

REVIEW ARTICLE

Human Leishmaniasis: Clinical, Diagnostic, and Chemotherapeutic Developments in the Last 10 Years

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The current interest in leishmaniasis stems from the importance of this disease with respect to travel medicine, veterans of Operation Desert Storm, humanitarian concerns, and infection with human immunodeficiency virus. Herein, I review aspects of leishmaniasis that are of practical value to practitioners, including presentation, diagnosis, and chemotherapy; I will emphasize advances in chemotherapy over the last 10 years. Amphotericin B and its new lipid formulations are now competitive with pentavalent antimony as primary therapy for visceral leishmaniasis. Pentamidine, paromomycin, and adjunctive therapy with interferon- γ are secondary regimens for the treatment of this condition. High-dose long-term regimens of antimony have been shown to be highly effective for the treatment of cutaneous leishmaniasis. Preliminary evidence of efficacy has been observed with short courses of pentamidine for the treatment of *Leishmania braziliensis* complex disease and topical paromomycin/methylbenzethonium chloride for the treatment of *Leishmania major* disease.

The leishmaniasis are visceral, cutaneous, and (rarely) mucosal diseases due to infection with species of the genus *Leishmania*. These diseases are prevalent where sandfly vectors and mammalian reservoirs exist in sufficient numbers to permit frequent transmission. In 1992, the worldwide incidence of visceral leishmaniasis was estimated to be at least 100,000 cases, and that of cutaneous leishmaniasis was estimated to be at least 300,000 cases [1].

The leishmaniasis have been considered tropical afflictions that together constitute one of the six entities on the World Health Organization/Tropical Disease Research list of most important diseases. Nevertheless, there are now prominent publications on leishmaniasis in the general medical literature. The increased interest in leishmaniasis in industrialized countries is probably due to the importance of travel medicine in this era of international travel, the importance of leishmaniasis as a medical problem encountered during the Persian Gulf War, the inclusion of visceral leishmaniasis as a complication of AIDS, and the concentration of immunologists on this immunologically important experiment of nature, in which microorganisms survive solely within phagolysosomes.

With the increased concern about leishmaniasis has come a strengthened desire to replace standard therapy—i.e., repeated injections of moderately toxic antimony (Sb)—with shorter courses of nonparenteral, less toxic, and more effective agents.

A lengthy review of chemotherapy for leishmaniasis was published in 1988 [2], and shorter reviews of chemotherapy have also appeared [3, 4]. The primary purpose of the present review is to provide an update on chemotherapy for leishmaniasis by reviewing the progress that has been made during the 10 years since 1987. In addition, I review the presentations of leishmaniasis as well as advances in its diagnosis.

Clinical Leishmaniasis

Visceral Disease

Leishmania multiply only within the phagocytic cells of the mononuclear phagocyte system. Visceral leishmaniasis (kala-azar) results from multiplication of leishmania within the mononuclear phagocytes. The disease is primarily caused by *Leishmania donovani* in the Indian subcontinent and Africa, *Leishmania infantum* in Mediterranean regions, and *Leishmania chagasi* in the New World; of these species, at least *L. infantum* and *L. chagasi* are closely related [5].

Classic visceral leishmaniasis presents as fever, hepatosplenomegaly, and pancytopenia. Patients report a history of fever, which is present at the time of medical consultation in all but a few cases [6]. On physical examination, the spleen, which is enlarged to a greater extent than the liver, is typically appreciated 5–15 cm below the left costal margin; however, it should be noted that splenomegaly may be absent in 5% of cases [7]. Hemoglobin concentrations of 5–9 g/dL, WBC counts of 2,000–4,000/mm³, and platelet counts of 100,000–200,000/mm³ are typical, although lower, more abnormal values can be observed in cases of more-extensive disease. Recently reported unusual presentations are acute hepatitis [8], bacteremia [9], and Guillain-Barré syndrome [10].

