Chemotherapy of leishmaniasis and trypanosomiasis

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No real breakthroughs in the treatment of leishmaniasis and African trypanosomiasis have been reported in the past year. Treatment with benznidazole for American trypanosomiasis in asymptomatic children has proved to be worthwhile, and a new experimental compound (D0870), possibly enabling the eradication of the parasite in a mouse model, has been reported.

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Current Opinion in Infectious Diseases 1997, 10:469–474

ISSN 0951-7375

Introduction

An overview of the current state of developments in research on the biochemistry of the trypanosomids has been published in book form in 1997 [1••]. The organisms causing African trypanosomiasis, Chagas' disease and leishmaniasis have many biochemical features in common, but are different from related mammalian metabolic pathways, being attractive targets for inhibitory therapeutic agents. The targets which may be exploited are mainly in the following priority areas: sterol biosynthesis, proteinases, biosynthesis of the polyamines (such as trypanothione), and the glycolytic pathway [1••,2]. Key researchers in the field discuss current and promising lead compounds, and the mechanisms of drug resistance in trypanosomatids [1••]. This book also contains chapters of practical value for clinicians on the current public health status and a summary of the chemotherapy [3] of human leishmaniasis and trypanosomiasis.

Leishmaniasis

No real breakthroughs were reported last year. The progress in the chemotherapy of cutaneous, mucosal and visceral leishmaniasis during the past 10 years is concisely but comprehensively reviewed by Berman [4••].

Pentavalent antimonial drugs

The first documented case of reversible peripheral neuropathy associated with parenteral sodium stibogluconate therapy for American cutaneous leishmaniasis has been reported, possibly because of a drug interaction between amitriptyline and sodium stibogluconate [5]. Although New World cutaneous leishmaniasis caused by *Leishmania (Vianna) brasiliensis* is preferably treated with systemic antimonials, in particular to prevent the late mucosal lesions, this regimen is difficult to apply for many practical reasons. Oliveira-Neto *et al.* [6] treated 74 patients from the Rio de Janeiro state in Brazil, with single ulcerative lesions on the trunk or the extremities, with intralesional meglumine antimonate in one to two sessions. Eighty per cent of the patients healed after a 12-week interval. Extensive follow-up over 5–10 years, with regular laryngological examination, disclosed no relapses nor the development of mucosal lesions. The prevailing subpopulations of parasites in that study showed a very low tendency to metastasize and, according to the authors, locally injected antimony was the therapy of choice in this region.

Research using animal models to test more effective and targeted delivery systems of the pentavelent antimonial drugs to the macrophages is in progress [7–10].

Amphotericin B

Conventional amphotericin B is the second line therapy for antimonial refractory visceral and mucosal leishmaniasis. The new amphotericine lipid formulations are rapidly cleared from the circulatory system by the mononuclear phagocyte system, accumulating in the macrophages, which are capable of killing the intracellular parasites. Although it is unlikely that the new formulations will intrinsically be more effective than amphotericin itself, they have the advantage of being less toxic (a dramatic decrease of renal disturbances). Because higher daily doses may be given, the total dose can be given over a brief interval of 5–10 days, counterbalancing the high cost of the agent with the shorter duration of hospitalization [4••]. Experience with these new formulations in the treatment of cutaneous and mucosal leishmaniasis is, except for some isolated case reports, very limited. In an open study in Brazil [11], five out of six patients with mucosal leishmaniasis (*L. (V.) braziliensis*) unresponsive to glucantime therapy, were successfully treated with AmBisome® with a follow-up period ranging from 26–38 months. Disadvantages of this treatment were the need for parenteral administraiton, but mostly the high cost [11]. Because of the variable susceptibility according to the geographical area, and the varying efficacy of the different lipid-amphotericin formulations, the need for further studies is obvious, before general conclusions may be drawn. Systemic, but not locally injected, liposomal amphotericin B (AmBisome®) showed activity against experimental cutaneous leishmaniasis with *Leishmania major* in a mouse model [12].

