prevent relapse. However, at these doses this highly cationic drug has a number of side-effects and it is not clear whether the anti-

cryptosporidial activity is direct or indirect. Paromomycin has also been used in veterinary medicine for cryptosporidiosis (Haberkorn, 1996).

Two other coccidian parasites of importance to humans, which cause intestinal infection and diarrhoea, are *Isospora belli*, which is frequently found in AIDs patients, and *Cyclospora cayatensis*, which has been reported from travellers. These infections respond to the trimethoprim-sulphamethoxazole combination, although for AIDS patients the treatment of *I. belli*, a parasite so far only described in humans, requires maintenance therapy.

Some of the most successful and chemically novel antiprotozoals have been developed as prophylactics for the avian coccidia, *Eimeria* spp. A range of polyether ionophores (Fig. 3) and synthetic drugs has been developed over the past 25 years but extensive use and delivery in foodstuffs has led to resistance. Three drugs developed recently which have activity against the intracellular developmental stages of *Eimeria* are diclazuril (a benzeneacetonitrile), semduramycin (a polyether antibiotic) and toltazuril (a triazenetrione) (Haberkorn, 1996).

Microsporidiosis is probably a transient infection in immunocompetent humans, particularly travellers to the tropics. With a few exceptions, the most serious disease occurs in the immunocompromised humans. Eight species of microsporidia have been identified in immunocompromised humans and two species are responsible for up to 33% of cases of chronic diarrhoea in AIDS patients. The predominantly intestinal parasite, *Encephalitozoon intestinalis*, has been shown to be sensitive to the benzimidazole albendazole (Fig. 1), although the drug is less effective against *Enterocytozoon bieneusi* infections (Lecuit *et al*. 1994). Another species of microsporidian, *Encephalitozoon hellem*, which predominantly infects mucosal surfaces and the cornea, is known to respond to albendazole and fumagillin.

Helminthiases (*other than human filariasis*)

The treatment of the majority of intestinal helminth infections, in particular those caused by nematodes and cestodes, and of tissue nematodes, for example *Trichinella spiralis* and larva migrans, has been transformed since the 1960s by the use of benzimidazoles (Fig. 1) along with the imidothiazoles, levamisole and tetramisole, and the pyrimidines, pyrantel and morantel. The structure and activities of the benzimidazoles used in human and veterinary medicine have been described in detail (Townsend & Wise, 1990; Campbell, 1990). Resistance to benzimidazoles was observed within 3 years of their introduction and is now a worldwide problem in veterinary medicine (Lacey & Gill, 1994: Roos, this volume) but not in human medicine. Albendazole, a more recently developed benzimidazole carbamate (Fig. 1), has proved to be a useful addition, being effective against *Echinococcus multilocularis*, partially effective against *E. granulosus* (Cook, 1990), and useful in the treatment of onchocerciasis (Cline *et al*. 1992) and human cysticercosis (*Taenia solium*) (Webbe, 1994). Another member of this group of drugs, triclabendazole, originally developed for veterinary use, has been used to treat human *Fasciola hepatica* infections. *Strongyloides stercoralis*, a nematode that can maintain autoinfection in the human host for over 50 years and can produce hyperinfection in immunosuppressed individuals, has proved difficult to treat. Thiabendazole, the standard treatment for many years, is not so effective in the hyperinfective syndrome and has been replaced by albendazole. Ivermectin has been considered as an alternative treatment in patients with chronic strongyloidosis as well as for hyperinfection in AIDS patients but has a higher efficacy and lower toxicity than the benzimidazoles (Daltry *et al*. 1994; Gann, Neva & Gam, 1994; Ottesen & Campbell, 1994).

Praziquantel, an orally available heterocyclic pyrazinoisoquinoline with a chemical structure dissimilar to that of any other antiparasitic drug (Fig. 4), has activity against a wide range of trematodes and cestodes, most importantly as a successful treatment for schistosomiasis caused by *Schistosoma mansoni*, *S. haematobium* and *S. japonicum* (Brindley, 1994) but also for fascioliasis, paragonimiasis and cysticercosis (Webbe, 1994). Despite 20 years of intensive use there is no confirmed resistance to praziquantel, although there have been reports of reduced efficacy to treatment in Senegal and Egypt. Whether this is caused by acquired resistance or other factors, for example dosage or host immunity, is a matter of considerable interest (Brindley, 1994; Fallon *et al*. 1996). There is a clear need for a strategy to avoid resistance to such a useful drug; it has been suggested that the use of chiral praziquantel (the laevo-enantiomer is more active against *S. japonicum* than the dextro-enantiomer) might retard resistance (Shu-Hua & Catto, 1989; Brindley, 1994), and strategies to discover alternative drugs are being pursued (Cioli *et al*. 1995). Other antischistosomal drugs, used before the advent of paraziquantel, are still available despite their limited efficacy (oxamniquine for *S. mansoni* and metrifonate for *S. haematobium*), clinical resistance (hycanthone and oxamniquine), and toxicity (niridazole and hycanthone).