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Symptomatic visceral leishmaniasis is commonly fatal if untreated. The death rate associated with this condition, even with treatment, can be approximated from reports of the Sudanese and Indian epidemics. In one large study, 336 (11%) of 3,076 Sudanese patients died of unspecified causes [11]. In a smaller series, five (6%) of 83 patients died [7]. Four of these five patients died of anemic heart failure, gastrointestinal bleeding, or liver failure before treatment was begun or on initiation of treatment. One patient died suddenly during treatment with stibogluconate. In 1990, government records from Bihar, India, showed 54,650 cases of leishmaniasis and 589 deaths (1%) [12].

Three other clinical presentations of visceral leishmaniasis have been described or have become better appreciated in the last decade.

Mild visceral disease that does not progress to classic kala-azar. There were no cases of classic visceral disease among the 500,000 United States soldiers deployed to the Arabian peninsula during Operation Desert Storm, but nine cases of mildly symptomatic visceral infection have been reported [13, 14]. Four of the nine patients were afebrile, three of the nine patients did not have organomegaly, and all had normal hemoglobin concentrations. Instead, these patients variously complained of fevers, chronic fatigue, malaise, cough, intermittent diarrhea, or abdominal pain. One patient did not have any symptoms or signs, and the diagnosis was suspected because of an elevated titer of serum antibody. One patient presented 2 years after the last possible exposure.

These patients had disease reminiscent of the "subclinical" disease previously described in Brazilian children [15]. In a survey of children who had titers of antibodies to *Leishmania* that were higher than a defined cutoff, investigators observed that approximately one-quarter of these children were completely asymptomatic, one-half had subclinical disease that was characterized by mild symptoms (malaise, diarrhea, and poor tolerance of work or play) and intermittent hepatomegaly, and one-quarter had disease that progressed to classic kala-azar either slowly (in 5 months) or rapidly (in 1 month) [15].

The experience during Operation Desert Storm reemphasizes the fact that in both developed countries and the developing world, inapparent disease and mild disease are the most frequent forms of visceral leishmaniasis. Thus, unexplained constitutional symptoms that appear within 2 years after exposure to an area where leishmaniasis is endemic might warrant investigation for infection with leishmania.

Visceral leishmaniasis as a component of AIDS. The coexistence of HIV and visceralizing *Leishmania* species in southern Europe and in a few other areas where leishmaniasis is endemic has resulted in a large number of dually infected individuals and a substantial literature on visceral leishmaniasis in the HIV-infected population. As of 1994, ~300 cases of dual infection had been reported in Europe (by 1997, the total number of cases was ~1,000), and the number of cases of visceral disease among HIV-infected patients in Europe is now approximately

equal to the number of such cases among non-HIV-infected patients [16].

Some investigators have concluded that the clinical presentation of visceral leishmaniasis in HIV-infected hosts is comparable to the classic presentation in non-HIV-infected hosts [16, 17], except that involvement of the gastrointestinal tract, from the esophagus to the rectum, is frequent in HIV-infected hosts [18–20]. Other authors have concluded that hepatosplenomegaly is frequently absent in HIV-infected patients [21, 22]. CD4 cell counts, although depressed, can be relatively high (e.g., mean count in one study, $147 \times 10^6/L$ [23]) compared with those for patients who have other opportunistic infections.

In contrast to the relative frequency of HIV-associated visceral leishmaniasis, only ~10 cases of HIV-associated cutaneous leishmaniasis have been reported [24–30]. Because some coinfecting patients have antibodies to *Leishmania* species, and some do not, it is believed that the former patients develop leishmaniasis as a result of reactivation of preexisting latent leishmaniasis by the HIV infection, whereas the latter patients develop leishmaniasis after they become infected with HIV [16].

Visceral leishmaniasis was previously recognized as a complication of immunosuppression in fewer than 30 non-HIV-infected patients [31]. A case of visceral leishmaniasis in a renal graft recipient has recently been reported [32]. The association of kala-azar with HIV infection emphasizes the fact that visceral leishmaniasis is an opportunistic infection that now affects patients in the developed world.

