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KINETOPLASTIDA: NEW THERAPEUTIC STRATEGIES

CROFT S.L.*

Summary:
New formulations and therapeutic switching of the established drugs, amphotericin B and paromomycin, together with the discovery of miltefosine, have significantly improved the opportunities for treatment of visceral leishmaniasis (VL) chemotherapy. However, for human African trypanosomiasis (HAT), Chagas disease and cutaneous leishmaniases there has been limited progress. For HAT, a novel diamidine, parfuramidine, is in phase III clinical trial for early-stage disease, but for the treatment of late-stage disease there are no new drugs and combinations of eflornithine with melarsoprol or nifurtimox have been the focus of clinical studies. For Chagas disease, different classes of compounds that have validated biochemical targets, sterol biosynthesis methylases and cysteine proteases, are in various stages of development. The genome sequences that are now available for the pathogens that cause the leishmaniases and trypanosomiases, and new methods for rapid validation of targets, are part of the solution to discover new drugs. The integration of medicinal chemistry, pharmacokinetics, project planning and interaction with the pharma/biotech sector are essential if progress is to be made. Although there are financial constraints, the appearance of new funding sources and not-for-profit product development partnerships offers hope for drug development.

KEY WORDS : visceral leishmaniasis, cutaneous leishmaniasis, human African trypanosomiasis, Chagas disease, drugs, chemotherapy.

The drugs used for treatment of the leishmaniases and the trypanosomiases (see Table I) are fraught with problems of toxicity, variable efficacy, parenteral administration or length of treatment. For the trypanosomiases and cutaneous leishmaniasis, there are limited drugs or treatments in clinical development. In contrast, for visceral leishmaniasis there has been some progress with the availability of liposomal amphotericin B, miltefosine and paromomycin for treatment in India. Although considerable advances in the identification, validation and characterization of drug targets has come with the completion of the genomes for *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major* (Berriman *et al.*, 2005) and new tools such as RNAi (Balana-Fouce & Reguera, 2007) have been developed, this is only one early part of the long and complex process of drug discovery and development.

* Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK.
E-mail: simon.croft@lshtm.ac.uk

New and established pharmacophores, based upon synthetic and natural product chemistry, have to be brought into place along with improved screening technologies to identify hits (Frearson *et al.*, 2007). Appropriate models of infection and early pharmacokinetic studies to evaluate leads, also need to be integrated into the process, as seen at academic centres in USA (www.ucsf.edu/mckerrow) and UK (www.dundee.ac.uk/biocentre). The support of public-private partnerships like the Drugs for Neglected Diseases initiative (www.dndi.org) and interaction with the commercial sector is also required. Further development will depend upon the expertise in lead optimization, toxicology and pharmacology available in the pharmaceutical and biotech sectors and CROs with the associated requirement for high level funding (see for example www.dndi.org). Clinical trials capacity is also required and is being addressed in India, Africa (www.sti.ch) and South America. The issues around drug production and delivery also need to be addressed; the example of paromomycin delivery for VL in India provides some model for the future (www.iowh.org).

LEISHMANIASIS

There have been different rates of development and there are different issues associated with drug development for visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL); these two manifestations will therefore be treated separately.

VISCERAL LEISHMANIASIS

Pentavalent antimonials, the standards drugs for 60 years, are now almost obsolete in India due to drug resistance (Croft *et al.*, 2006), but are still useful in the rest of the world. The introduction of generic brands has reduced costs. Amphotericin B, normally considered a second line drug, is now first line in Bihar state, India. Although a number of amphotericin B lipid formulations, developed during the 1980s for treatment of systemic mycoses in immunocompromised patients, have proved effective in the treatment of VL, only one of

	Drugs	Comments
Visceral leishmaniasis		
First line drugs	Sodium stibogluconate (Pentostam and SSG)	Generic sodium stibogluconate (SSG) from Albert David (India) has made treatment cheaper. Sanofi-Aventis have also reduced the price of Glucantime
	Meglumine antimoniate (Glucantime)	
	Amphotericin B (Fungizone)	
	Liposomal amphotericin B (AmBisome)	This has proved to be the most effective and least toxic lipid formulation for VL
	Miltefosine	Registered in India and available on private market. Cheaper price available *via* WHO
Clinical trials	Paromomycin	In phase IV trials in India
	Sitamaquine	Completed phase II trials in India
	Other amphotericin B formulations	Further pre-clinical research for development of cheaper formulations
Cutaneous leishmaniasis		
First line drugs	Sodium stibogluconate (Pentostam)	
	Meglumine antimoniate (Glucantime)	
	Amphotericin B (Fungizone)	For complex manifestations, like mucosal leishmaniasis
	Pentamidine	For specific forms in South America
	Paromomycin (topical formulation)	
Clinical trials	Paromomycin (topical formulations, phases II and III)	Formulations from WRAIR (Washington) and FioCruz (Belo Horizonte) on trial
	Miltefosine (oral, phase III)	There appears to be differences in species sensitivity. Registered in Colombia
	Imiquimod (topical immunomodulator, phase II)	As an adjunt therapy to antimonials. Reported not to be effective in Iran
Human African trypanosomiasis		
• ***Haemolymphatic stage***		
First line drugs	Pentamidine	
	Suramin	
Clinical Trial	Parfuramidine (DB289)	Phase III trials in DRC and Sudan
• ***CNS stage***		
First line drugs	Melarsoprol	
	Eflornithine	
Clinical Trial	Nifurtimox and eflornithine co-administration	Phase III trial with DNDi and WHO TDR
Chagas disease		
First line drugs		
• ***Acute stage***	Nifurtimox	
	Benznidazole	
• ***Indeterminate stage***	No treatment	
• ***Chronic stage***	No treatment	
Clinical trials	Benznidazole	BENEFIT trial for indeterminate stage