A major change in the use of anthelmintics for human infections is a move from the treatment of the infected individual to treatment of populations. The broad-spectrum effects of mebendazole, albendazole and praziquantel make them suitable for mass treatment programmes in Africa, Central and South America and Asia, where parasite burden can be high. A major consideration in these programmes has been the design of a cost-effective approach linked to the impact on child development through education programmes (Bundy & Guyatt, 1995).

Mass treatment programmes should also consider the development of resistance. The way these drugs have been used over many years to treat individuals may explain the low level of resistance currently found in humans. In contrast, in livestock these drugs have been used on a widescale population basis and widespread resistance is found (Roos, this volume). Improved strategies for the use of anthelmintics in animal populations, including drug combinations and new drug delivery systems, are required (McKellar, 1994).

Filariasis

The clinical treatment of filariasis has been dominated by two drugs, the simple piperazine derivative, diethylcarbamazine, and the complex macrocyclic lactone antibiotic, ivermectin. (Fig. 5). Ivermectin, a semi-synthetic derivative of avermectin, is the drug of choice for onchocerciasis because it is active against skin dwelling first stage larvae (microfilariae) of *Onchocerca volvulus* at exceedingly low doses, 150 μg/kg bodyweight. This drug has revolutionized the treatment of onchocerciasis and led to strategies for disease control through mass prophylaxis by annual single doses (Ottesen & Campbell, 1994). Nevertheless, drugs with activity against adult *Onchocerca* are limited. The reported activity of ivermectin against adult *Onchocerca* probably results from its effects on the uterus of females, which cause embryonic development to arrest (Chavasse *et al*. 1993). Currently, suramin, a drug first synthesized in 1916, remains the only recommended macrofilaricide for onchocerciasis.

Ivermectin has also had an impact on the treatment of lymphatic filariasis. Low doses (10–20 μg/kg) kill *Wuchereria bancrofti*, whereas higher doses are required to clear microfilariae of *Brugia* spp. The long-term effect of high dose treatment on both types of lymphatic filariasis suggests that ivermectin is having a macrofilaricidal effect. The older compound, diethylcarbamazine (DEC), first introduced in 1947, is still the recommended drug for the treatment of lymphatic filariasis. It affects adult worm viability and fecundity, but its use is limited by severe host hypersensitivity to the dying microfilaria (Mazzotti reaction). Recent studies with DEC have concentrated upon single-dose use for lymphatic filariasis and also combination studies with ivermectin (Moulia-Pelat *et al*. 1993). As both drugs can be given orally there is a possibility of mass treatment at doses with low side effects (Ottesen & Campbell, 1994).

DEC is also effective in the chemotherapy of another species of filaria, *Loa loa*. It is active against all stages of the parasite and also has weak prophylactic properties. Ivermectin reduces loiasis microfilaraemia by 90%, but more slowly than DEC and this possibly reduces the risk of acute inflammatory reactions. The combined use of ivermectin with DEC has been considered in cases of high microfilaraemia where DEC alone could be dangerous (Ottesen & Campbell, 1994). However, the priority is to find a macrofilaricide. Many biochemical and molecular targets have been identified (Ginger, 1991), but success has proved to be elusive.

DRUG DESIGN

Molecular and biochemical approaches

The majority of antiparasite drugs were developed for chemotherapy without knowledge of their mode of action, including the anti-folates (Sneader, 1985). Even for some well known drugs, like chloroquine and pentamidine, the precise mechanisms of action are still not clear. In the past two decades the strategy has been reversed (Cohen, 1979; Wang, 1984), and the imperative has been to define metabolic differences between parasite and host, identify biochemical targets for drugs and design specific inhibitors to these targets. This rational approach to chemotherapy has not been fully exploited yet but this volume contains many examples of the characterization of molecular, biochemical and physiological drug targets (see reviews by Nare *et al*., Coombs & Mottram, Hunter, Martin *et al*.; Schmatz, Toulmé *et al*. and Urbina, in this volume); a considerable volume of data has been accumulated that could be used in drug design.