Antimony and amphotericin B formulations have been used for the initial treatment of visceral leishmaniasis in HIV-infected patients. Although use of the amphotericin B formulations is associated with a higher initial cure rate (100%) than is use of antimony (50%) [22, 33], relapses typically occur, even when liposomal amphotericin B is used [33]. Since it is unlikely that initial therapy will eliminate all organisms, maintenance regimens are needed to prevent relapses. Antimony and amphotericin B are generally used and should probably be reserved for initial and subsequent treatment courses. Therefore, the secondary parenteral agents pentamidine and paromomycin, alone or perhaps combined with putatively effective drugs such as interferon, ketoconazole/fluconazole, or allopurinol, are the choices for maintenance therapy.

There will likely be so few evaluable cases treated with any one combination regimen that it will generally not be possible to determine if an adjunctive drug contributes significantly to efficacy. Since leishmania multiply slowly and effective antileishmanial agents can be toxic, maintenance therapy should be given intermittently. An example of a successful maintenance regimen is included in a case report by Lortholary et al. [34]; this regimen included pentamidine (2 mg/kg) plus interferon (175 μg), three times per week, 1 week per month.

Sudanese disease ("killing disease"), including post-kala-azar dermal leishmaniasis (PKDL). Visceral leishmaniasis is endemic in Sudan, where the incidence of classic visceral leish-

maniasis was recently estimated to be 4% [35]. There has been an explosive increase in the number of cases of visceral leishmaniasis during the 1990s [36, 37]. In the 10 years before 1995, it was estimated that 100,000 persons died of visceral leishmaniasis in southern Sudan [11].

The large number of cases that occurred during this period included previously rare clinical features such as the sensation of burning feet and other neurological symptoms (in 46% of 111 cases of kala-azar [38]), leishmanial cholecystitis [39], and congenital leishmaniasis [40]. A notable feature of kala-azar in Sudan was PKDL, seen in 56% of the cases [41].

The disease was clinically similar to that already known in India. Macules and papules first appeared around the mouth. Those that did not heal spontaneously became more dense and spread to the rest of the face, upper chest and back, upper arms, or over the entire body and included nodules and ulceration. However, in contrast to the interval of years between an episode of visceral disease and the onset of cutaneous lesions for Indian patients with PKDL, the interval between the end of treatment of kala-azar and the onset of cutaneous disease for the Sudanese patients ranged from 0 to 180 days (mean interval, 56 days).

The epidemic of kala-azar in Sudan illustrates that an explosive increase in severe kala-azar can occur in the developing world.

Cutaneous Disease

Cutaneous leishmaniasis results from multiplication of *Leishmania* in the phagocytes of the skin. The disease is primarily caused by members of the *L. mexicana* complex (*L. mexicana mexicana*, *L. mexicana amazonensis*, and *L. mexicana venezuelensis*) and the *L. braziliensis* complex (*L. braziliensis braziliensis*, *L. braziliensis panamensis*, and *L. braziliensis guyanensis*) in the New World [5], and by *L. tropica* and *L. major* in the Old World. In the classic course of this disease, lesions first appear as papules, progress to ulcers, then spontaneously heal with scarring over months to years. The lesions may uncommonly be nodular; nodules were the sole lesions in 10% of 182 cases of cutaneous disease in Colombia [41a].

In cases of disease due to *L. braziliensis*, lymphadenopathy may accompany the skin lesions (77% of cases in one report) or precede the skin lesions by 1–2 months [42, 43]. Investigations of personnel stationed at military units in areas of endemicity for defined intervals showed that the cutaneous lesions first appeared from 2 days to 78 days (median interval, 17 days) after exposure [44].

The use of placebo in drug trials has made it possible to evaluate the natural history of cutaneous leishmaniasis during the time before the controls received treatment. In one study of disease due to *L. mexicana mexicana* in Guatemala [45], 22 (88%) of 25 lesions healed in a median observation time of 14 weeks (range, 6–44 weeks). In contrast, only 22% of

L. braziliensis lesions healed during a similar median time of 13 weeks [45].

The results of these reports suggest that at least in the New World, patients may exhibit lesions as early as a few weeks after exposure, and that nodular lesions and lymphadenopathy as well as the more typical ulcers should be evaluated for the presence of *Leishmania*. In contrast to *L. braziliensis* lesions, lesions due to *L. mexicana mexicana* generally heal rapidly.

