Expert Opinion

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Clinical status of agents being developed for leishmaniasis

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Leishmaniasis, which exists in both visceral and cutaneous forms, is currently treated with intramuscular antimony or intravenous amphotericin B. The primary unmet need is for oral therapy. Of the several drugs in clinical development, miltefosine is unique in being an oral agent with efficacy against both forms of the disease. Sitamaquine is an oral agent with substantial but not sufficient efficacy against visceral disease. Oral fluconazole has been shown to be more effective than placebo in one instance: for *Leishmania major* cutaneous disease from Saudi Arabia. Paromomycin is in widespread trial. Topical paromomycin formulations are being tested for cutaneous disease, and intramuscular paromomycin is in Phase III trial for Indian visceral disease. The most likely replacements for present therapy are oral miltefosine for many of the visceral and cutaneous syndromes, intramuscular paromomycin for visceral disease and topical paromomycin for some forms of cutaneous disease.

Keywords: fluconazole, imiquimod, leishmaniasis, miltefosine, paromomycin, sitamaquine

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1. Background

1.1 Leishmaniasis

Leishmaniasis is caused by parasites of the genus *Leishmania*. Leishmaniasis is generally a zoonotic disease (a disease maintained in animals and spread from one mammalian host to another via the bite of a vector). If a person intrudes into the *Leishmania* cycle and is bitten by an infected female sandfly, he/she can become infected with the parasite. *Leishmania* are obligate intraphagocytic microorganisms. After inoculation into the skin, the organisms rapidly locate to the phagolysosomes of the mononuclear phagocyte system. The parasites multiply within the phagolysosomes, resulting in the associated clinical symptomatology. Multiplication in the macrophages of the skin causes cutaneous disease whereas multiplication in the macrophages of the spleen/liver/bone marrow causes visceral disease. As an obligate intramacrophage microorganism, effective immunity is based on T helper type 1 (T_H1) immune mechanisms rather than T_H2 immune mechanisms.

Leishmania donovani, *L. infantum* and *L. chagasi* are the cause of visceral disease. *L. major* and *L. tropica* are the primary causes of disease in Asia, India and the Mediterranian regions of Europe and Africa (the Old World). *L. Vianna* (v) *braziliensis*, *L. v guyanensis*, *L. v peruviana*, *L. v panamensis*, *L. mexicana* (m) and *L. m amazonensis* are the primary species found in Central America and South America down to San Paolo (the New World).

The disease incidence published in 1992 may still be accurate today. The estimate was ~ 100,000 new cases annually of visceral disease and 300,000 cases annually of cutaneous disease [1], although recent World Health Organization (WHO) estimates are 500,000 cases of visceral disease and 1,500,000 cases of cutaneous disease [101].

Cutaneous leishmaniasis classically first manifests as a papule that then evolves into an ulcer. In cutaneous leishmaniasis, cellular immune mechanisms are operative. In the Old World, cutaneous disease due to *L. major* generally cures rapidly: 60% of lesions were self-cured in 3 – 4 months after seeking medical attention in

one study [2]. When the disease is caused by *L. tropica*, it is thought to take longer to cure. Cases of cutaneous disease due to *L. mexicana* also resolve in 3 months but only 10 – 40% of the cases of the *L. v* group (such as *L. v braziliensis* and *L. v panamensis*) re-epithelialise in this period of time [3,4].

Visceral disease is associated with hepatosplenomegaly, fever and pancytopoenia. Cellular immune mechanisms are not operative and visceral leishmaniasis progresses over days to a few months and can finally lead to death.

1.2 Present antileishmanial agents

The classic treatment for all forms of leishmaniasis is by pentavalent antimonials (Sb) in the form of sodium stibogluconate (Pentostam™) or meglumine antimonite (Glucantime™). To be absorbed, antimonials must be delivered parenterally, either intravenously or intramuscularly. The standard dose for cutaneous disease is 20 mg Sb/kg/day for 20 days and is 20 mg/kg/day for 28 days for visceral disease. The secondary agent is amphotericin B. The disadvantages of antimony and amphotericin B have been recently summarised [5]. For antimonials, there is ≥ 40% clinical resistance and associated toxicity for visceral disease after long-term use. Adverse effects such as myalgia, arthralgia, anorexia, hyperamylasaemia and rises in liver function enzymes are common. Although amphotericin B cures ~ 100% of visceral patients, the adverse effects of fever/chills and elevations of kidney function tests are frequent, and liposomal amphotericin B is too expensive for widespread use.

