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Chemotherapy of trypanosomiases and leishmaniasis

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New formulations, therapeutic switching of the established drugs amphotericin B and paromomycin, and the serendipitous discovery of miltefosine have markedly improved leishmaniasis chemotherapy in the past 21 years. The situation for the two trypanosomiases has been less encouraging. Apart from the introduction of eflornithine for the treatment of late-stage human African trypanosomiasis, with its serious limitations in terms of cost and difficulty of administration, no new drugs have been incorporated into the chemotherapeutic arsenal in the past 25 years, despite important advances in knowledge of the biology of the etiological agents and the pathophysiology of these diseases. In the case of Chagas disease, several classes of compound that target the validated biochemical pathways of the parasite (e.g. inhibitors of sterol biosynthesis and cysteine proteases) are in the pipeline. With the availability of complete genome sequences for all three pathogens, and methods for rapid validation of targets, it is hoped that much-needed amelioration will occur soon. Financial constraints continue to represent a major hurdle to drug development. However, the appearance of not-for-profit product-development partnerships offers a new paradigm for bringing new drugs to patients.

Available drugs – then and now

It is more than disappointing to reflect that the start of a review of the chemotherapy of human African trypanosomiasis (HAT) and Chagas disease is hardly different today from how it was 21 years ago. The drugs used for treatment of these two diseases (Table 1), with all their associated problems of toxicity, variable efficacy, parenteral administration or length of treatment, are the same now as then. Even those currently in clinical trials suffer limitations. It is only in the treatment of leishmaniasis that some clear advances have been made, although most of these have been painfully slow and are based on studies and proof of principle established 21 years ago. Considerable advances in the validation and characterization of drug targets and in new chemistry are now making a difference and offer hope for the coming years. If this knowledge is matched by improved models of disease and drug-development processes, in addition to improved funding opportunities, we might be able to put arsenical and antimonial drugs onto the museum shelf once and for all.

Leishmaniasis

Of the standard drugs recommended 20 years ago, pentavalent antimonials are now almost obsolete in India because of drug resistance [1] but they are still useful in the rest of the world, where the introduction of generic brands has reduced costs. Amphotericin B, a second-line drug in 1984, has moved to the forefront in India. In 1979, groups in London and Liverpool showed that the encapsulation of antimonial drugs in liposomes enhanced anti-leishmanial efficacy in experimental visceral leishmaniasis (VL) models. By 1985, this approach had been adopted (although later abandoned) by Wellcome for the development of a sodium-stibogluconate–liposome formulation, and it had been demonstrated that amphotericin B liposomes worked in both VL and cutaneous leishmaniasis (CL) experimental models. A range of amphotericin B lipid formulations, developed independently during the 1980s for treatment of systemic mycoses in immunocompromised patients, has also proved to be effective in the treatment of leishmaniasis. One of these – the liposomal formulation AmBisome (http://www.ambisome.com/), which was first shown to cure a case of VL in 1991 – is registered for treatment of VL, and a single-dose therapy of 5 mg kg^{-1} has been shown to cure 90% of patients in India suffering from this disease [2]. High cost limits the wider use of AmBisome for treatment. Paromomycin, originally an oral aminoglycoside for intestinal infections, had already shown efficacy as a topical treatment for CL by 1985 and as a parenteral drug for VL by 1990. Amazingly, it has taken until 2005 for Phase III clinical trials for paromomycin against VL to be completed in India [3] – a reflection of the limited funding available. For CL, there remains a search for more-effective and less-irritant topical formulations than those originally developed [4], and some are undergoing clinical trials [5]. As with paromomycin, the antileishmanial activity of miltefosine (Figure 1) was identified at Wellcome and, again, its activity was proven by 1985 [6]. This lysophospholipid analog, a surprising antiprotozoal found more by serendipity than by design, is the first oral treatment produced for VL [7], is effective against CL [8] and has been registered for treatment of pathological conditions in India (in 2002) and Colombia (in 2005), respectively.