Mucosal Disease

Although mucosal leishmaniasis is defined as infection of the mucosal membranes of the nose and mouth, infection is initially evident in the nasal mucosa [46] and then may involve the oropharynx and larynx. Mucosal disease had been thought to be a late sequel of New World cutaneous disease, but in recent drug trials, mucosal metastasis was reported in the first year after the appearance of the cutaneous lesion [47]. Mucosal disease characteristically does not heal spontaneously and evolves slowly (mean time, 3 years) before first being brought to medical attention [47].

Immunologic Correlates of Clinical Disease

A major reason for interest in leishmaniasis is the immunologic polarity of the disease: cure is associated with the presence of cellular immune responses, while continued disease is associated with the absence of such responses. For example, classic cutaneous leishmaniasis eventually heals spontaneously and is characterized by positive responses to *Leishmania* skin-test antigens and generally high values for *Leishmania* antigen-induced lymphocyte transformation in vitro [48]. In the rare cases of diffuse cutaneous leishmaniasis in which nodules do not heal with time, the leishmanin test and lymphocyte transformation are routinely negative [48]. Frank visceral leishmaniasis does not heal spontaneously, and skin tests and in vitro lymphocyte transformation are negative in cases of acute kala-azar but convert to positive after successful chemotherapy [49–51].

In spite of the polarity of gross clinical and immunological parameters and an equally dichotomous situation in inbred mice, analysis of more-subtle immunologic parameters reveals a less polar situation for both cutaneous and visceral disease.

PCR has been used to determine cytokine mRNA in biopsy specimens from patients with classic cutaneous disease, diffuse cutaneous disease, or mucosal disease. The mRNA determinations included those for the Th1 cytokines IFN- γ and IL-2, which augment cell-mediated immune responses by activating macrophages, and those for the Th2 cytokines IL-4, IL-5, and IL-10, which inhibit some cell-mediated responses. It is surprising that mRNA for both the Th1 cytokines and the Th2 cytokines was present in specimens of lesions of all three diseases, although the mRNA for IL-4 tended to be present in higher quantities in lesions of diffuse and mucosal disease [52, 53].

With respect to the lesions of classic cutaneous disease, chronic lesions (those present for ≥ 4 months) and early lesions (those present for ≤ 2 months) did not differ in terms of IFN- γ levels, although chronic lesions contained significantly higher levels of IL-10 [54].

Another approach has been to determine the levels of chemokines (mediators that attract phagocytes and lymphocytes.) in leishmanial lesions. Classic cutaneous disease is associated with the chemokine monocyte chemoattractant protein-1, which may stimulate macrophage microbicidal mechanisms, whereas diffuse disease is associated with the chemokine macrophage inflammatory protein-1 α [55].

In one study of visceral leishmaniasis, IFN- γ was present and IL-4 was undetectable in *Leishmania* antigen-stimulated peripheral blood mononuclear cells from patients with active disease; the levels of both cytokines were higher after administration of successful chemotherapy [56]. Analysis of cytokine patterns in bone marrow [57] and lymph nodes [58] showed that both IFN- γ and IL-10 were present in the lesions of acute visceral disease and that their levels diminished after cure, although the loss of IL-10 was more dramatic than the loss of IFN- γ .

In summary, the clinically polar forms of leishmaniasis have been found to be associated with both Th1 and Th2 cytokines, which is surprising. For only one form of disease, kala-azar, and one cytokine, IL-10, can a reasonable association between clinical course and cytokine levels be made. IL-10 can suppress the production of IFN- γ as well as the cellular response to this interferon [58].

The fact that high levels of IL-10 are present during visceral disease, whereas much lower levels are present after successful chemotherapy, correlates with the inability to kill *Leishmania* within macrophages, the lack of skin-test responses, the lack of in vitro lymphocyte transformation during disease, and the reversal of these parameters after chemotherapy. These correlations suggest that therapy with IFN- γ , IL-2, anti-IL-10, or IL-12 (which increases the number of Th1-producing cells) could be effective for kala-azar.