The fact that the standard agent (antimony), secondary agent (amphotericin B) and another employed agent (pentamidine) are all parenteral signifies that the primary clinical need is an effective oral agent. Miltefosine (hexadecylephosphocholine) is the leading oral agent, with sitamaquine (WR-6026), the azoles (fluconazole, ketoconazole and itraconazole) and a purine analogue (allopurinol) also being studied (Table 1). For cutaneous disease, topical formulations are attractive, with the major candidates being paromomycin chemotherapy and imiquimod immunotherapy. For visceral disease, intramuscular paromomycin fills a niche. Although this is another parenteral formulation, it may be almost as effective as amphotericin B and is less toxic, does not require intravenous administration and, as an older agent, may be inexpensive.

2. Miltefosine

2.1 Present status of miltefosine

Miltefosine (Figure 1) was originally developed as an oral agent for cancer. Although not sufficiently effective for cancer, clinical safety data generated from the cancer studies proved invaluable when laboratory evidence of efficacy for leishmaniasis was later found, and the clinical development programme moved rapidly.

Visceral leishmaniasis was first investigated. A 1998 pilot study of a 4-week treatment showed that low doses of 50 or 100 mg every other day were ineffective, high doses (200 or 250 mg/day) were not well tolerated but mid-doses (100 or 150 mg/day) were effective and tolerated, at least for the five patients of each group [6]. A large Phase II study confirmed the effectiveness and tolerance of 100 – 150 mg/day for 4 weeks in 30 patient groups. The simplest regimen, 100 mg/day (2.5 mg/kg/day for these 40-kg patients) cured 97% of the patients [7].

The Phase III trial compared miltefosine 2.5 mg/kg/day for 4 weeks in ~ 300 patients with the standard of care, amphotericin B 1 mg/kg every other day, for a total of 15 injections over 4 weeks in ~ 100 patients [5].

At the end of therapy, all of the patients who had repeat splenic aspiration were parasitologically negative and demonstrated initial cure. At a 6-month follow up, nine miltefosine patients (3%) and none of the amphotericin B patients (0%) had relapsed parasitologically. Of the miltefosine patients, 3% were lost prior to the 6-month follow up, so the intent-to-treat final cure rate in the miltefosine group was 94% and the per-protocol final cure rate in that group was 97%. Of the miltefosine patients, ~ 25% had previously failed treatment with pentavalent antimony and can be regarded as clinically resistant to antimony. The cure rate in the previously treated group was equal to that in the naive group.

Miltefosine tolerance in the visceral leishmaniasis population could be well assessed with the ~ 300 patients of this trial, with the data from the ~ 100 amphotericin patients being available to correct for side effects due to the disease itself. Vomiting was seen in 38% of the miltefosine patients (versus 20% of the amphotericin B patients). In 75% of the cases, vomiting lasted for 1 – 2 days. All of the episodes were common toxicity criteria (CTC) grades 1 and 2 (1 and 2 – 5 episodes per day, respectively). Diarrhoea was seen in 20% of the miltefosine patients (versus 6% for amphotericin B). Again 75% of the episodes lasted 1 – 2 days, and all but one episode was CTC grades 1 or 2 (an increase of 2 – 3 and 4 – 6 stools/day, respectively).

Liver function tests for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) showed a somewhat increase during week 1 of therapy, before falling during the second week and then decreasing further as the disease resolved. Mean values of renal function tests (blood urea nitrogen [BUN] and creatinine) did not change significantly, although one patient had a sizeable creatinine elevation that was most likely due to drug administration.

Because of preclinical concern about male reproductive capacity, in this study, male patients were followed to determine the number of live and healthy births to their sexual partners. In the miltefosine group, there were 48 healthy births to the partners of 80 male patients (0.6 births/patient). In the amphotericin B group, there were 12 healthy births to 20 such partners (0.6 births/patient).

The Phase III study established the efficacy and tolerability of miltefosine 2.5 mg/kg/day for 4 weeks under supervised clinical conditions, for ≥ 12 year old Indian visceral