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Table 1. Drugs in use and on trial in 1985 and 2005

Visceral leishmaniasis	**1985**	**2005**
First-line drugs	Sodium stibogluconate (Pentostam); meglumine antimoniate (Glucantime)	Sodium stibogluconate (Pentostam, generic sodium stibogluconate); meglumine antimoniate (Glucantime)
	Amphotericin B (Fungizone)	Amphotericin B (Fungizone) Liposomal amphotericin B (AmBisome)
	Pentamidine	Pentamidine
Clinical trials	Allopurinol (Phase II)	Miltefosine (oral, Phase IV, registered in India) Paromomycin (Phase III) Sitamaquine (oral, Phase II) Other amphotericin B formulations
Drugs in preclinical development	–	–
Cutaneous leishmaniasis	**1985**	**2005**
First-line drugs	Sodium stibogluconate (Pentostam); meglumine antimoniate (Glucantime)	Sodium stibogluconate (Pentostam); meglumine antimoniate (Glucantime)
	Amphotericin B (Fungizone)	Amphotericin B (Fungizone)
	Pentamidine	Pentamidine Paromomycin (topical formulations)
Clinical trials	Paromomycin (topical formulation, Phase II)	Miltefosine (oral, Phase III)
	Allopurinol riboside (Phase II)	Paromomycin (other topicals, Phase II) Imiquimod (topical immunomodulator, Phase II)
Drugs in preclinical development	–	–
Human African trypanosomiasis	**1985**	**2005**
Haemolymphatic stage		
First-line drugs	Pentamidine	Pentamidine
	Suramin	Suramin
Clinical trials	–	DB 289 (Phase III)
Central nervous system stage		
First-line drugs	Melarsoprol	Melarsoprol Eflornithine
Clinical trials	–	Nifurtimox in combination with eflornithine (Phase III)
Drugs in preclinical development	–	–
Chagas disease	**1985**	**2005**
First-line drugs	–	–
Acute stage	Benznidazole; nifurtimox	Benznidazole; nifurtimox
Indeterminate stage	–	–
Chronic stage	–	–
Clinical trials	Allopurinol (acute stage, Phase II)	Benznidazole (indeterminate stage)
Drugs in preclinical development	–	Antifungal triazoles (posoconazole, ravuconazole, TAK-187) Cruzipain inhibitor (K777)

The successes in leishmaniasis chemotherapy during the past 21 years have been based on reformulation and screening rather than rational design and an improved understanding of immunology. The two hopes for rational design in the 1980s – the pyrazolopyrimidines (allopurinol and derivatives) that disrupt purine salvage and nucleic acid biosynthesis, and the inhibitors of C14-α demethylase and sterol biosynthesis (antifungal azoles) – proved to have disappointing efficacy, as mitigated by pharmacokinetic properties [9] and biochemical routes that enable bypass in parasites [10]. A variety of research approaches has led to recent reports of novel compounds with promising *in vivo* activities: (i) on biochemical pathways such as isoprenoid biosynthesis, leading to the identification of anti-leishmanial bisphosphonates [11]; (ii) on natural-product screening, leading to the identification of maesabalides [12]; (iii) and on chemical derivatization, leading to the identification of novel quinolines [13].

Human African trypanosomiasis

Unprecedented advances in the understanding of the biology of the African trypanosome during the past 21 years have been accompanied by dismal progress in exploiting this information to develop new drugs [14]. Admittedly, the ornithine decarboxylase inhibitor eflornithine was registered to treat late-stage *gambiense* disease in 1990 following first reports of treatment in 1984 [15]. The lower incidence and severity of adverse effects compared with those caused by melarsoprol have led some to advocate that eflornithine become the first-line treatment for late-stage HAT [16]. However, difficulties in manufacturing, the requirement of high doses and a need for prolonged intravenous infusion make eflornithine expensive and difficult to distribute and administer in rural Africa. Its availability as a trypanocide is dependent on commitments made to Medecins Sans Frontieres (http://www.msf.org/) and the World Health Organization [WHO (http://www.who.int/en/)] by the manufacturing company sanofi-aventis (http://en.sanofi-aventis.com/index.asp). No other compound has been registered in the past 21 years.