Diagnostic Methods

Since the association of *Leishmania* with clinical leishmaniasis early in the 20th century, the diagnosis of leishmaniasis has been made by classic microbiological methods. Samples of infected tissue are obtained, and the organisms are either seen in Giemsa-stained impression smears of tissue or cultured from tissue. The techniques for obtaining cutaneous tissue and evaluating it for the presence of *Leishmania* species have been reviewed by Kalter [59]. Aspirates of infected mucosal tissue and aspirates or biopsy specimens of involved visceral tissues (e.g., the spleen, liver, or bone marrow) are similarly subjected to microscopic examination and culture. Cultured organisms can be identified to the species level, usually by use of isoenzyme electrophoresis [60].

Histopathology is rarely positive for patients with the New World form of cutaneous disease. Either Giemsa-stained smears or culture was found to be more sensitive than histopathology in a Guatemalan report [61] and a Colombian [62] report (table 1). The sensitivity of cultures was improved by $\sim 20\%$ when three specimens were obtained [61]. In reports from the Old World, histopathology and staining were more sensitive (table 1) [63, 64]. In general, both staining and culture should be performed, since smear-negative lesions can be culture positive [61], and culture-negative lesions can be smear positive [61, 62].

Culture is more sensitive than microscopy for diagnosing mucosal disease [62] (table 1). For visceral disease, both microscopy and culture generally yield positive results if splenic aspirates are obtained, and splenic tissue is superior to bone marrow and lymph node specimens for making this diagnosis (table 2). (The high specificity of diagnosis based on evaluation of one splenic aspirate is partially due to the fact that splenic aspiration is the positive control: patients with negative splenic aspirates are generally judged not to have kala-azar.) Visceral leishmaniasis is a multiorgan disease; in one study, nasopharyngeal swabs were found to be positive by stain and by culture in 28% and 36% of patients, respectively [67].

Therefore, the limitations of classic diagnostic methods are the requirement for tissue, the fact (which is not surprising) that repeated tissue sampling improves sensitivity, and the need to train laboratory personnel specifically in leishmanial diagnostic techniques (i.e., recognition of shrunken leishmania in histopathologic slides and in Giemsa-stained smears, and culture of parasites).

In recent years, attempts have been made to use PCR, serological tests, and skin tests to eliminate the need for tissue samples and to perform monoclonal antibody staining of tissue smears to improve sensitivity.

PCR was not highly sensitive (60%–80% sensitivity [69–71]) or specific (50% specificity [69]) when performed on biopsy specimens of cutaneous leishmanial lesions from patients in Colombia [69], Peru [70], or the Old World [71]. In a recent report, PCR was found to be highly sensitive (86%) for the identification of *L. major* lesions in Sudanese patients, but its specificity was not investigated [64]. In one study of patients with visceral disease from India, Kenya, and Brazil [72], assay of blood samples with use of PCR demonstrated high sensitivity (90%) and high specificity (100%). In another study from India, there was a large discrepancy between patients whose samples were positive by Giemsa staining, ELISA, or PCR [73].

Interest in the use of PCR should not obscure the fact that there are serious difficulties with this technique. For the diagnosis of cutaneous disease (for which the need is for a sensitive and specific test), a large number of control skin biopsy specimens must be obtained from healthy individuals to verify the specificity of PCR. For the diagnosis of visceral disease (for which classic techniques are already highly sensitive and spe-

Table 1. Comparison of the sensitivities of the methods used for the diagnosis of cutaneous and mucosal leishmaniasis.

Reference	Country	No. of lesions	Percentage of lesions positive by indicated method			
			Histology	Giemsa stain	Culture	Skin test
[61]	Guatemala	>37	18	70	38	ND
[62]	Colombia	>140	14	22	58	ND
[63]	Tunisia	8	ND	100	100	ND
[64]	Sudan	22	73	55	ND	ND
[65]	Colombia	>100	ND	ND	ND	~85
[66]	Ethiopia	12	ND	ND	ND	83, 50 ⁺ (ag1, ag2)
[62]	Colombia*	9	25	14	78	100

NOTE. ND = not done.

* Mucosal disease.

cific and for which there is a need to eliminate aspiration of the viscera), PCR is unlikely to be a competitive technique until it has been shown that blood (rather than tissue) contains sufficient leishmanial DNA for detection. For the diagnosis of either disease, clinical laboratory personnel will have to be familiar with the routine use of PCR for diagnosing other diseases so that equipment and technical training are available.