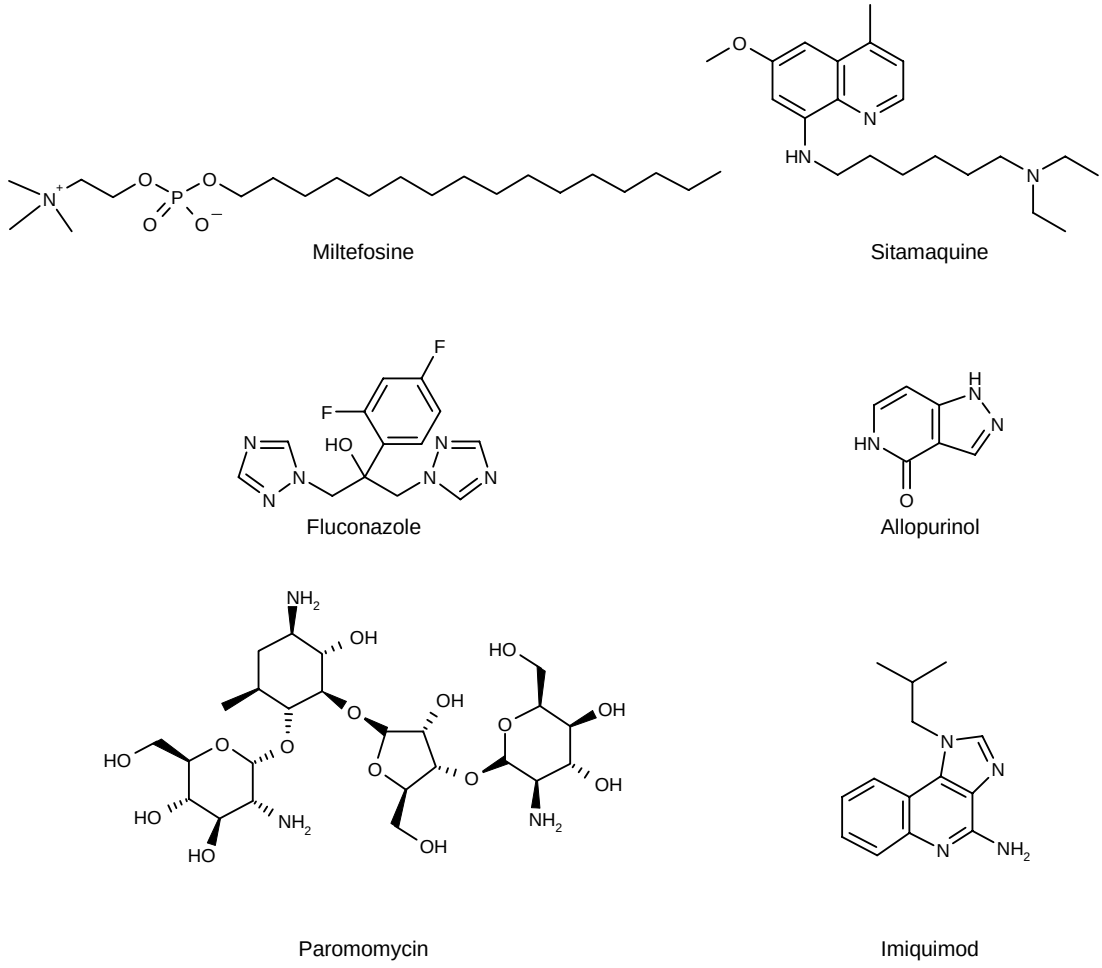

Figure 1. Antileishmanial agents in clinical development.

leishmaniasis patients with mild-to-moderate disease including those who were clinically resistant to antimonial therapy.

The indications for miltefosine were extended to the paediatric population in India via another trial. The childhood trial employed the adult dose, 2.5 mg/kg/day, for 28 days to 80 patients of 2 – 11 years of age [8]. One patient died early due to intercurrent pneumonia. The other 79 patients were initially cured. A total of three patients relapsed by the end of 6 months of follow up and the final cure was 75 of the 79 evaluable patients (95%).

HIV-coinfected patients have been receiving miltefosine on an individual basis in Europe. The combined experience by the end of 2004 has been reported [9]. A total of 39 patients of mean weight ~ 60 kg received initial treatment of 100 mg/day for a mean of 55 days. Of the 25 patients who showed initial cure or improvement, 22 received a second course of therapy lasting a mean of 48 days. Of the 15 who responded to the second course of therapy, nine patients received a third course and four patients received a fourth course. These results suggest that miltefosine provides initial responses in many patients but, in conformity with the relapses that are seen when patients are treated with standard antileishmanial agents, most of the miltefosine patients relapse.

Miltefosine has also been used in cutaneous leishmaniasis; 2.5 mg/kg/day for 4 weeks was administered to 89 randomised patients and 44 placebo patients who acquired disease in Colombia or Guatemala [10]. In essence, two separate studies were performed: one against *L. panamensis* disease in Colombia, one against combined *L. braziliensis* and *L. mexicana* disease in Guatemala. In Colombia, the per-protocol cure rates were 91% for miltefosine and 38% for placebo. These values are similar to historical values for the antimony standard of care and for placebo, respectively. In Guatemala, the per-protocol cure rates were 53% for miltefosine and 21% for placebo. The disparate efficacy results indicated that miltefosine is a useful oral agent against cutaneous

leishmaniasis caused by *L. v panamensis*, at least in Colombia, but less useful than antimony against disease in Guatemala.