Oral treatment

Many oral antifungal agents inhibit the biosynthesis of ergosterol, which is also the main constituent of leishmanial membranes. Their use is therefore biochemically rational for the treatment of leishmaniasis. The antifungal azoles (ketoconazole and itraconazole) are sterol 14-alpha-demethylase inhibitors. Terbinafine, from the allylamine group, is a squalene epoxidase inhibitor, and acts on an earlier step in the ergosterol synthesis pathway. The advantages of these compounds are the single daily oral dose and the low toxicity. Contradictory reports of both success and failure of itraconazole treatment for cutaneous leishmaniasis continue to be published. Itraconazole treatment (7 mg/kg/day for 21 days) in 65 patients with cutaneous leishmaniasis caused by *L. major* gave a low response rate in a randomized double blind study in Iran [13], with 59% complete healing 1 month after the end of treatment against 44.3% complete healing in the 66 placebo treated patients. The authors briefly discuss earlier clinical trials with itraconazole, underlining the fact that many studies were open trials, conducted in small groups, using diverse and ill-defined criteria. Leishmaniasis in other geographical regions or caused by other species, however, needs further investigation. In a small double blind randomized study in India [14], oral itraconazole (200 mg a day for 6 weeks) seems to be promising (seven out of 10 healed versus one out of 10 in the placebo group at 3 months follow-up), but these results need confirmation in a larger series of patients. Experimental studies with terbinafine have now been followed by the first reports about the clinical efficacy of this compound. In an open pilot study in the Asir region of Saudi Arabia [15], terbinafine 250–500 mg/day was administered for 4 weeks to 14 patients with cutaneous leishmaniasis (*Leishmania tropica*), resulting in complete cure in four (28.5%), partial cure in six (43%) and complete failure in four patients (28.5%) at 6 months follow-up. The authors speculated whether a higher dose, a longer duration, or possibly topical treatment, might result in higher cure rates. In Sudan the combination of terbinafine (250 mg/day) plus itraconazole (200 mg/day) for 4–8 weeks in nine patients with post kala-azar dermal leishmaniasis (*Leishmania donovani*) failed in eight [16]. A more than 300-fold increase in susceptibility of cultured *L. brasiliensis* promastigotes to azoles was measured in the presence of terbinafine, attributed to the combined effect of squalene and the methylated sterol precursors on the physical properties of the membrane of the cell, leading to loss of cell viability [17]. Combination therapy with azoles and terbinafine in the treatment of human *L. brasiliensis* infections deserves further study.

Allopurinol contines to give controversial results for the treatment of cutaneous leishmaniasis. Allopurinol (20 mg/kg per day for 28 days) as monotherapy was ineffective in Colombian cutaneous leishmaniasis (*Leishmania panamensis*) [18], but the addition of stibogluconate increased the cure rate for cutaneous leishmaniasis from 39% to 71% in another randomized, controlled study in southern Colombia [19].

Atovaquone, already approved for human use as an anti-protozoal drug in AIDS patients, and currently being investigated in malaria, has minimal toxicity and can be taken orally. In mice, oral atovaquone halted the parasite replication in the liver, and the combination of a suboptimal (leishmanistatic) dose of pentavalent antimony combined with atovaquone proved to be leishmanicidal for *L. donovani* [20]. These findings suggest a potential role for this oral agent in visceral leishmaniasis as an adjunct to conventional antimony treatment [20]. Promastigotes of *Leishmania chagasi* are susceptible to atovaquone *in vitro*, but atovaquone did not show a significant effect on *L. chagasi* in a Syrian hamster model [21].

Local treatment

Local treatment with paromomycin, although cheap, simple, well tolerated and without the side effects of conventional treatments, continues to give controversial results. After initial studies in which it was found to be effective, others gave disappointing results or failed to show any benefit [4••]. An ointment containing paromomycin (15%) and methylbenzethonium chloride (12%) in white soft paraffin, administered twice a day for 15 days, for the treatment of cutaneous leishmaniasis in Turkey (in a randomized clinical

trial of topical paromomycin (n=40) versus oral ketoconazole) gave a low cure rate of 37.5%, with incomplete healing in 20% and complete failure in 42.5% of patients [22]. A 50-year-old woman with leishmaniasis recidivans, mimicking lupus vulgaris, for 45 years, was healed with a paromomycin-methylbenzethonium chloride applied topically twice a day for 3 months [23]. In 53 patients in Honduras with cutaneous leishmaniasis, paromomycin (10%) plus ureum (10%) in white soft paraffin ointment administered three times a day for 4 weeks, was found to be ineffective [24]. Topical paromomycin (15%) plus ureum (10%) in white soft paraffin twice a day for 4 weeks in 116 patients in Iran accelerated the clinical recovery, with a parasitological reduction at days 29 and 45, but not on day 105 [25] (preliminary data). In an earlier report of the same group [26], using paromomycin (15%) and methylbenzethonium chloride (12%), there was no clear clinical benefit at any stage after treatment. The development of improved formulations for aminosidine (and other antileishmanial compounds) with enhanced transdermal penetration is clearly a research priority. A patient from Italy with cutaneous leishmaniasis for more than 12 months with no tendency to self healing (and numerous parasites still detectable in smears and biopsy), who had not responded to topical therapy with paromomycin, was treated in Germany with paromomycin 10% in unguentum cordes under an occlusive plastic foil, changed three times a week [27,28]. The lesion started to heal within 1 week and was completely epithelialized at the end of the fourth week. Occlusive paromomycin seems to be a promising approach [27,28], but, evidently, further studies are warranted. Al-Majali *et al.* [29] followed 215 patients with one to four lesions of cutaneous leishmaniasis in Jordan (*L. major* and *L. tropica*) after treatment with liquid nitrogen (cryosurgery) in one to three sessions with an interval of 3 weeks. The treatment was effective and well tolerated in all of the patients, except one failure, who was subsequently treated with pentavalent antimony.