Serological tests are rarely performed to diagnose cutaneous leishmaniasis; as suggested by previous data, the sensitivity (67%–76%) and specificity of these tests are low [44, 74]. On the other hand, serological tests generally are highly sensitive for diagnosing visceral disease. For the three major areas where leishmaniasis is endemic (i.e., the Indian subcontinent [75], East Africa [76, 77], and Brazil [78]), an ELISA or direct agglutination test (DAT) had sensitivities of >90%. The problem with serological tests for diagnosing visceral disease has been their specificity: false-positive results have been obtained in cases of asymptomatic leishmanial infections or other infectious diseases.

Alteration of the antigens used for DAT [79] and for ELISA [78, 80] have recently been reported to be successful in markedly eliminating false-positive results. Use of the altered DAT antigen resulted in a sensitivity of 92% and a specificity of 100% for 50 Sudanese patients [79]. The recombinant used for the ELISA, antigen r39, is of particular interest. This antigen is not very sensitive, but this lack of sensitivity can be an advantage in diagnostic testing. The antigen has the sensitivity

for identifying 95%–100% of cases of classic kala-azar but not for identifying nonleishmanial infection other than some cases of toxoplasmosis [81].

This lack of reactivity may also be the reason that titers of antibody detected by ELISA diminish after successful chemotherapy has been administered [81]. Therefore, r39-based ELISA has potential for the diagnosis of classic kala-azar and for the recognition of treatment failures. On the other hand, the antigen's lack of sensitivity means that leishmanial syndromes in which antibody generation is modest (i.e., mild visceral disease in Brazil and cutaneous disease) are not recognized with use of r39 [80].

The leishmanin test can be highly specific and sensitive for diagnosing cutaneous and mucosal leishmaniasis, as has been shown for the antigen made in Colombia [65] (table 1). However, the Ethiopian report demonstrated that patients who are skin-test positive when one test antigen is used may not be positive when another antigen is used (table 1) [66]. The development of a useful skin test will require the choice of an immunologically cross-reactive antigen so that the antigen is sensitive to disease from multiple regions of endemicity and will require commitment to good manufacturing practices so that the antigen is reproducible.

The staining of tissue with monoclonal antibodies has dramatically increased diagnostic sensitivity to >90% (up from ~40% with Giemsa staining) in studies of American cutaneous disease [82, 83]. The problem with monoclonal antibody stain-

Table 2. Comparison of the sensitivities of methods used for the diagnosis of visceral leishmaniasis.

Reference	Country	No. of lesions	Percentage of lesions positive by indicated method			
			Giemsa stain	Culture of spleen	Culture of bone marrow	Culture of lymph node
[67]	Kenya	64	97	92	ND	ND
[68]	Sudan	84	ND	96	70	58

NOTE. ND = not done.

ing is that the internal architecture of leishmania is not as apparent as it is with Giemsa staining, and a trained examiner is needed to differentiate *Leishmania* from host debris.

At present, cutaneous, mucosal, and visceral leishmaniasis must be diagnosed by classic parasitological methods: examination of Giemsa-stained tissue smears and culture of tissue. When the diagnosis of cutaneous and mucosal disease proves dilemmatic, a skin test antigen that has been proven to have good sensitivity in the region in which the patient was exposed could be used in deciding for or against treatment. When the diagnosis of visceral leishmaniasis proves dilemmatic, a serological test with proven sensitivity and specificity in the region of exposure might similarly be used in deciding for or against treatment.

Routine serological studies are positive for only ~50% of HIV-infected patients [17, 22]. However, the immune deficits in these patients result in an increased probability that monocytes in the peripheral circulation are parasitized with leishmania. The sensitivity of culture of the buffy coat of peripheral blood was 53% in one study [84] and 67% in another [85], and this technique can be recommended to aid in the diagnosis if an HIV-infected person is suspected of having visceral leishmaniasis.

