Leishmaniasis– current chemotherapy and recent advances in the search for novel drugs

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The chemotherapy currently available for leishmaniasis is far from satisfactory. Resistance to the pentavalent antimonials, which have been the recommended drugs for the treatment of both visceral (VL) and cutaneous leishmaniasis (CL) for >50 years, is now widespread in India. Although new drugs have become available in recent years for the treatment of VL, including AmBisome®, the excellent but highly expensive liposomal formulation of amphotericin B, and the oral drug miltefosine, which has now been registered in India, treatment problems remain. Parenteral and topical formulations of the aminoglycoside paromomycin offer potential treatments for VL and CL, respectively. The search for new drugs continues, with bisphosphonates, for example, risedronate and pamidronate, and plant derivatives such as licochalcone A and quinoline alkaloids being reported to have activity against experimental animal infections. The immunomodulator imiquimod has proved to be an adjunct for CL therapy. Many potential drug targets have been identified in biochemical and molecular studies, and some have been validated. Attempts to exploit these targets are in progress.

The leishmaniases are a complex of diseases caused by at least 17 species of the protozoan parasite *Leishmania*. The parasite exists in two forms: the flagellated promastigote in the female phlebotomine sandfly vector; and the amastigote in the mammalian host. Amastigotes are obligate intracellular parasites of macrophages (and rarely other cell types), where they survive and multiply within a phagolysosome compartment. Leishmaniasis has traditionally been classified in three different clinical forms, visceral (VL), cutaneous (CL) and mucocutaneous leishmaniasis (MCL), which have different immunopathologies and degrees of morbidity and mortality. Most VL caused by *Leishmania donovani* is fatal if untreated, whereas CL, caused by species such as *Leishmania major*, *Leishmania mexicana*, *Leishmania braziliensis* and *Leishmania panamensis*, frequently self-cures within 3–18 months, leaving disfiguring scars. Human leishmaniasis is distributed worldwide, but mainly in the tropics and sub-tropics, with a prevalence of 12 million cases and an approximated

incidence of 0.5 million cases of VL and 1.5 million cases of CL (http://www.who.int/tdr/diseases/leish/diseaseinfo.htm) (Table 1). Leishmaniasis, in particular *Leishmania infantum* infection, is also an important disease of dogs in Mediterranean countries and Brazil. These general aspects of leishmaniasis and overall control strategies have been reviewed recently [1,2].

The main drug treatments recommended for both VL and CL were first introduced over 50 years ago. However, the position is changing and some new therapies are becoming available. Unfortunately, it now seems clear that the previous ambition to develop a single drug or drug formulation to be effective against all forms of leishmaniasis was too optimistic. Not only do *Leishmania* spp. differ intrinsically in their drug sensitivity (Box 1), but also the visceral and cutaneous sites of infection impose differing pharmacokinetic requirements on the drugs to be used.

The target for chemotherapy is the intracellular amastigote that survives and divides in tissue macrophages, whereby causing the disease (Figure 1). The amastigote resides within a parasitophorous vacuole, which resembles a secondary lysosome with a pH of 4.5–5.0. The acidic environment has implications for the amastigote's strategies for nutrient acquisition and ion homeostasis [3]. These involve a variety of transporters that could mediate drug uptake or drug efflux, and so play a part in determining the parasite's susceptibility to chemotherapy. Current evidence, although far from comprehensive, suggests that different *Leishmania* spp. not only reside in different macrophage types, but also have differing adaptations that facilitate intracellular survival [4]. Such species variations could account for some differences in drug susceptibility and make it imperative that appropriate laboratory models are used for assessing drug efficacy.