American trypanosomiasis

The currently recommended drugs, nifurtimox and benznidazole, although approximately 60% effective in the acute phase [30•], have many disadvantages [3]: limited or no activity in the indeterminate and chronic forms because of insufficient power to eradicate the parasite; the problem of different drug sensitivities of the different *Trypanosoma cruzi* strains in different geographical areas; the long duration of treatment; and serious side effects. The production of nifurtimox (Lampit®) has now been discontinued [3].

Clinical therapy

Andrade *et al.* [31•] performed a prospective, randomized, double blind, placebo-controlled clinical trial in 112 children living in the north of Goias (Central Brazil), who were presumably in their first years of infection. Benznidazole 7.5 mg/kg a day divided into two doses for 60 days was administered to 58 children, 7–12 years old. The environment of the children was maintained free of transmission during the follow-up period of 3 years (and thereafter). Benznidazole was approximately 56% effective in causing the disappearance of specific antibodies measured by the chemiluminescent antigen trypomastigote enzyme-linked immunosorbent assay, used as an accepted surrogate measure of parasite clearance. Only one child was withdrawn, because of a moderate papular rash, illustrating the lower toxicity in children than in adults, even at higher doses. Assessment of the preventive effect of benznidazole treatment on the progression from infection to disease and its long term safety would be possible only through extended follow-up [32]. The authors cited earlier reports on the effect of benznidazole in chronic chagasic animal models, and in patients with chronic Chagas' disease (fewer ECG changes, lower frequency of deterioration in the clinical condition). They concluded that these findings may justify the recommendation of treatment for asymptomatic seropositive children as a public health policy, a statement supported by other experts [33•], especially in countries that are also attempting to rule out the possibility of reinfection by eliminating the vectors. Luquetti [30•] summarized the discussions of a meeting of 13 experts from several states of Brazil on the etiological treatment of Chagas' disease. Besides the useful practical guidelines in this report, there is consensus that benznidazole treatment is indeed indicated in recent chronic infections (those infected less than 10 years previously), therefore practically all children with positive serological reactions should be treated. There is no consensus about the antiparasitic treatment in the chronic phase. One author is more cautious, stating that, for the time being, benznidazole should not be routinely given in patients with early chronic Chagas' disease [34].

Experimental therapy

Urbina *et al.* [35•] report on the activity of D0870 on *T. cruzi*, a bis-tri-azole derivative currently being developed as a systemic antifungal agent. Next to inhibition of the ergosterol biosynthetic pathway at the level of C14-alpha-demethylase (with the consequent depletion of essential endogenous sterols or the accumulation of toxic intermediates, or both), it blocks cell division. In a murine model of long term disease there was 80–90% protection of the animals from death (50% of the controls died) with 80–90% parasitological cures (15–30% spontaneous cures in controls; nifurtimox and ketoconazole were not significantly different compared to controls). D0870 could therefore be useful in the treatment of human long term Chagas' disease [33•,35•] and clinical for *T. cruzi* treatment could begin within the next two years [36]. In a brief but informative overview Docampo and Schmunis [33•] stress the need for chemotherapeutic agents that are effective against all strains of *T. cruzi* and conclude that sterol biosynthesis inhibitors continue to be important potential chemotherapeutic agents against Chagas' dissease, especially synergistic combina-

tions of sterol biosynthesis inhibitors (e.g. azoles plus allylamines; azoles plus lovastatin).

Kinnamon *et al.* [37] examined the effectiveness of the 8-aminoquinolines in reducing 14-day parasitaemias of 4–6-week-old albino mice, compared with nifurtimox as the reference drug. Of 77 primaquine analogues, one compound (WR254261) was 14-fold as efficacious [37] and of 40 non-primaquine analogues, another compound (WR229238) was found to be 13-fold as efficacious as nifurtimox [38]. Certain compounds of these series therefore warrant further evaluation.