Treatment of Leishmaniasis

Pentavalent Antimonials

Organic antimonials were first used in 1912, soon after the recognition in 1904 that *Leishmania* species were the cause of leishmaniasis [2]. Although the pentavalent antimonial sodium stibogluconate (Pentostam; Wellcome Foundation, London) was first recognized as clinically effective in 1947 (table 3) [2], stibogluconate and meglumine antimoniate (Glucantime; Rhône Poulenc, Paris) are still the mainstay of therapy for all the leishmaniasis. Although stibogluconate produced in India is widely used in that country, there have been no formal comparisons of Indian stibogluconate with Pentostam or with Glucantime.

As the leishmaniasis became more extensively treated and more carefully studied, treatment failures with Sb and clinically resistant isolates became recognized. Alternatives to Sb have recently been found for some syndromes (see below). Nevertheless, the mainstay of chemotherapy is still the pentavalent antimonials.

The primacy of pentavalent antimonial therapy has been maintained by increasing the dosage for syndromes for which the cure rate has been found to be low. A review of the data in 1992 [92] showed that for Kenyan [86] and Indian [87] kala-azar and for Panamanian cutaneous disease [88], Sb at a dosage of 20 mg/(kg·d) was more effective (cure rate, >90%) than Sb at a dosage of 10 mg/(kg·d) (table 3), and it was recommended that the daily dose be 20 mg/kg. Because a 28-day course of treatment was successful in Kenya, and a 40-day

course was being administered in India, it was recommended that the duration of therapy for kala-azar be 28 days and perhaps 40 days in regions where clinical resistance had been observed.

Even though no studies of cutaneous disease had compared a regimen of 20 mg/(kg·d) for 20 days with a regimen of 20 mg/(kg·d) for a shorter period of time, a 20-day course of therapy was found to be effective, while a shorter course might not be; therefore, a 20-day course of treatment was recommended for cutaneous disease requiring treatment. Mucosal leishmaniasis is frequently resistant, even after 28 days of therapy (table 3) [91]. Nevertheless, treatment for 28 days was chosen for mucosal leishmaniasis because of insecurity concerning the toxicity of more lengthy treatment regimens.

There have since been attempts to decrease the duration of treatment with antimony for cutaneous disease by administering antimony at a dosage of 20 mg/(kg·d) for a shorter period—10 days. This regimen was successful against *L. braziliensis* disease in Guatemala (table 3) [90].

Clinical response to antimonials is initially rapid in cases of cutaneous leishmaniasis, but clinical parameters do not completely normalize until months after treatment has ended. In cases of kala-azar, patients become afebrile in 4–5 days of the initiation of therapy [93], and patients generally feel much better by the end of the first week. Nevertheless, by the end of a treatment course of about 4.5 weeks, the spleen size decreases by only ~50% [93]. However, 2 months after the end of therapy the spleen size returns to the approximate size noted at 1-year follow-up, which is probably the preinfection baseline size [93]. In a study of cutaneous disease due to *L. braziliensis*, 32% of lesions completely reepithelialized by the end of a 3-week treatment course; 60% reepithelialized within 6 weeks; and ~90% had reepithelialized 13 weeks after treatment [89].

These data indicate that treatment should not be continued until all visceral parameters normalize or until the cutaneous lesion reepithelializes but that it should be continued for a period defined by previous experience to ultimately result in clinical cure. If by the end of that time, the rate of resolution of clinical parameters is much slower than that summarized above, initial clinical failure should be anticipated, and alternative therapy considered.

The time at which relapse may occur has been suggested in recent papers. Of 65 Kenyan patients with visceral leishmaniasis who were treated with various antimonial regimens, 14 (22%) relapsed: 13 of these patients relapsed 2 months after discharge from the hospital, and one patient relapsed 6 months after discharge [94]. At the time of relapse, the patients' spleens had barely declined in size from that observed on admission, and the platelet counts were lower than they had been on admission. The investigators suggested that patients with kala-azar need to be followed up for only 6 months to verify that no relapse has occurred and that the platelet count as well as spleen size are useful prognostic indicators [94].

Netto et al. [95] followed up cases of cutaneous and mucosal leishmaniasis due to *L. braziliensis* for 4–5 years. Among

Table 3. Efficacy of pentavalent antimonial regimens for the treatment of leishmaniasis.