Table I. - Drugs in use or on clinical trial in 2007.

these, the liposomal formulation AmBisome, has become a standard. It is registered for the treatment of VL in various countries, its use is described by a WHO working group (Bern *et al.*, 2006) and a single dose therapy of 5 mg/kg has been shown to cure 90 % patients in India (Sundar *et al.*, 2003). A significant reduction in price negotiated by WHO with the producers (Gilead) will have an impact, but AmBisome will remain an expensive treatment. A parenteral formulation of the aminoglycoside paromomycin, has moved slowly through clinical trials over the past decades, more recently showing 94 % efficacy (15 mg/kg for 21 days) in phase III clinical trials in India (Sundar *et al.*, 2007) and was registered there for VL in 2006. Like paromomycin, the anti-leishmanial activity of the phospholipid derivative, miltefosine (Fig. 1) was first identified at the Wellcome laboratories, UK. (Croft & Engel, 2006). This drug has provided the first oral treatment for VL and the first to undergo phase IV studies (Bhattacharya *et al.*, 2007).

Other opportunities remain. Rational approaches, pyrazolopyrimidines (allopurinol and derivatives) that disrupt purine-salvage and nucleic acid biosynthesis, and the inhibitors of 14-α demethylase and sterol biosynthesis (antifungal azoles), proved to have disappointing efficacy, due to poor pharmacokinetic properties