During this time, however, the incidence of HAT has risen sharply, and those engaged in treating this disease have resorted to trying to improve the use of currently registered drugs. Pharmacokinetic studies of melarsoprol led to the successful testing of a shortened ten-day course

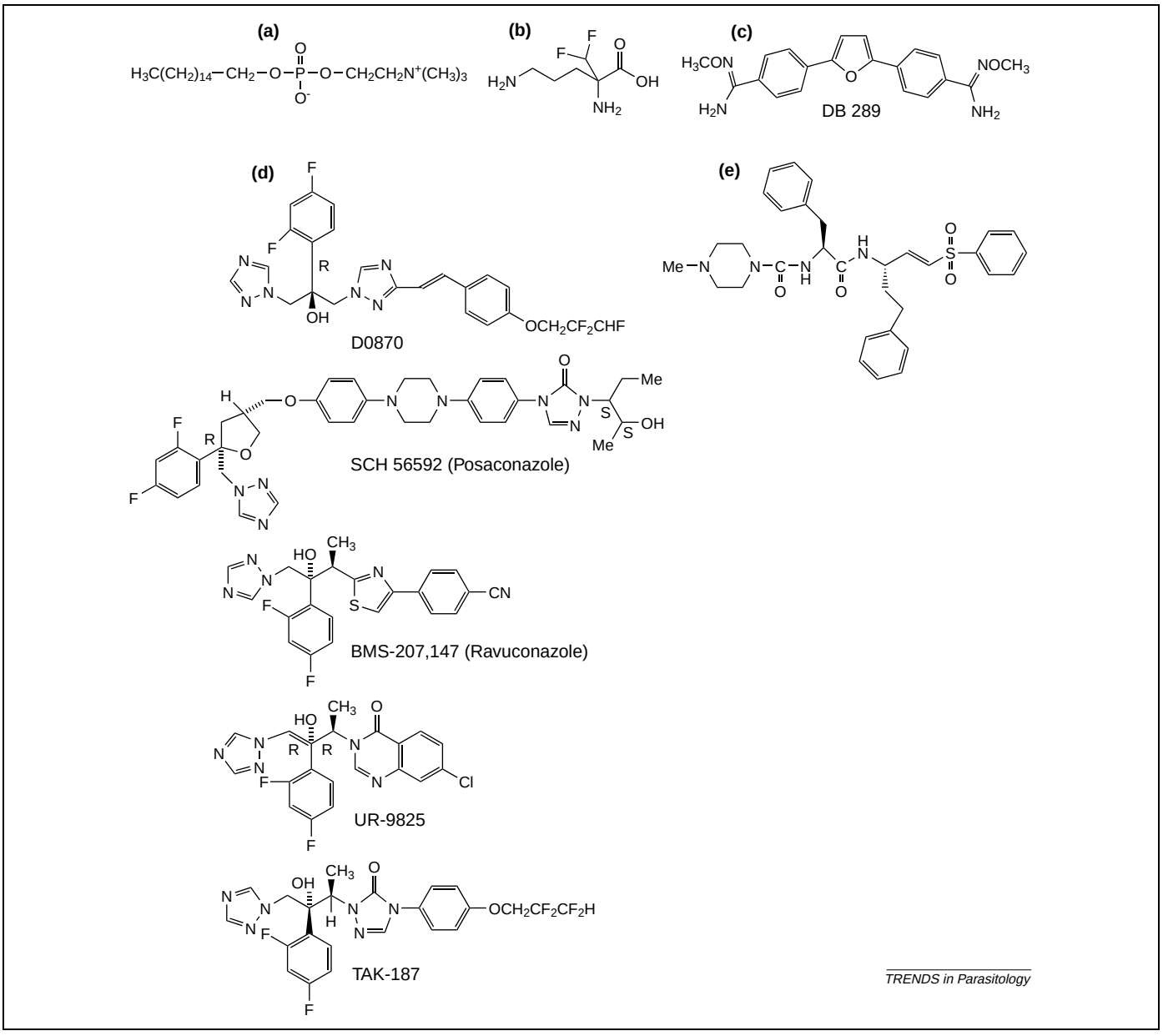

Figure 1. New drugs in clinical or preclinical development for the trypanosomiases and leishmaniasis. **(a)** Miltefosine, an alkylphosphocholine originally developed as an anticancer agent, has been developed for treatment of both VL and CL by Zentaris (http://www.zentaris.net/cms/front_content.php?idcat=17) and the WHO TDR. **(b)** Eflornithine (difluoromethylornithine) is an irreversible inhibitor of ornithine decarboxylase and polyamine biosynthesis that was originally developed as an anticancer agent but is now used for the treatment of stage 2 *Trypanosoma brucei gambiense* infection. **(c)** The diamidine DB 289 is a prodrug of 2,5-bis(4-amidinophenyl)furan (DB75 or furamidine) and is currently in Phase III clinical trials for treatment of stage 1 *T. b. gambiense* infection in Central Africa (in development with the North Carolina University and Gates Consortium). **(d)** New triazole derivatives are inhibitors of *Trypanosoma cruzi* sterol C14α sterol demethylase that have potent and selective activity and pharmacokinetic properties. They can eradicate the parasite from animal models of both acute and chronic Chagas disease. These derivatives are currently in development as systemic antifungal agents. **(e)** N-methyl-piperazine-urea-F-hF-vinyl-sulfone-phenyl (K-777) is a specific, irreversible inhibitor of cruzipain, an essential cysteine protease present in all stages of *T. cruzi*. K777 kills *T. cruzi in vitro* and *in vivo*.