Cutaneous leishmaniasis patients suffer only from a skin ulcer and are systemically normal. Because this was the first blinded trial of miltefosine in an essentially 'normal population', the trial permitted determination of the inherent clinical tolerance of this drug. Nausea was reported by 27% more miltefosine than placebo patients. Vomiting but not diarrhoea was also specifically attributable to miltefosine, and was experienced by 32% of the patients compared with 5% of the placebo patients. Of the miltefosine patients, ~75% who vomited had only 1 – 2 episodes during the 28-day therapy course, and no patient stopped therapy for this reason.

Creatinine was more frequently elevated in the miltefosine group compared with the placebo group but almost all of the creatinine elevations were mild. AST and ALT were not more frequently elevated in the miltefosine group compared with controls. The mild changes in laboratory parameters suggests that in the cutaneous leishmaniasis population, in contrast to visceral leishmaniasis patients who have systemic disease, routine recording of laboratory parameters need not be performed.

By March 2005, miltefosine was registered in India for visceral disease in patients ≥ 2 years of age at a dose of 2.5 mg/kg/day for 28 days, for visceral disease in Germany for immunocompetent and also HIV-coinfected patients at a dose of 100 mg/day ≤ 67 kg (150 mg/day for patients > 67 kg) for 28 days and longer for HIV-coinfected patients and in Colombia for visceral and cutaneous disease.

2.2 Future development issues for miltefosine

In spite of registration of miltefosine for syndromes seen in India, Germany and Colombia, an important question has been raised: 'how broadly applicable will miltefosine therapy be for the diversity encompassed by human leishmaniasis, which includes several clinical syndromes, caused by ~ 21 leishmanial species in 88 countries?' [11]. The further development programme for miltefosine is intended to determine the general utility of this product.

Will miltefosine be as effective against visceral disease in Brazil due to *L. chagasi*, in Mediterranian regions due to *L. infantum*, and in Africa due to *L. donovani*, which may differ from Indian *L. donovani*? Formal studies in Brazil and in Ethiopia are underway.

Will miltefosine be able to be administered for sufficient periods of time to suppress *Leishmania* effectively in HIV-coinfected patients, and will relapsing parasites show miltefosine resistance? Formal efficacy, tolerance and resistance data will be needed from centres in which patients can receive long-term therapy and from which parasites can be obtained for evaluation in preclinical models.

Will miltefosine be valuable for the myriad of cutaneous syndromes across the world? Investigators in the several endemic regions will need to formally study ~ 40 patients in each region in order to approximate efficacy.

Will miltefosine be as effective and safe under routine clinical conditions as under formal study? For visceral disease, a large Phase IV post-registration trial of miltefosine 2.5 mg/kg/day for 4 weeks is underway in India and Nepal.

Will strict reproductive contraception be maintained in female patients for the period of miltefosine administration and ~ 8 half-lifes (total of 2 – 3 months) following administration?

3. Sitamaquine

Sitamaquine (Figure 1) was originally synthesised during World War II as a potential replacement for the antimalarial drug primaquine. Sitamaquine is an analogue for primaquine, which has respectable antileishmanial activity in preclinical models, and the screening of sitamaquine in the hamster model infected with *L. donovani* revealed extraordinary efficacy.

The initial Phase II clinical study was conducted in Kenya [12]; 16 patients were entered. A total of eight patients received 0.75 – 1 mg/kg/day for 14 days; one patient was cured and the others showed a decrease in parasite counts. The next eight patients received 1 mg/kg/day for 28 days; four patients were cured and the rest showed sizeable decrements in parasite counts.

With this encouraging data, further Phase II work was undertaken in Brazil [13]. The first cohort received sitamaquine 1 mg/kg/day for 28 days and further cohorts received progressively higher doses of 1.5, 2, 2.5 and finally 3.25 mg/kg/day. Efficacy data were surprising in several ways. In contrast to the experience in Kenya, the 1 mg/kg/day dose was unsuccessful in Brazilian patients, with none of four patients cured. Also, although efficacy increased at 1.5 mg/kg/day (one out of six cured [17%]) and 2 mg/kg/day (four out of six cured [67%]), efficacy did not continue to increase at 2.5 mg/kg/day (one out of five cured [20%]) and the one patient who was administered 3.25 mg/kg/day did not cure.