The demands for new antileishmanial drugs have been fed in recent years by the demonstration of acquired resistance to the pentavalent antimonial drugs, the first-line chemotherapy. The most severe problem reported to date is in Bihar, India [5]. The concept of drug resistance in leishmaniasis is not straightforward – sensitivity to drugs has to be evaluated carefully and considered in relation to the differences in intrinsic drug sensitivity between

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Table 1. Factfile: leishmaniasis statistics[a]

	Statistic/information	Reference
Geographical location	Worldwide tropical and sub-tropical regions	www.who.int/tdr/diseases/leish
Population at risk in 2003	350 million	www.who.int/tdr/diseases/leish
Number of deaths in 2002	59 000	www.who.int/tdr/diseases/leish
Global case fatality rate in 2002	NK	–
Number of new cases in 2002	1.5–2.0 million	www.who.int/tdr/diseases/leish
Number of multidrug-resistant cases in 2002	Resistance to antimonials only	www.who.int/tdr/diseases/leish
Global disease burden in 2002 (DALYs)	2 307 000	www.who.int/whr/2002/whr2002_annex3.pdf
Current drugs available	Pentostam™, Glucantime, amphotericin B, pentamidine, AmBisome®	www.who.int/tdr/diseases/leish
Drugs undergoing clinical trials	Miltefosine (Phase 3 for VL and Phase 4 for CL)	www.who.int/tdr/diseases/leish
Drugs undergoing development	Paromomycin (Phase 3)	www.who.int/tdr/diseases/leish

[a]Abbreviations: CL, cutaneous leishmaniasis; DALYs, disability-adjusted life years; NK, not known; VL, visceral leishmaniasis.

species and situations where leishmaniasis is an anthropotic disease as opposed to a zoonotic disease (Box 1). However, there is a difficulty when a standard drug regimen ceases to effective. Treatment efficacy is also compromised when there is immunosuppression, in particular due to HIV co-infection. This can lead to exacerbation of disease or emergence from latent infection – the depleted immune capability means that standard chemotherapy is frequently unsuccessful [6].

Current recommendations

The drugs currently recommended for the treatment of leishmaniasis [7] include the pentavalent antimonials sodium stibogluconate [Pentostam™, GlaxoSmithKline (GSK); http://www.gsk.com] and meglumine antimoniate (Glucantime, Aventis, http://www.aventis.com), amphotericin B (Bristol-Myers Squibb; http://www.bms.com/) and its lipid formulation AmBisome® (Gilead; http://www.gilead.com/), and pentamidine (Aventis) (Table 2 and Figure 2). The antimonials were first introduced in 1945 and remain effective treatments for some forms of leishmaniasis, but the requirement for up to 28 days of parenteral administration, the variable efficacy against VL and CL, and the emergence of significant resistance are all factors limiting

the drugs' usefulness (Box 1). Recent interest in this class of drugs has focused largely on confirming the efficacy of low-cost generic drugs [8] and elucidating, at last, the mechanism of action and how parasites have become resistant to the drugs. The usefulness of the diamidine pentamidine as an antileishmanial drug has been limited by its toxicity. Nevertheless, since its introduction in 1952, it has had value for VL, CL and also diffuse CL, although normally it is used as a second-line drug when antimonials have proved ineffective. The polyene antibiotic amphotericin B has proved to be highly effective for the treatment of antimonial-resistant *L. donovani* VL [9] and cases of MCL that have not responded to antimonials, but it is an unpleasant drug because of its toxicity and the need for slow infusion parenteral administration over four hours. However, antileishmanial chemotherapy has benefited from the development of lipid-associated formulations of amphotericin B, which have reduced toxicity and an extended plasma half-life in comparison to the parent drug, for the treatment of fungal infections. The formulations in either lipid (AmBisome®, involving unilamellar liposomes, and Abelcet®, incorporating a lipid complex) or cholesterol (Amphocil®, as a colloidal dispersion) have all
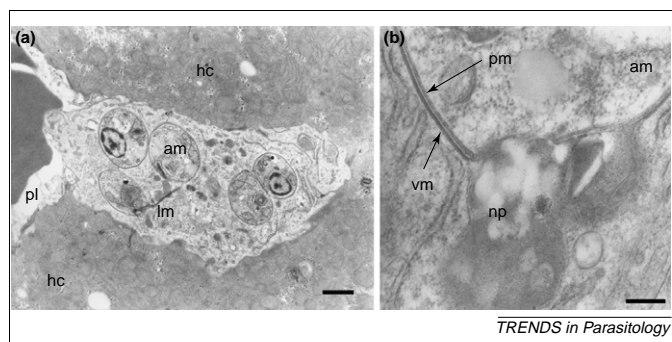