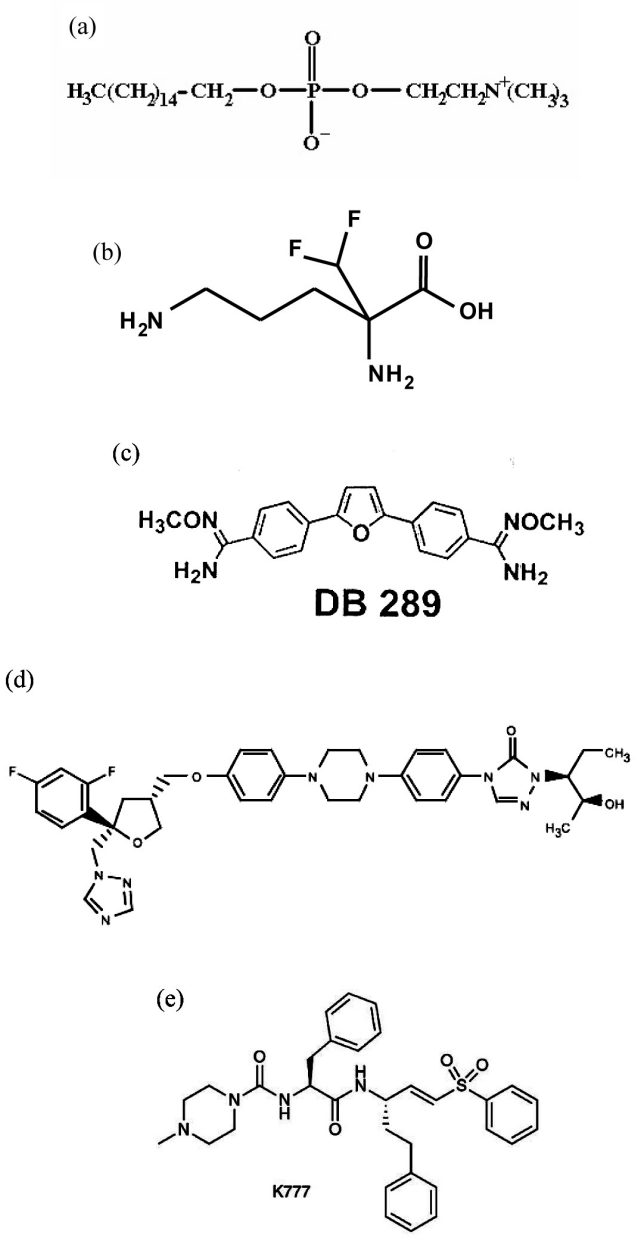

Fig. 1. – New drugs in clinical or pre-clinical development for the trypanosomiases and leishmaniasis
(a) Miltefosine, an alkylphosphocholine, originally developed as an anti-cancer agent, subsequently developed for treatment of both VL and CL by Zentaris AG (Germany) and WHO/TDR.
(b) Eflornithine, difluormethylornithine, an irreversible inhibitor of ornithine decarboxylase and polyamine biosynthesis, originally developed as an anti-cancer agent, but used for the treatment of CNS stage *T. b. gambiense* infection.
(c) DB 289, a diamidine, pro-drug of 2,5-bis(4-amidinophenyl)furan (DB75 or furamidine), currently in phase III clinical trials for treatment of haemolymphatic stage *T. b. gambiense* infection in central Africa, in development with N. Carolina University Gates Consortium.
(d) Posaconazole, a triazole derivative, that inhibits *Trypanosoma cruzi* sterol C14α sterol demethylase and can eradicate the parasite from animal models of both acute and chronic Chagas disease. Registered as a systemic antifungal agent.
(e) K777 (N-methyl-piperazine-urea-F-hF-vinyl-sulfone-phenyl), a specific inhibitor of cruzipain, an essential cysteine protease of *Trypanosoma cruzi*, with trypanocidal activity, both *in vitro* and *in vivo*.

(Shapiro *et al.*, 1991) or biochemical routes permitting bypass in intracellular parasites (Roberts *et al.*, 2003). However, allopurinol remains a component of treatment of canine VL and the triazole posoconazole was effective in rodent models (Al-Abdely *et al.*, 1999). Natural product screens have identified chalcones (Zhai *et al.*, 1999), maesabalides (Maes *et al.*, 2004), and novel quinolines (Nakayama *et al.*, 2006) with activity in rodent VL models, although metabolic and toxic liabilities have limited the progress of lead compounds. Other opportunities lie in understanding of metabolic pathways and enzyme targets, with studies on isoprenoid biosynthesis leading to studies on bisphosphonates (Yardley *et al.*, 2002), and on kinases to paullones (Grant *et al.*, 2004).

Cutaneous leishmaniasis (CL)

In comparison to VL there are limited proven options for CL (see Table I). Pentavalent antimonials have proved inconsistent in their effectiveness across the different *Leishmania* species (Croft *et al.*, 2006), and pentamidine and amphotericin B are limited to specific types of CL (see Alvar *et al.*, 2006). Paromomycin in various topical formulations has variable efficacy (see Garnier & Croft, 2002), and there is a continuing search for more effective and less irritant topical creams and gels (Ben-Salah *et al.*, 2005). Oral miltefosine also has some variable, species dependent effectiveness against CL (Soto *et al.*, 2004: Yardley *et al.*, 2005) and is now registered for this indication in Colombia (2005). Further studies are required to define effectiveness against different forms of this disease.

HUMAN AFRICAN TRYPANOSOMIASIS

Significant advances in our understanding of the biology of *Trypanosoma brucei* have not yet led to new drugs (Berriman *et al.,* 2005). Since the registration of the ornithine decarboxylase inhibitor, eflornithine, in 1990 for late stage *gambiense* disease (Burri & Brun, 2003) no novel treatments have been introduced. The lower incidence and severity of adverse effects of eflornithine when compared to melarsoprol, has led some to advocate that this drug should become the first line treatment for late stage HAT (Chappuis *et al.*, 2005). The requirement for high doses and prolonged intravenous infusion, however make the drug expensive and difficult to distribute and administer in rural Africa. Its availability as a trypanocide is dependent upon commitments made to MSF and WHO by the manufacturing company (Sanofi-Aventis). There have been other approaches to improve the use of currently registered drugs. Pharmacokinetic studies of melarsoprol led to the successful testing of a shortened

10 day course (rather than 21-35 days) which improves patient compliance and reduces hospital costs (Schmidt *et al.*, 2004). Studies aimed to modify dosing with eflornithine are also underway with clinical studies on co-administrations with melarsprol or nifurtimox recently reported. These studies are essential in the face of the increased incidence of treatment failure with melarsoprol in some HAT foci (Brun *et al.*, 2001). Field studies to determine the cause of these failures are of importance and are in progress.