Another unanticipated finding was nephrotoxicity in two patients who were administered 2.5 mg/kg/day and the patient administered 3.25 mg/kg/day. In the dog model, dosing had to stop at 3 mg/kg/day because of methaemoglobin formation, but there was no evidence of nephrotoxicity in dogs. In a toxicity study in HIV patients without leishmaniasis, methemoglobinaemia (> 20%) was seen in three of the six subjects who received 150 mg/day (~ 2.5 mg/kg/day) but nephrotoxicity was not observed [14]. Thus, methaemoglobinaemia, expected for a primaquine analogue and seen in dog studies and a non-*Leishmania* clinical study, was not seen in the visceral leishmaniasis patients but nephrotoxicity, not expected from any previous work, was seen in visceral leishmaniasis patients receiving ≥ 2.5 mg/kg/day.

Further Phase II evaluations were undertaken in Kenya (the region of initial sitamaquine success) and in India. These studies have not been published as of August 2005. An indication of what at least the Kenyan data are likely to show

Table 1. Antileishmanial agents in clinical development.

Drug	Present status	Suggested further trials
Miltefosine (oral)	2.5 mg/kg/day for 28 days is registered for visceral leishmaniasis in India, visceral leishmaniasis (including in immunocompromised patients) in Germany and cutaneous leishmaniasis and visceral leishmaniasis in Colombia	Test versus visceral leishmaniasis in other regions (Africa and South America). Test versus ML. Test versus cutaneous leishmaniasis in other Old and New World regions
Sitamaquine (oral)	Sitamaquine 2 mg/kg/day for 28 days is 65 – 85% effective for visceral leishmaniasis. A dose of 2.5 mg/kg/day is nephrotoxic	Combine with another anti-visceral leishmaniasis agent in an attempt to demonstrate higher efficacy and also lack of nephrotoxicity
Fluconazole (oral)	Fluconazole 200 mg/day for 6 weeks accelerates the cure of *Leishmaniasis major* from Saudi Arabia	Shorter course (200 and 400 mg/day for 3 weeks) versus *Leishmaniasis major*. Trials against cutaneous leishmaniasis in other regions.
Allopurinol (oral)	Allopurinol 20 mg/kg/day for 28 days is ineffective as sole agent for Colombian cutaneous leishmaniasis	Formal study of combinations with other antileishmanial agents
Imiquimod (topical)	In combination with antimony, accelerates the cure of Peruvian cutaneous leishmaniasis	Repeat above study for cutaneous leishmaniasis from other regions
Paromomycin (topical)	Paromomycin plus MBCL and paromomycin/WR have efficacy in single trials	Evaluation of paromomycin plus MLBL and paromomycin/WR in a representation of endemic regions for cutaneous leishmaniasis
Paromomycin (intramuscularly)	15 mg/kg/day for 20 days is effective for Indian visceral leishmaniasis, ineffective for South American cutaneous leishmaniasis	Test for visceral leishmaniasis in other regions (Africa and South America)

MBCL: Methylbenzethonium chloride; ML: Mucosal leishmaniasis; WR: Walter Reed Institute of Research.

is implied in a case report from this site [15]. Doses of 2 – 3 mg/kg/day are implied to give 80 – 85% efficacy, with nephrotoxicity at the 'higher doses'.

The metabolism of sitamaquine in humans is virtually unknown. Only 5% of sitamaquine clinical metabolites are identified [13]. Whatever molecular species are present in visceral leishmaniasis patients, the therapeutic index appears to reach a plateau at ~ 2 mg/kg/day, with higher doses providing renal toxicity without increased efficacy. The utility of sitamaquine depends on the value of an oral product that is ~ 80% curative at a dose just lower than that that is toxic to the kidneys. It is unlikely that such a product can be used by itself, not just because of individual failures but also because a ~ 20% failure rate creates a condition for a generation of drug resistance in the community. Whether the good, but not outstanding, efficacy of sitamaquine can be employed in combination with another drug is likely to depend on whether combined renal toxicity can be avoided.

4. Azoles

Leishmania and fungi share features of sterol biosynthesis; for both, the final demethylated sterol is ergosterol rather than the mammalian cholesterol. For both, amphotericin B, which intercalates with ergosterol, is an excellent antimicrobial agent. In 1981, the antifungal agent ketoconazole was shown to be active in the test tube against *Leishmania* [16] and was also later shown to inhibit *Leishmania* sterol demethylation in fungi [17].