Figure 1. The membrane barriers that antileishmanial drugs have to penetrate. **(a)** Drugs in plasma (pl) gain access to *Leishmania donovani* amastigotes (am) in liver macrophages (Kupffer cells) (lm), between hepatocytes (hc), by firstly entering the low pH parasitophorous vacuole (probably by passive diffusion) and then entering the parasite itself via membrane transporters, endocytosis or diffusion. Scale bar = 2 μm. **(b)** Amastigote (am) within phagolysosomal vacuole, showing vacuole membrane (vm) and parasite membrane (pm) barriers that have to be crossed by drugs. An alternative route via fusion of an endosome containing nanoparticles (np) is also illustrated. Scale bar = 0.2 μm.

Box 1. Drug sensitivity and resistance

Drug treatment of leishmaniasis is complicated by variation in efficacy as a result of:

(i) Intrinsic variation in drug sensitivity

There is at least a threefold to fivefold difference among *Leishmania* spp. in sensitivity to antimonials, paromomycin, azoles and miltefosine [56].

(ii) Acquired drug resistance

Up to 60% VL patients in Bihar State, India, do not respond to pentavalent antimonials. *Leishmania donovani* amastigotes derived from isolates of non-responsive patients ($ED_{50} = 7.4$ mg ml^{-1}) had a threefold lower sensitivity to antimonials in the *in vitro* macrophage model than those derived from isolates of patients that did respond to antimonials ($ED_{50} = 2.4$ mg ml^{-1}) [57]. Drug resistance has great public health importance for anthroponotic *Leishmania donovani* and *Leishmania tropica*; most other forms of leishmaniasis are zoonotic and so spread of drug resistance is less likely.

(iii) Immune status

Antimonials have reduced activity in the absence of a T-cell immune response [33]. The absence of immune response has an impact in treatment of diffuse cutaneous leishmaniasis cases, and HIV and visceral leishmaniasis co-infection cases [34].

Table 2. Actions of antileishmanial drugs

Generic name of drug (chemical type)	Mechanism of action[a]	Ref.
Pentavalent antimonials: Meglumine antimoniate (Glucantime) Sodium stibogluconate (Pentostam™)	Structure of sodium stibogluconate is still not known despite its use for over 50 years. Activated within the amastigote, but not in the promastigote, by conversion to a lethal trivalent form. Activation mechanism not known. Antileishmanial activity might be due to action on host macrophage.	[52–54]
Amphotericin B (polyene antibiotic)	Complexes with 24-substituted sterols, such as ergosterol in cell membrane, thus causing pores which alter ion balance and result in cell death.	[43]
Pentamidine (diamidine)	Accumulated by the parasite; effects include binding to kinetoplast DNA. Primary mode of action uncertain.	[41]
Paromomycin (aminoglycoside antibiotic) (also known as aminosidine or monomycin)	In bacteria, paromomycin inhibits protein synthesis by binding to 30S subunit ribosomes, causing misreading and premature termination of mRNA translation. In *Leishmania*, paromomycin also affects mitochondrion.	[21]
Miltefosine (hexadecylphosphocholine)	Primary effect uncertain, possible inhibition of ether remodelling, phosphatidylcholine biosynthesis, signal transduction and calcium homeostasis.	[15]
Sitamaquine (8-aminoquinoline, originally WR6026)	Unknown, might affect mitochondrial electron transport chain.	[18]
Imiquimod (imidazoquinoline)	Stimulates nitric oxide production from macrophages.	[35]

[a]For further details, see reviews in Refs. [41,53,55]. For chemical structures, see Figure 2.

been in clinical trials for VL and/or MCL. AmBisome® is the best tested of these formulations, has proved to be effective [10] and has been approved by the Food and Drug Administration (http://www.fda.gov/) [11], but high cost has limited its use. Approaches to reduce cost include: (i) efficacy trials of single-dose AmBisome® treatment for VL, with 90% cure rate reported to date [12]; (ii) the use of cheaper liposomal formulations, already tried for VL [13]; and (iii) the development of alternative nanoparticle and/ or lipid formulations or chemical derivatives, which have proved effective in experimental models [14].

