**Kinetoplastida: New Therapeutic Strategies**

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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 leishmaniasis 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 essential if progress is to be made. Although there are financial constraints, there is some hope for drug development.

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

The drugs used for treatment of the leishmaniasis 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 have 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.

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**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 (Groft 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

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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.
However, allopurinol remains a component of treatment of canine VL and the triazole posaconazole 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 paulolones (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 efflornithine are also underway with clinical studies on co-administrations with melarsoprof or nifurtimox recently reported. These studies are essential in the face of the increased incidence of treatment failure with melarsoprof 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 (Anse de 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 TRYPAansomiasis (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 benzimidazole, 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 posaconazole, 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 sulfone, 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 aci-docalcisomes, 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.

REFERENCES