In-vitro methods may allow the screening of large numbers of candidate compounds against *T. cruzi* [39,40]. Purine-pyrimide analogues were tested in a culture system using mammalian host cells (testing the intracellular replication by the parasite and the host-cell infection rate). Allopurinol, and the anti-HIV agents zidovudine, 2′3′-dideoxyinosine (ddI), and 2′,3′-dideoxyadenosine (ddA) among others were found to be very potent inhibitors of amastigote growth [39]. Almeida *et al.* [41] described two patients with Chagas' disease reactivation after heart transplantation, who were effectively treated by allopurinol 600 mg and 900 mg per day, respectively, for 2 months, who remained well during the reported follow-up period of 12 and 3 months, respectively.

African trypanosomiasis

During the past years there have only been limited advances in the chemotherapy of African sleeping sickness. Studies in the search to reduce toxic doses, and investigations on the synergistic effects of existing drugs are still ongoing. The state of the art for the treatment of human African trypanosomiasis has recently been reviewed in a number of excellent papers [1••,42,43].

Clinical therapy

Trypanosoma brucei gambiense

Doua *et al.* [44] described the results of pentamidine treatment in 58 patients in the Ivory Coast with early central nervous system involvement (white blood cell count more than 3 and less than 20 per mm^3, with or without trypanosomes in the cerebrospinal fluid). Post-treatment follow-up of 52 patients for more than 2 years indicated a cure rate of 94%. Although the rate of relapse is slightly higher (6%) than with melarsopol (3.7%) [45], pentamidine has many fewer side effects. Khonde *et al.* [46] reported the results of a 7-day course of eflornithine for relapsing *T. gambiense* sleeping sickness in 47 patients. The only confirmed relapse after the 7-day treatment was in a child. Simarro and Asumu [47] described for the first time a case of Gambian trypanosomiasis cured by the synergistic effect of melarsoprol and eflornithine administered simultaneously, after both of them given separately had failed. Jennings [48] had clearly demonstrated this synergism in a murine model as far back as 1988.

Trypanosoma brucei rhodesiense

Combination treatments were also applied for East African trypanosomiasis. Foulkes [49] examined the synergism of metronidazole and suramin in the treatment of arsenical refractory Rhodesian sleeping sickness. Further studies of this oral and cheap adjunct therapy have yet to confirm the results obtained in one patient. Taelman *et al.* [50] reported a case study of a successful combination treatment with suramin and eflornithine in late stage Rhodesian trypanosomiasis. Recently, however, one of the authors reported a cure failure in three out of six more patients treated with exactly the same dose of suramin and eflornithine (Clerinx, personal communication).

Experimental therapy in murine models and in-vitro determination of antitrypanosomal activity of new compounds

Compounds based on the structure of methylglyoxal bis(guanilhydrazone) were evaluated for trypanocidal activities on African trypanosomes by Bacchi *et al.* [51]. Compound CGP 40215A, an inhibitor of adenosylmethionine decarboxylase, was effective in curing laboratory model infections when used at a dose of 5–25 mg/kg/day. In combination with eflornithine, CGP 40215A was strongly synergistic in curing an animal model central nervous system infection. Sufrin *et al.* [52] enhanced the antitrypanosomal activity of purine nucleosides by their conversion to O-acetylated derivatives, and the authors recommended the routine preparation of these compounds for further in-vitro and in-vivo screening.

Topical chemotherapy (transcutaneous)

Jennings and colleagues [53,54] successfully tested the use of topical chemotherapy for experimental murine African trypanosomiasis with cerebral involvement. They combined melarsoprol with 5-nitroimidazoles (megazol, fexinidazole or Mk-436). The compounds were administrated as a gel by the addition of hydroxypropylcellulose.

Conclusion

At present, there is still a need for new, safe and effective drugs against all forms of leishmaniasis and trypanosomiasis, preferably oral drugs, with low production costs for the endemic areas, without any risk of harm for the environment (as is the case in the manipulation of arsenic for the production of melarsoprol), and with the capacity to eradicate the parasites. Recent biochemical and molecular studies revealed potential chemotherapeutic targets, and may in the future provide more specific and effective drugs. Many years may evolve between discovery and delivery. In the meantime, it is therefore important to improve the use of existing treatments and, as in the case of leishmaniasis, to develop new formulations of the existing antileishmanial compounds (parenteral, oral, intradermal and transdermal).

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