Perhaps the most significant recent advance has been the effective oral treatment of VL by using miltefosine, an alkylphosphocholine originally developed as an anticancer drug (see Ref. [15]). The antileishmanial activity of miltefosine was initially discovered in the mid-1980s, and the subsequent demonstration of its efficacy in several experimental models (see Ref. [15]) led in the mid-1990s to clinical trials and co-development of miltefosine for leishmaniasis by a partnership between Asta Medica (now Zentaris) and WHO/TDR. After a Phase 3 trial, in which 282 out of 299 (94%) VL patients were cured with an
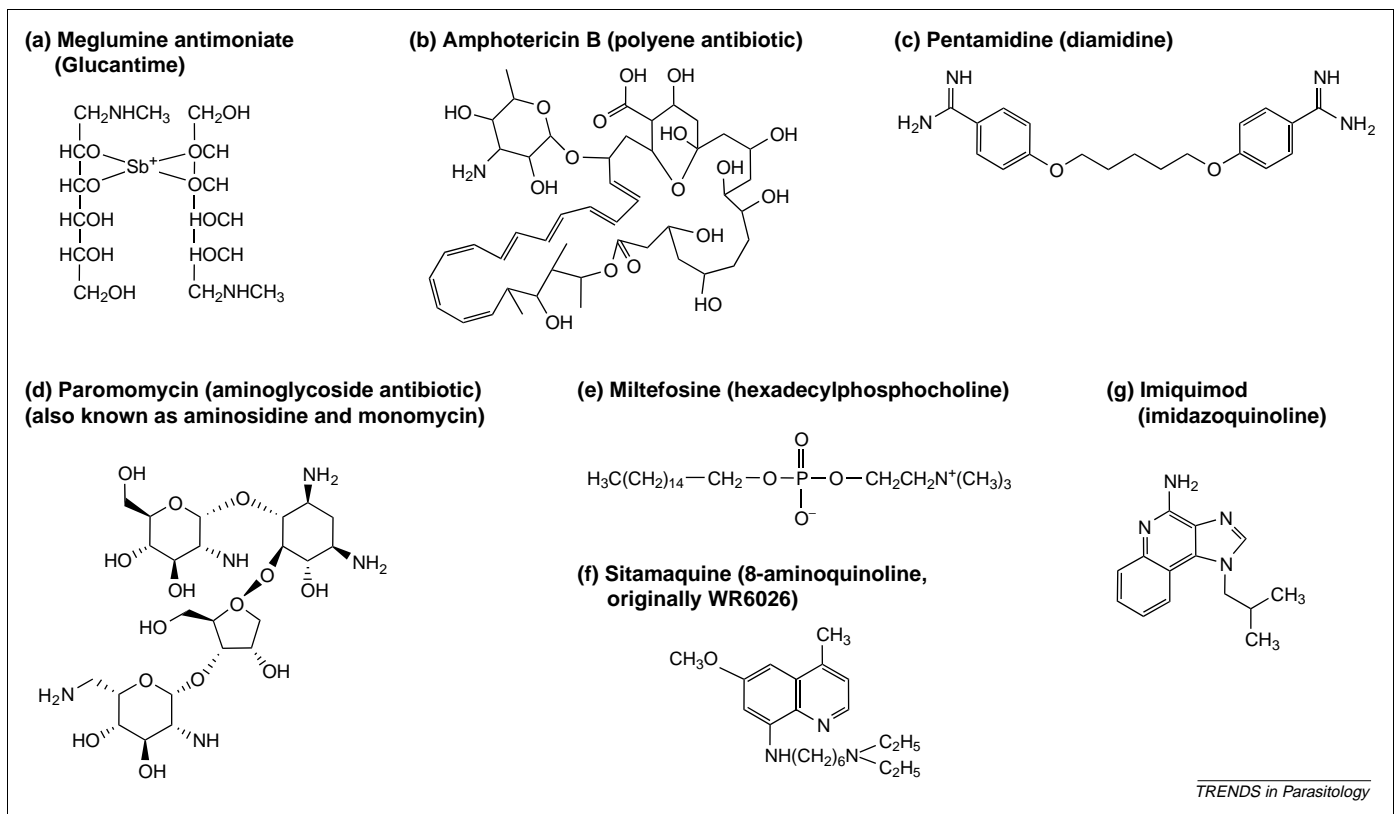

Figure 2. Chemical structures of antileishmanial drugs. See Table 2 for more information on these drugs.