The number of known potential chemotherapeutic targets in protozoan parasites is due to increase dramatically as the sequencing of the genomes of *P. falciparum*, *T. cruzi*, *L. major* and *T. brucei* is completed in the coming years. Advances in computing and bioinformatics will help to distinguish molecular differences between parasite and host. The problem comes in interpreting the differences and identifying which will make valid drug targets (Wang, this volume).

The way novel drugs are identified has also been influenced by new techniques in medicinal chemistry. Combinatorial chemistry has opened up the possibility of generating millions of variants of a core structure in small quantities and at low cost. This approach was first applied in antimicrobial chemotherapy using peptide chemistry (Blondelle, Pérez-Payá & Houghten, 1996) and is how beginning to make an impact on antiparasite chemotherapy; for example, libraries of trypanothione reductase inhibitors and dihydrofolate reductase inhibitors have been synthesized (Marsh *et al*. 1996). An essential adjunct to this approach to exploit the potential of this large chemical diversity is the use of robotically controlled assays of recombinant target enzymes. The increasing sophistication of chemistry and the concerns of drug regulatory bodies about toxicity are turning attention to chiral chemistry (Hutt &

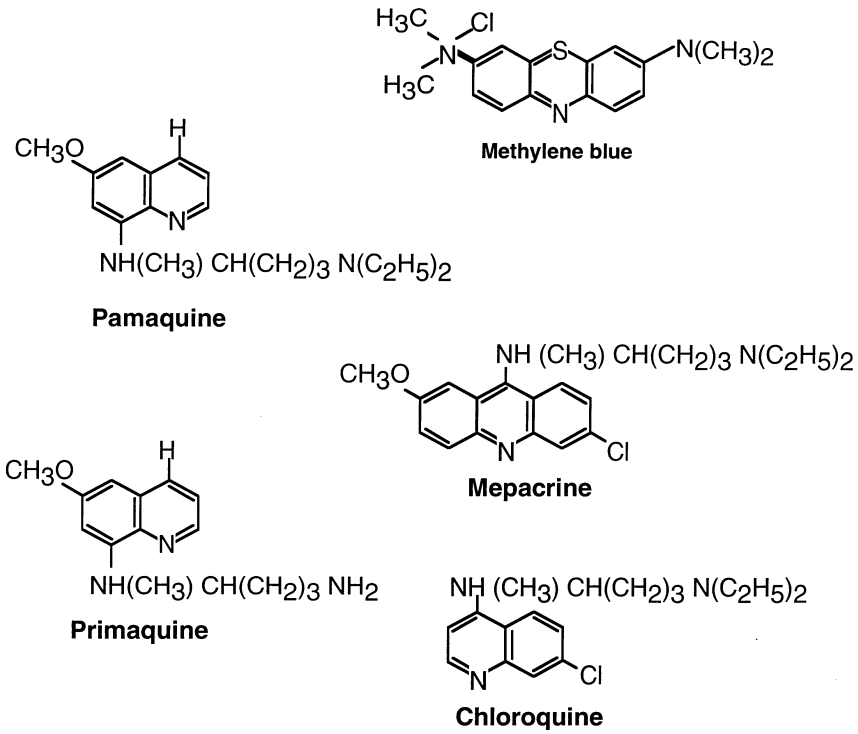

Fig. 8. Development of quinoline antiprotozoals.

O'Grady, 1996). In antiparasite chemotherapy, the importance of chiral chemistry is exemplified by differences in the activities of the enantiomers (mirror-image stereoisomers with identical intramolecular distances between non-bonded atoms and physico-chemical properties) quinine and epiquinine against *P. faciparum* (Karle *et al*. 1992), (−) and (+) praziquantel against *S. mansoni* (Shu-Hua & Catto, 1989) and of levamisole and tetramisole against nematodes. The bis-triazole, D0870, is an enantiomer of the racemic compound, ICI 195,739, and has both greater antifungal and anti-*T. cruzi* activity than the parent compound (Urbina, this volume).