of treatment (rather than 21–35 days), which improves patient compliance and reduces hospital costs [17]. Studies aimed at modifying the dosing of pentamidine and eflornithine are also underway. The increased incidence of treatment failure with melarsoprol in some HAT foci is of growing concern [18]. Field studies to determine whether these failures relate to drug resistance brought about by loss of the P2 transporter, which contributes to the uptake of both melarsoprol and diamidine-class drugs, are in progress.

Only one drug for treating HAT is currently undergoing clinical trials. The orally available prodrug DB289 (Figure 1) is converted systemically into another diamidine (DB75) that is active against early-stage disease [19]. The blood–brain barrier remains a potent hindrance to reaching parasites within the brain of patients with late-stage HAT [20]. The number of diamidines that has emerged from a consortium led by the University of North Carolina (http://www.pathology.unc.edu/faculty_labs/tidwell_lab/tidwellhome.html) and funded by the Gates Foundation (http://www.gatesfoundation.org/default.htm) is remarkable, and it is hoped that other prodrugs that are active against late-stage disease will also emerge and move to clinical development. Trypanosomes are highly

sensitive to selected nitroheterocyclic compounds. Because the nitrofuran nifurtimox and the nitroimidazole benznidazole are registered for use against *Trypanosoma cruzi* infection, efforts to extend their use to African trypanosomes have been made. Clinical trials suggest that neither drug is sufficiently active alone to indicate its use for treatment of HAT. Other nitroheterocyclic compounds such as megazol stimulated interest until genotoxicity issues halted their development [21], although other compounds of this class might yet be useful [22].

Overall, the lack of advances in the treatment of HAT highlights the great gap that has emerged between basic and clinically oriented science during the past 21 years: a gap that must now be closed [23].