There is only one drug currently in clinical trials. The orally available prodrug, parfuramidine (DB289) (Fig. 1), is converted systemically into another diamidine (furamidine, DB75) that is active against early stage disease (Ansede *et al.*, 2004). The blood brain barrier remains a challenge in drug design to ensure sufficient drugs reaches parasites within the brain of late stage patients. A large number of diamidines have been synthesized through a consortium led by the University of North Carolina (funded by the Bill and Melinda Gates Foundation) and other pro-drugs from the same series active against late stage disease have emerged. Trypanosomes are highly sensitive to selected nitroheterocyclic compounds that have shown activity against CNS stage infections in experimental models. Although clinical studies have suggested that the nitrofuran nifurtimox is insufficiently active alone for treatment of HAT, studies have ensured that this drug is tested in combination (*ibid*) and that other potent and less genotoxic compounds are sought (Stewart *et al.*, 2004 and www.dndi.org).

Research on unique metabolic targets in trypanosomes (Berriman *et al.,* 2005), including that around thiol metabolism, in particular on trypanothione reductase, on energy metabolism, in particular on glycosomal enzymes, and on polyamine metabolism, with interesting novel compounds identified but without any clear candidates for lead optimization emerging (see Luscher *et al.*, 2007). The lack of advances in treatment for HAT highlight the need for closer integration of chemistry and biology efforts and improved understanding of the pharmacokinetic and pharmacodynamic requirements of the ideal drug.

SOUTH AMERICAN TRYPANOSOMIASIS (CHAGAS DISEASE)

Similar to above, despite the impressive advances in our knowledge about the biology of *T. cruzi* (El-Sayed *et al.,* 2005), the only drugs currently available are the nitrofuran nifurtimox and the 2-benzimidazole benznidazole, which were developed 1960's and 1970's. These drugs are active in the acute stage of the disease (up to 80 % efficacy) but of limited efficacy against established chronic stage disease, require long courses of treatment (60 days) and have severe side effects. With the reduction of transmission of Chagas disease in several foci in S. America, there has been greater focus on the needs for treatment of indeterminate and early chronic phases. Thorough experimental studies show that persistence of parasites, coupled with an imbalanced immune response that could include autoimmune reactions, generate sustained inflammatory responses in infected tissues producing the characteristic lesions of chronic Chagas disease (Tarleton *et al.*, 2001). Significant reduction of *T. cruzi* from infected patients appears, therefore, to be essential to prevent disease progression and to avert its irreversible long-term consequences. Studies with benznidazole have shown that it has some efficacy in early chronic infections (Sosa-Estani *et al.*, 1998), and a long term clinical trial (BENEFIT) is now underway to determine the extent of use of this drug for this indication (http://clinicaltrials.gov/show/NCT00123916). There are several rational approaches to the treatment of Chagas disease that have identified novel compounds or the potential for therapeutic switching. The potential of specific ergosterol biosynthesis inhibitors that act at the level of C14α sterol demethylase, has long been known, and some like ketoconazole entered clinical studies decades ago. However, studies by Urbina and colleagues have shown that new anti-fungal triazole derivatives (Fig. 1), for example posoconazole, have very high potency against *T. cruzi*, and are capable of curing chronic infections in mice (Molina *et al.*, 2000). One of this drug class will certainly enter clinical trials. Other novel ergosterol biosynthesis inhibitors that act at the level of squalene synthase or oxidosqualene cyclase (Urbina & Docampo, 2003) also show potential. Inhibitors of cruzipain, an essential protease specific to the parasite (Cazzulo *et al.*, 2002) and one particular vinyl sulphone, K777, is in pre-clinical development (Doyle *et al.*, 2007). Other studies have identified inhibitors of targets on the isoprenoid biosynthesis pathway, including farnesyl transferase and farnesyl pyrophosphate synthase. N-alkyl-bisphosphonates, inhibitors of farnesyl that selectively accumulate in the parasite's acidocalcisomes, also have activity in experimental models (Garzoni *et al.*, 2004).

DISCUSSION

The recent publication of the genome sequences of the pathogens that cause leishmaniasis and trypanosomiasis helps to identify both similarities as well as differences in potential drug targets. The subtle differences between the parasites in their metabolic adaptations, the required pharmacokinetic properties of drugs for the different sites of infection, and

the different approaches required for acute and chronic infections, indicates that we are not in the business of discovering one drug. Each of these three diseases will require several drugs or formulations of drugs for the treatment of all their manifestations. The discovery and development of these new drugs will require: *i)* increased input from the disciplines of chemistry, pharmacology, toxicology and pharmaceutics, and *ii)* further development of suitable disease models and methods for progressing leads and candidate drugs through pre-clinical studies. The limited progress in drug development of the past decades is part of history. Changes in donor patterns, in incentives in the new not-for-profit model of drug development, in the engagement of the pharmaceutical industry bode well for the future. But this should not be taken for granted and the responsibility for a sustained effort in this field requires effective teams, prioritization where necessary and decisions.

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