The attractive biochemical rationale for the azoles has led to a considerable number of clinical reports on ketoconazole and the newer antifungal azoles/triazoles such as fluconazole and itraconazole.

4.1 Ketoconazole

In a non-placebo-controlled study, ketoconazole was shown to have moderate activity against cutaneous disease in Panama. Of 21 patients (76%), 16 administered 600 mg/day for 28 days were cured, a value similar to that of standard of care antimony (13 of 19 patients [68%]) with whom the ketoconazole group was randomised [18].

In reports against Old World cutaneous disease, ketoconazole (600 and 800 mg/day for ~ 6 weeks) cured all of the 21 patients in an uncontrolled study of presumed *L. major* in Kuwait [19] but 400 mg/day for 10 weeks cured only 4 of 19 patients with presumed *L. tropica* disease in India [20]. For visceral disease in India, only 33% of the patients responded to 600 mg for 4 weeks versus 82% of antimony cases [21].

4.2 Fluconazole

Although the *in vitro* efficacy of fluconazole (Figure 1) has not been well demonstrated, the inherent interest in inhibitors of ergosterol biosynthesis, and the fact that fluconazole is 10 times more concentrated in the skin compared with

plasma, led to the evaluation of fluconazole 200 mg/day for 6 weeks in a placebo-controlled trial for *L. major* in Saudi Arabia [2]. There was a statistical difference between the cure rate at 3 months between the fluconazole (79%) and placebo groups (34%), although the difference diminished by 4 months (~ 90 versus ~ 70%, respectively); thus, fluconazole accelerated the rapid natural healing rate of *L. major* disease. Two case reports with fluconazole for Old World cutaneous leishmaniasis have subsequently appeared: an Afganistani woman with *L. tropica* infection was cured by the same regimen of 200 mg/day for 6 weeks [22] and a Kosovan child was cured with 100 mg/day for 3 weeks [23]. Fluconazole has also been evaluated for visceral disease. A total of 11 patients were given ~ 12 mg/kg/day (adults) or 18 mg/kg/day (children) for 30 – 45 days. All six of the initial responders relapsed within 2 months [24]. In a later report from India, 12 of 20 children were cured with 5 mg/kg/day for 30 days [25]. Fluconazole plus allopurinol has been used to successfully suppress visceral leishmaniasis in two immunosuppressed patients [26].

4.3 Itraconazole

In contrast to fluconazole, itraconazole has not been effective for cutaneous leishmaniasis. In the New World, ~ 25% of Colombian [27] and Ecuadorian [28] patients were cured. In the Old World, 59% of Iranian *L. major* patients were cured with itrazonazole 200 mg/day for 8 weeks but 53% of the placebo patients were also cured [29], and eight of nine patients with Sudanese PDKL did not respond to itraconazole 200 mg/day plus terbinafine 250 mg/day for 4 weeks [29]. Dogra *et al.* [31] found that 7 out of 10 cases of Indian cutaneous disease responded to itraconazole 100 mg/day for 6 weeks compared with 1 of 10 placebos.

5. Purine analogue

The purine analogue allopurinol (Figure 1) has a strong biochemical rationale as an antileishmanial agent because it is phosphorylated by the parasites to form an analogue of ATP. In 1992, Colombian investigators reported that the antimony cure rate was 36%, the cure rate with antimony plus allopurinol in combination was 74%, and the cure rate for a non-randomised allopurinol alone group was 80% [32]. To investigate the activity of allopurinol alone in Colombia (in a randomised trial), a randomised comparison of antimony, allopurinol and placebo was performed. The efficacy of antimony was 93%, the efficacy of allopurinol was 33% and the efficacy of placebo was 37% [33]. Reports of combinations containing allopurinol have since been published. A subsequent report by Martinez *et al.* [34] supported the efficacy of allopurinol plus antimony (71%) compared with antimony alone (39%), but the several reports from Martinez suffer from the very low efficacy of antimony standard of care compared with what is expected of antimony, as well as the 93% reported by Velez *et al.* [33]. In Peru, allopurinol plus antimony did not improve on the 75% cure rate of antimony alone [35]. In the Old World, allopurinol and antimony in combination has been used for recalcitrant cases of *L. major* [36].