South American trypanosomiasis (Chagas disease)

In 1985, autoimmune phenomena were widely believed to be the primary factors leading to the pathology associated with the chronic phase of Chagas disease [24]. Recent investigations point to persistence of parasites, coupled with an imbalanced immune response that could include autoimmune reactions [25], generating sustained inflammatory responses in infected tissues that underlie the characteristic lesions of chronic Chagas disease [26]. These findings indicate that the elimination of *T. cruzi* from infected patients might be a prerequisite to arrest the evolution of the disease and to avert its irreversible long-term consequences. Unfortunately, despite the impressive advances in understanding the biology of *T. cruzi*, the only drugs currently available against this organism are those that were already registered 21 years ago: nifurtimox and benznidazole, which were developed empirically in the 1960s and 1970s. These compounds are active in the acute stage of Chagas disease (up to 80% efficacy), and benznidazole has also recently been shown to be efficacious in early chronic infections [27] but of limited efficacy against established chronic-stage disease. The side effects of both nifurtimox and benznidazole can be severe.

Rational approaches to the treatment of Chagas disease are underway. Among the most advanced agents in development are new triazole derivatives (Figure 1): specific inhibitors of ergosterol biosynthesis that function at the level of C14-α sterol demethylase. These drugs are poised to enter clinical trials soon [28]. Inhibitors of cruzipain – an essential protease specific to the parasite [29] – and N-alkyl-bisphosphonates – inhibitors of farnesyl pyrophosphate synthase that selectively accumulate in the acidocalcisomes of the parasite [28,30] – are rapidly advancing in preclinical development. Several other promising approaches (e.g. inhibitors of trypanothione synthesis and metabolism [31], hypoxanthine guanine phosphoribosyl transferase inhibitors [28] and novel inhibitors of ergosterol biosynthesis that function at the level of squalene synthase or oxidosqualene cyclase [28]) are also undergoing preclinical investigation.

Concluding remarks

The past 21 years have seen considerable advances in knowledge of the biochemistry and cell biology of the three pathogens that cause leishmaniasis and trypanosomiases; these advances are exemplified by the recent publication of the genome sequences of these organisms [32]. Thus, all potential drug targets are now accessible, and methods are available for the rapid validation and characterization of targets. However, the discovery and development of new drugs also require increased input from the disciplines of chemistry, pharmacology, toxicology and pharmaceutics to complement these advances in molecular biology, and further development of suitable disease models and methods for progressing leads and candidate drugs through preclinical studies – a problem also identified for other diseases [see US Department of Health and Human Services Food and Drug Administration Report, March 2004 (http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html)]. The limited progress in drug development during the past 21 years, however, has been caused mainly by both the lack of economic incentives in the current, for-profit, model of drug development for the pharmaceutical industry to be fully engaged and the absence of alternative models with the appropriate expertise. One estimated cost for the development of a new chemical entity (NCE) for the market is US$800 million; typically, thousands of compounds are tested for a single treatment before an NCE that is both safe and effective is marketed [33]. The US$800 million (and rising) figure reflects the high attrition rate, the demands for complex treatments for diseases of the developed world and the fact that the productivity of the pharmaceutical industry has steadily declined; it is currently more than tenfold lower than in the 1970s [34]. One analysis of this inadequate development of drugs to treat tropical diseases, which afflict mostly poor rural populations of the world, showed that only 1% of the NCEs introduced into the market in the 25 years preceding 2000 was developed to treat these diseases. Only 10% of the current global heath R&D effort is directed towards addressing the medical needs of 90% of the human population: a result of both market failure and inadequate public policies in endemic countries [35,36]. However, the cost of drug development for tropical infectious diseases should be significantly lower. Increased awareness of this situation has led to the formation of several not-for-profit product-development partnerships (PDPs) such as the Drugs for Neglected Diseases initiative (http://www.dndi.org/), the Medicines for Malaria Venture (http://www.mmv.org/pages/page_main.htm) and the Institute for OneWorld Health (http://www.oneworldhealth.org/) that, along with the WHO Tropical Diseases Research Programme (TDR), aim to address this misbalance in the world biomedical research and development effort. The PDPs propose alternative drug RandD models, fostering effective collaborations between the public and private sectors and including groups from endemic countries in which most health research and health services are funded and conducted by the public sector with a lower cost of production and services [37].

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