Table 3. Antileishmanial drugs in discovery and development[a]

Late discovery phase	Pre-clinical development	Phase 1/Phase 2	Phase 3	Phase 4
Bisphosphonates (risedronate and pamidronate) for VL and CL	PX-6518, until 2002 (Tibotec, Belgium).	Sitamaquine for VL (GSK, UK)	Paromomycin for VL (WHO/TDR with Institute for One World Health, USA)	Miltefosine for VL in India (Zentaris, Germany and WHO/TDR)
Licochalcone A derivatives for VL and CL (with LICA Pharmaceuticals, Denmark)		Paromomycin topical (WRAIR, USA)	Miltefosine for CL (Zentaris, Germany)	
Propylquinolines		Imiquimod (topical) for CL in combination with antimonials		
Azoles: posoconazole (Schering-Plough) for CL		Azoles: itraconazole, ketoconazole, fluconazole		

[a]For more details, see text and Ref. [55]. The different phases in drug discovery and development are: (I) Late discovery phase, leads that have shown promising efficacy *in vivo* in animal models; (ii) pre-clinical development, pharmacokinetic, toxicological and chemical analyses; (iii) Phase 1, exploratory study in healthy human volunteers; (iii) Phase 2, efficacy and dose-range studies in infected patients; (iv) Phase 3, efficacy, safety and tolerance studies in comparison to standard drug leading to registration; and (v) Phase 4, post-registration to monitor performance. Abbreviations: CL, cutaneous leishmaniasis; VL, visceral leishmaniasis; GSK, GlaxoSmithKline; WRAIR, Walter Reed Army Institute of Research.

oral dose of 2.5 mg kg^{-1} of miltefosine daily for 28 days [16], miltefosine was registered in India in March 2002 for oral treatment of VL. Over 700 patients, including many who were refractory to antimonials, have now been successfully treated [16]. It remains to be seen whether miltefosine has similar efficacy against VL in other endemic areas such as Sudan, and against *L. infantum* (also known as *Leishmania chagasi*) VL in South America and the Mediterranean area. Miltefosine has also proved to be active against CL in a clinical trial in Colombia [17] and further trials against this disease type are planned. The major limitation of miltefosine is teratogenicity and this excludes its use in women of child-bearing age. Miltefosine is also being considered for the treatment of canine leishmaniasis, a disease for which no drugs are currently effective. Similar compounds, for example, an oleyl-phosphocholine formulation, have proved to be effective in dogs*. Effective treatment of canine leishmaniasis would be beneficial not only for the dogs concerned, but also in reducing an important animal reservoir of the parasites that could be transmitted to humans via sandflies. Clearly, the use of similar drugs for treating canine and human leishmaniasis could increase the likelihood of drug resistance emerging, although this should not happen if mass treatment programmes are avoided.

There are several other potential drugs at various stages of development (Table 3). Another oral drug that might have an impact on VL is the 8-aminoquinoline derivative sitamaquine, currently in development with GSK [18]. The antileishmanial activity of this compound was first identified in the 1970s at the Walter Reed Army Institute of Research (WRAIR, http://wrair-www.army.mil/). Several small Phase 1/2 clinical trials have been completed with varying levels of success, for instance, 67% of patients were cured of *L. chagasi* in Brazil when treated with 2 mg kg^{-1} daily for 28 days [19], and 92% were cured of VL when treated with 1.7 mg kg^{-1} daily for 28 days in Kenya†. Sitamaquine is rapidly metabolized, forming

desethyl and 4-CH$_2$OH derivatives, which might be responsible for its activity. Toxicity appears to be relatively mild, it causes mild methemglobinaemia, and further studies are planned.