6. Topical agents

6.1 Paromomycin

In the early 1990s, El-On *et al.* [37] showed that Israeli *L. major* treated with 15% paromomycin (Figure 1) plus 12% methylbenzethonium chloride (MBCL) in a base of soft white paraffin twice daily for 10 days cured lesions more rapidly (100% cure rate at 21 – 30 days) than was seen with untreated lesions on the same patients (100% cure rate at 51 – 60 days). In an attempt to eliminate the stinging due to the high concentration of MBCL, 15% paromomycin plus 10% urea, again in soft white paraffin, was formulated. The paromomycin plus urea formulation was not more effective than placebo for *L. major* in Tunisia or in Iran. After twice daily dosing for 14 days, there was a 70% cure rate at 13 weeks in both the treatment and placebo groups in Tunisia [38] and a 68% cure rate in both the treatment and placebo groups at 13 weeks in Iran [39]. A later study in Iran randomised patients between 2 and 4 weeks of active therapy. At the end of 4 weeks, the 4-week therapy was more effective (74% cure) than the 2-week therapy (59% cure) [40]. Recent Iranian trials compared paromomycin plus urea with intralesional antimony. In one study, intralesional antimony was more effective (~ 40% failure rate) than topical therapy (~ 73% failure rate) [41], whereas in another study, both of the therapies were equally effective (~ 67% cure rate) [42].

In the New World, paromomycin plus MBCL did not improve the cure rate of a low, partially effective dose of antimony alone in Colombia [43]. The efficacy of 10 days of paromomycin plus MBCL plus 7 days of antimony was 58% compared with the 53% efficacy of 7 days of antimony alone. However, in neighbouring Ecuador, by 12 weeks after the beginning of a 10-day treatment period, the cure rates after paromomycin plus MBCL (79%) and after paromomycin plus urea (70%) were close to that after antimony (92%) [44]. In Guatemala, paromomycin plus MBCL (twice daily for 20 days) had a very high efficacy rate (86%) compared with placebo (39%) [45].

To aid penetration of paromomycin to the lesion, the Walter Reed Institute of Research (WR) has formulated 15% paromomyin in a complex base that is now in clinical trial. Initial results of 'paromomycin/WR' in Colombia were mildly encouraging. Although the cure rate was not increased in the active cream group compared with the placebo cream group, the time to cure in the active group was shorter than that in the placebo group [46]. A larger placebo-controlled study against *L. major* in Tunisia has concluded, with apparently statistically significant results (Grogl, personal communication, 29 June 2005).

6.2 Imiquimod

Imiquimod (Figure 1) stimulates toll-like receptor 7 on macrophages and dendritic cells and thereby induces a T_H1-type immune response with increases in TNF-α, IFN-α and -γ, and IL-2. This immunomodulator is registered for the topical treatment of venereal warts and so is easily obtainable. Imiquimod has been shown to add to the efficacy of antimony against Peruvian cutaneous leishmaniasis. In the first trial, 12 patients who had failed antimonial therapy were administered antimony (standard dose daily for 20 days) plus topical imiquimod (every other day for 20 days). Of the 12 patients, 10 were cured at the 6-month follow up [47]. In the next trial, 20 naive patients received topical antimony plus imiquimod and were randomised against 20 patients who received topical antimony plus placebo [48]. A total of 3 months after therapy, there was a 72% cure rate in the combination group versus 35% in the antimony-alone group. At 6 months, the cure rates were 72 versus 50%, and at 12 months the cure rates were 72 versus 75%; thus, imiquimod increased the cure time, although not the cure rate, in Peruvian disease.

Imiquimod by itself appears not to be sufficiently effective. Against Old World disease in Damascus, topical therapy three times/week caused regression of of the lesions in 2 – 4 weeks in 10 of 12 patients, but by 8 weeks all of the lesions showed progression [49].

7. Parenteral agent (paromomycin)

Paromomycin is an aminoglycoside registered in Europe at a dose of 15 mg/kg/day for 10 days. Unlike the other clinical aminoglycosides, paromomycin has broad antiparasitic activity, and oral (nonabsorbable) paromomycin is registered for the treatment of intestinal amebiasis.

Intramuscular paromomycin is in active trial for visceral leishmaniasis. In the initial study in Kenya, 14 – 16 mg/kg/day for a mean of 19 days cured 15 of 19 patients [50]. A subsequent combination of paromomycin (15 – 17 mg/kg/day) plus antimony for 2.5 – 3 weeks cured 18 of 22 Indian patients [51] and 60 of 67 Sudanese patients [52].

Dose ranging of paromomycin as a sole agent has also been reported. Jha *et al*. [53] found that the cure rates after 12, 16 and 20 mg/kg/day for 21 days were 77, 93 and 97%, respectively, of 30 patients per group. In a parallel study, Thakur *et al.* found more uniform cure rates: 90, 89 and 86% cure rates for the three respective dose groups [54]. The overall conclusion from the Jha and Thakur reports is that the optimum dose is likely to be ~ 16 mg/kg/day for 21 days [53,54].