Pharmacological approaches

Drug design does not stop, nor for that matter did it start, with molecular and biochemical targets. Paul Ehrlich, the founding father of modern chemotherapy, took the first steps in the rational design of antiparasite drugs. Ehrlich's studies as a pathologist taught him that certain chemicals, in particular acidic azo dyes, are differentially accumulated by cells, tissues and microorganisms. In the first study of selective toxicity in the treatment of an infectious disease, Guttmann and Ehrlich in 1891 used methylene blue to cure two cases of malaria. This dye was known to stain the blood stages of the recently discovered *Plasmodium* parasites at concentrations that did not affect leucocytes. When experimental models of malaria became available in the 1920s, methylene blue provided the starting point for the design of more effective antimalarials, the quinolines and the acridines (Sneader, 1985), compounds that retain or have enhanced selective uptake by *Plasmodium*-infected cells (Fig. 8). The concept of a magic bullet, a drug targeted to a specific site of disease, is often attributed to Ehrlich; in fact, he outlined his ideas within the more complex concept of a poisoned arrow (Ehrlich, 1913). Salvarsan (arsphenamine), the first drug developed for the treatment of syphylis, was represented as having amino groups for attachment to the parasite receptor, the benzyl group as the shaft of the arrow and the trivalent arsenic group as the poison, the toxophore. Ehrlich's background in immunology (indeed, he described what we term as drug resistance as the pathogens' 'immunity' to a drug) helped him to develop the concept that drugs, like antibodies, need receptors for their selectivity. 'Parasites are only killed by those materials to which they have a certain relationship, by means of which they are fixed by them', the famous … *corpora non agunt nisi fixata*. Within these ideas he outlined another area of drug design, the concept of the pro-drug: 'salvarsan and mercury salts are not intended to act directly on the parasites, but indirectly, owing to the fact that they excite the organism to the formation of specific antisubstances' (Ehrlich, 1913).

The elements of drug design that relate to accumulation by cells, activation by parasite or host cells, and distribution, have been used in several approaches by medicinal chemists and parasitologists to improve the targeting of drugs to pathogens or to the infected compartments in host mammalian cells. The physico-chemical properties of a drug can

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have a profound effect on its uptake and accumulation and this has been clearly shown for antibacterials (Tulkens, 1991) as well as antiparasite drugs. The distribution of the antimalarial, chloroquine, to the acidic food vacuole of the *Plasmodium* trophozoite is closely related to the pK values of the basic groups on the side-chain of this 4-aminoquinoline (Ward, Bray & Hawley, this volume). Other antiprotozoal activities that have been partly explained by their lysosomotropic properties include those of the amino acid and dipeptide esters and bis(benzyl)polyamine analogues that accumulate in the *Leishmania* phagosome (Rabinovitch, 1989; Baumann, McCann & Bitonti, 1991). Lipophilicity, one element of quantitative structure activity relationship (QSAR) analysis, has been used in the design of trypanocidal drugs (Booth *et al*. 1987) and has provided an explanation for the activity of tetracycline derivatives against *Giardia* (Katiyar & Edlind, 1991). Recent research has also demonstrated that studies on the interaction between drugs and surface receptors/transporters can play an important role in drug design; for example, the permeability pathways and choline transporters in *P. falciparum*-infected erythrocytes (Elford, Cowan & Ferguson, 1995; Vial, 1996), and the purine and glucose transporters in *T. brucei* (Carter, Berger & Fairlamb, 1995). Carrier molecules can also be incorporated into drug design to aid targeting to receptors on infected cells or parasites. One example of this is the targeting of glycosylated allopurinol riboside to the mannose/fucose receptor on macrophages infected with *Leishmania* (Negre *et al*. 1992), and another is primaquine linked to lactosylated BSA to the asialoglycoprotein receptor on hepatocytes infected with *Plasmodium* (Holfstenge *et al*. 1986).

Drug design to improve the pharmacokinetics (the absorption, metabolism, tissue distribution and excretion) of drugs is receiving increasing attention (Gumbleton & Sneader, 1994) and the pharmacokinetic properties of drugs are being given higher priority in drug screening. Analysis of physicochemical properties of drugs can be used to help to improve oral bioavailability (Navia & Chaturvedi, 1996). In other areas of pharmacokinetics, the importance of chemical structure in drug metabolism by the host was recently demonstrated in the design of antimalarial naphthoquinones: atovaquone is not metabolized by human liver microsomes, whereas its forerunner as a drug candidate, the compound 58C, could be metabolized by human microsomes, although not by rodent microsomes (Hudson, 1993).

The importance of bringing pharmacology into the early stages of drug development and the importance of this aspect of molecular design and its role in strategies to combat drug resistance should not be underestimated. Indeed, drug discovery depends upon the effective integration of chemistry, biochemistry, biology and pharmacy (Omura, 1986); in antiparasite chemotherapy there is also a peculiar economic dimension because of the impact of parasitic diseases in developing countries where drugs are not affordable (Gutteridge, this volume).

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