Paromomycin (PM), an aminoglycoside antibiotic, was originally identified as an antileishmanial in the 1960s and has been used in clinical trials for both VL and CL. Although development of the parenteral formulation of PM, a drug with poor oral bioavailability, for VL has been slow, several Phase 2 trials in India and Kenya have been promising, with 90% of patients cured of VL following treatment with 15 mg kg^{-1} daily for 20 days, including antimony-refractory cases [20]. Funding for Phase 3 clinical trials and pharmaceutical manufacture has recently been secured (Table 3). There are also encouraging findings on the use of PM as a topical treatment for CL. The report by El-On and colleagues in 1984 that a topical formulation containing 15% PM and 12% methyl benzethonium chloride (a skin-penetrating agent) was effective against experimental CL led to clinical trials. One such trial demonstrated that 77% were cured after 20 days treatment compared with 27% cured in the placebo group [21]. Other topical formulations with a lower level of skin irritancy have subsequently been on clinical trial, including one containing 15% PM with 10% urea and, more recently, another containing 15% PM with 0.5% gentamicin in a vehicle that contains 10 surfactant (WR279 396) that cured 64% of CL patients after 20 days treatment in Colombia [22]. These studies have highlighted the need for rational pharmaceutical design of formulations optimal for CL [23].

The antileishmanial activity of the purine analogue allopurinol was identified over 30 years ago and, because it had oral bioavailability and was widely used for other clinical indications, it entered clinical trials for VL and CL. However, the results were disappointing. Allopurinol is used as a substrate by various enzymes of the purine salvage pathway of trypanosomatids, and is selectively incorporated into nucleotide intermediates and nucleic acids in the parasite. In recent years, allopurinol has been considered as part of a maintenance therapy for canine leishmaniasis, against which it has suppressive activity [24].

* Eibl, H. (2002) Alkylphosphocholines: potent new drugs to cure leishmaniasis. Abstract on pp. 14–15, COST B9 Meeting on Lipids, membranes and protozoal parasites, held 1–2 February 2002, in Utrecht, The Netherlands.

† Wasunna, M.K. et al. (2001) Sitamaquine (SB-252220, WR6026) in the oral treatment of visceral leishmaniasis. Abstract no 125, Second World Congress on Leishmaniasis, held 20–24 May 2001, in Hersonissos, Crete.

A variety of compounds discovered to have antileishmanial activity are at various stages of development (Table 3). Two classes of compounds offer potential of 'therapeutic switching' or 'piggy-back' chemotherapy. These include the azoles that were developed as antifungal drugs. *Leishmania* resemble fungi in synthesizing 24-substituted sterols such as ergosterol, whereas mammals have just cholesterol. Azoles, such as ketoconazole, inhibit 14α-demethylase, a key enzyme in this sterol biosynthesis pathway. Ketoconazole, itraconazole and fluconazole have undergone several trials for CL and VL with equivocal results. In one controlled trial, ketoconazole was found to have some activity against *L. mexicana*, but not against *L. braziliensis* infections [25]. Some recent encouragement has been given by the oral activity of posoconazole in a *Leishmania amazonensis* experimental model [26]. Bisphosphonates, for example, risedronate and pamidronate, which are in widespread use in the treatment of bone disorders such as osteoporosis, have also shown activity against leishmaniasis in experimental models [27,28]. These studies followed the characterization of acidocalcisomes in trypanosomatids with high polyphosphate and pyrophosphate content, and the hypothesis that bisphosphonates could interfere with pyrophosphate metabolism, although it is now thought that the prime target might be farnesyl pyrophophate synthase – a key enzyme in isoprenoid biosynthesis [29].