A large Phase III comparison of paromomycin (15 mg/kg/day for 21 days) with the standard of care in India (amphotericin B), which was sponsored by the Institute for One World Health and Tropical Disease Research/World Health Organization, has now concluded and the final results will soon be known. Preliminary results that were presented at meetings suggest that paromomycin has efficacy not far below that of amphotericin B and is well tolerated in terms of the aminoglycoside toxicity parameters of ototoxicity and nephrotoxicity.

Note that intramuscular paromomycin is not effective for New World cutaneous leishmaniasis; 18 mg/kg/day for 14 days cured only 50% of Colombian patients [55]. Although this dose of 252 mg/kg is somewhat less than that being used in the Phase III Indian visceral leishmaniasis studies (315 mg/kg), a considerably lower dose (12 mg/kg/day for 14 days = 168 mg/kg) cured 45% of patients; therefore, it appears that increasing the dose above ~ 200 mg/kg does not lead to an increase in efficacy. The experience from Belize was similar. Paromomycin 14 mg/kg/day for 20 days (total dose = 280 mg/kg) healed only 10 of 17 lesions, whereas antimony healed 15 of 17 lesions [56].

8. Expert opinion and conclusion

The fact that the present therapy for the leishmaniases consists of parenteral antimony and parenteral amphotericin B indicates that oral agents are the primary unmet need for antileishmanial chemotherapy. Miltefosine is active for both visceral and cutaneous syndromes. Although miltefosine is effective against visceral disease in one endemic region (India) and cutaneous disease in one region (Colombia), this does not necessarily mean that miltefosine will be effective for leishmaniasis in all of the regions; for example, miltefosine was less effective than historical values of antimony in Guatemala. Nevertheless, at present, miltefosine is without competition as an oral agent with general efficacy for the leishmaniases and should be evaluated for disease in all regions.

Sitamaquine is an oral agent with substantial but not sufficient efficacy against visceral disease. Effort should be spent to find an agent with which to combine sitamaquine, such that the combination can have efficacy > 90% at sitamaquine doses that do not lead to nephrotoxicity.

Of the azoles, fluconazole is the only agent for which efficacy has been demonstrated in a placebo-controlled trial. It is difficult to generalise from this trial of Saudi Arabian *L. major*. Fluconazole might not be as effective against *L. major* from other regions. Because *L. major* rapidly self-cures, fluconazole might be a weak agent that is ineffective against cutaneous disease due to other species and that are more persistent. Fluconazole should be evaluated against *L. major* in other regions, and also against *L. tropica* and *L. mexicana*. If efficacy continues to be demonstrated, then shorter courses should also be evaluated.

A cogent development plan for imiquimod, in which this immunomodulator in combination with antimony was shown to be valuable first in antimony rescue cases and then in naive cases, has shown that imiquimod accelerates the antimony-induced cure of Peruvian cutaneous leishmaniasis. The general utility of a product that is only valuable in combination is not clear. The acceleration of cure time has to be balanced against the added cost and inconvenience of a second agent. Imiquimod may have a

role in the general treatment of antimony-resistant lesions, but only a small minority of lesions are clinically antimony resistant. Perhaps the greatest value of imiquimod is the proof-of-principle that immunomodulators can clinically benefit the leishmaniases.

Topical paromomycin formulations have been effective versus placebo in individual regions: Israel and Guatemala for paromomycin plus MBCL, and Tunisia for paromomycin/WR. An inherent disadvantage of topical treatment is that each lesion needs to be separately treated; thus, topical therapy is best suited for those patients who have one or a few lesions. However, many cutaneous leishmaniasis patients do have few lesions. The topical formulations need an advocate, a pharmaceutical firm that will sponsor trials against cutaneous leishmaniasis in several regions so that their general efficacy can be ascertained.

Intramuscular paromomycin is sponsored by a consortium consisting of the Institute for One World Health, Tropical Disease Research/World Health Organization, Drugs for Neglected Diseases and the manufacturer. Overall utility will, as for all drugs, depend on the sum of efficacy, toxicity, feasibility of administration (in this case intramuscularly) and cost. As for miltefosine, efficacy may vary with species, and the product will need to be tested against visceral leishmaniasis in Africa and Brazil as well as in India. The requirement for intramuscular administration is negative but the fact that paromomycin is an old drug and is likely to be available at a low cost is a positive factor.

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