Other leads have come from plant products. Licochalcone A from the Chinese liquorice plant *Glycyrrhiza* has shown reasonable oral efficacy in experimental models of VL and CL; synthetic oxygenated derivatives are also active [30]. One derivative, 35 m4ac, resulted in 97% suppression of *L. donovani* liver amastigotes in a hamster model when given at 20 mg kg^{-1} for six days intraperitoneally. The compounds appear to interfere with mitochondrial function. The 2-substituted quinoline alkaloids, from the Bolivian plant *Galipea longiflora*, have also shown oral activity in experimental VL and CL mouse models [31]. Saponins purified from the Vietnamese plant *Maesa balansae* and designated PX-6518 showed excellent activity after parenteral administration against VL and CL in rodent models [32]. However, the development of PX-6518 was halted as a result of unacceptable toxicity (L. Maes, 2003, PhD thesis, University of Antwerp, Belgium).

Immunomodulation

Cure of leishmaniasis, probably even during chemotherapy, appears to be dependent upon the development of an effective immune response that activates macrophages to produce toxic nitrogen and oxygen metabolites to kill the intracellular amastigotes [6,33,34]. This process is suppressed by the infection itself which downregulates the requisite signalling between macrophage and T cells, for example, the production of interleukin (IL)-12 or the presentation of major histocompatibility complex (MHC) and co-stimulatory molecules at the macrophage surface. Studies in the 1980s showed that biological immunomodulators such as interferon (IFN)-γ can provide a missing signal and enhance the activity of antimonials in the treatment of VL and CL. Recently, a new generation of immunopotentiating drugs have shown potential for leishmaniasis treatment. The imidazoquinoline imiquimod, an ingredient of the topical cream for genital warts known as Aladara™ (3M Pharmaceuticals; http://www.3m.com/), induces nitric oxide (NO) production in macrophages. Imiquimod was shown to have antileishmanial activity via macrophage activation in experimental models [35] and in clinical studies on CL in combination with antimonials [36]. This sensitivity of *Leishmania* amastigotes to NO was also exploited in a study using the NO generator nitroso-N-pencillamine (SNAP) topically on *L. brazilienisis* infections [37]. In one approach to restore signalling, the substituted benzaldehyde tucaresol, which stimulates a signal to CD4$^+$ T cells and promotes T helper cell (Th) type 1 cytokine production, showed activity in mouse VL models. A 5 mg kg^{-1} oral dose for five days proved effective, resulting in a 60% reduction in the number of *L. donovani* liver amastigotes in mice. [38]. On a different pathway, anisomycin restores signalling via CD40 and activates p38 mitogen-activated protein (MAP) kinase thus killing parasites in mouse models [39]. These results indicate that immunomodulatory drugs show promise as an adjunct to chemotherapy.

Rational approach: possibilities and problems

New antileishmanial drugs are required and the favoured approach adopted by many to achieve this goal is to identify potential drug targets (either through biochemical studies or, increasingly, by mining the *L. major* genome database), validating them either chemically or genetically [40], and then identifying inhibitors that can serve as lead compounds to enter a drug development process. Much of this approach is similar to that being applied with many other pathogens (see Cowman and Crabb; Biagini *et al*. this issue).

One of the major gaps in the drug development process for novel antileishmanials (and also drugs against other parasitic diseases) has been the chemistry–biology interaction to obtain lead compounds that can enter the development process. Many articles have been published (usually by researchers based in universities or government-funded research institutes, rather than scientists based in industry) showing that compound X inhibits enzyme Y *in vitro*. Few of these have been followed by work to establish whether the compound has useful activity against leishmaniasis in an animal model. In many cases, this could reflect simply that the compound shows no specificity and is toxic. More chemical syntheses are required to obtain analogues of the compound Xs or other enzyme inhibitors, so that lead compounds that can be progressed into drug development are discovered. However, there have been two other problems: (i) obtaining chemical libraries that can be screened to discover novel inhibitory compounds (such libraries are owned mainly by pharmaceutical companies with restricted access); and (ii) ensuring patent protection (which is expensive and not easily covered without partnerships with industry).

The development process itself is highly costly. As the resources available for the search for new antileishmanials are considerably more restricted than those allocated for diseases directly affecting the developed world, few

Table 4. Validated and potential antileishmanial drug targets[a]

Enzyme/pathway	Evidence	State of exploitation	Ref.
Trypanothione metabolism	Validation of trypanothione reductase as a drug target suggested by genetic experiments. Glutathionylspermidine synthetase and trypanothione synthetase also potential targets yet to be fully validated.	Many inhibitors of trypanothione reductase reported, yet none have been shown to be effective *in vivo* through inhibition of enzyme.	[41]
Cysteine peptidases	The Clan CA enzyme CPB validated by genetic manipulation and chemical means.	Inhibitors effective *in vivo*. One undergoing clinical trials against Chagas disease.	[42]
Sterol biosynthesis	Inhibitors of enzymes of sterol biosynthesis pathway (e.g. 14 α-demethylase) have antileishmanial activity *in vitro*.	At clinical phase of development (see Table 2), but parasite might be able to salvage the host's sterols to survive drug attack.	[43]
DHFR	A favoured target as the enzyme differs from the mammalian counterpart and homologous enzymes are targets for the antimalarial pyrimethamine and the antibacterial trimethoprim.	Many inhibitors reported, but *in vivo* activity is disappointing. Parasite might be able to overcome DHFR inhibition by alternative pathway involving PTR1.	[44,45]
PFT and NMT	Enzyme inhibitors are promising agents in other chemotherapy (e.g. as antifungals). Leishmanial *NMT* gene essential.	*NMT* gene characterized. Inhibitors show antiparasitic activity *in vitro*.	[46]
Glycolysis	Glycolysis considered essential, many enzymes enclosed in glycosomes.	Enzymes characterized and inhibitors discovered. Inhibitors with *in vivo* activity are elusive so far.	[47]
Polyamine metabolism	Ornithine decarboxylase is target of DFMO, an effective drug against African trypanosomiasis.	DFMO is ineffective against leishmaniasis probably due to polyamine salvage from host.	[48]
Protein kinase	Cyclin-dependent kinases essential to parasite.	Inhibitors have been discovered, active against amastigotes in macrophages.	[49]
Microtubules	Tubulin validated as drug target.	Parasite-specific agents required.	[50,51]

Abbreviations: CPB, a cysteine peptidase; DFMO, eflorinithine; DHFR, dihydrofolate reductase; NMT, *N*-myristoyl transferases; PTR, pteridine reductase; PNT, protein farnesyl transferase.

[a]This list is intended to give information on some of the areas under study and an impression of the current status of attempts to exploit them, but it is not all-inclusive.

candidate drugs have progressed into development over the years. The most fruitful recent approach has been to piggy-back upon the drug development programmes in other areas, such as anticancer and antifungal agents. The advent of miltefosine as a registered antileishmanial agent is a prime example of the success of this approach. Other examples have been detailed above.

Research aimed at identifying and validating new drug targets is in one respect the most *Leishmania*-specific part of the drug discovery process, although comparative studies on *Leishmania* and trypanosomes have been fuelled by the desire to find a broad-spectrum anti-trypanosomatid drug. The cell biology of *Leishmania* and mammalian cells differ considerably and this distinctness extends to the biochemical level. This provides the promise that many of the parasite's proteins should be sufficiently different from anything in the host to be successfully exploited as drug targets. Discovering new antileishmanial drugs should be considerably easier than discovering new anticancer drugs. Thus, from the scientific perspective (as opposed to the economic perspective), there is much cause for optimism. Indeed, over many years, a whole array of 'interesting drug targets' have been proposed – too many to list here. This claim has been made for many proteins that have been shown to differ from a mammalian counterpart in even a minor way. Unfortunately, few of these possible targets have been rigorously validated (i.e. evidence has not been provided that interfering with the action of the protein would kill the parasite). There are several approaches that can provide such evidence, the most powerful being chemical and genetic validation [40]. However, even these can be uninformative about the potential of proteins for chemotherapeutic exploitation. Notably, proteins involved in activating pro-drugs can be easily overlooked. The few

leishmanial proteins generally accepted as being validated drug targets are listed in Table 4, together with some other proteins that have been heralded as important potential targets, but for which fully convincing evidence needs to be produced. These can be classed as being in the early discovery phase. Time will tell which, if any, of these aspects of parasite biochemistry really can be exploited by the development of much needed novel antileishmanial drugs.

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