Reference	Type of disease, study site	Study design	Drug, dosage	No. of patients cured/total no. of patients (%)
[86]	Visceral Kenya	Open label, randomized	Stib, 20 mg/(kg · d) for ≤28 d	21/21 (100)
			Stib, 10 mg/(kg · d) for ≤28 d	12/20 (60)
[87]	India	Open label, randomized	Stib, 20 mg/(kg · d) × 40 d	62/64 (97)
			Stib, 20 mg/(kg · d) × 20 d	51/63 (81)
			Stib, 10 mg/(kg · d) × 40 d	45/61 (74)
			Stib, 10 mg/(kg · d) × 20 d	33/58 (57)
[11]	Sudan	Open label, nonrandomized	Stib, 20 mg/(kg · d) × 30 d	2,562/3,076 (83)
[88]	Cutaneous Panama	Blinded, randomized	Stib, 20 mg/(kg · d) × 20 d	19/19 (100)
			Stib, 10 mg/(kg · d) × 20 d	16/21 (76)
[89]	Guatemala	Blinded, randomized	Stib, 20 mg/(kg · d) × 20 d	24/25 (96)
[90]	Guatemala	Blinded, randomized	MA, 20 mg/(kg · d) × 10 d	19/21 (91)
[90]			MA, 20 mg/(kg · d) × 10 d	18/20 (90)
[46]	Mucosal Panama	Open label, nonrandomized	Stib, 20 mg/(kg · d) × 28 d	10/16 (63)*
[91]	Peru	Open label, nonrandomized	Stib, 20 mg/(kg · d) × 28 d	6/8 (75)*
				2/21 (10) [†]

NOTE. MA = meglumine antimoniate (Glucantime; Rhône Poulenc, Paris); Stib = stibogluconate (Pentostam; Wellcome Foundation, London).

* Mild disease.

[†] Severe disease.

62 cutaneous cases treated with Glucantime, there were six cutaneous relapses (10%) and two mucosal relapses (3%). Four of the cutaneous relapses occurred at the original site of disease, which suggests relapse rather than reinfection; these probable relapses occurred 2 months, 4 months, 6 months, and (remarkably) 30 months after the original treatment had been discontinued. The two cases of mucosal relapse occurred 5 months and 37 months after treatment had been discontinued [95].

Systemic toxicity caused by the antimonials may be best determined in patients treated for mucocutaneous leishmaniasis, which is a nonsystemic disease. Of 29 Peruvian patients with mucosal disease that was treated with Pentostam for 28 days, 83% had myalgias and/or arthralgias, 28% had abdominal symptoms, and 21% had headache [91]. These symptoms were first noticed during the second-third week of therapy [47]. Ten percent of the 29 patients had aspartate aminotransferase (AST) levels that rose to as much as three times the upper limit of normal, but most AST levels declined in spite of continued therapy [91].

Gasser et al. subsequently found that chemical pancreatitis (elevation of serum levels of amylase or lipase) was evident on examination of the stored sera from all of the Peruvian patients and of the sera from 16 (94%) of 17 prospectively followed-up patients treated with Pentostam for all forms of leishmaniasis [96]. If symptoms or the degree of the serum

abnormalities did not cause therapy to be stopped, the values of amylase and lipase generally started to decline despite continued therapy [96]. Gasser et al. concluded that Sb is the only chemotherapeutic agent that routinely causes pancreatitis during treatment and that pancreatic inflammation is probably the cause of the nausea and abdominal pains experienced by many patients.

Other side effects of antimonial therapy include isolated instances of decreases in the formed elements of the blood (RBCs, platelets, and WBCs [46, 91]) and of reversible peripheral neuropathy [97]. Although cardiotoxicity can occur with Sb treatment and may be the cause of sudden death [92], significant electrocardiographic changes (concave ST segments or QT_c prolongation) do not occur in patients given single courses of Pentostam (20 mg/[kg · d]) for up to 28 days [89, 91].

In summary, the dosage of antimonial therapy has been increased to 20 mg/(kg · d), given for 20–28 days, to increase efficacy. This daily dose and duration of treatment routinely result in chemical pancreatitis, symptomatic pancreatitis and musculoskeletal pain occur in one-third to two-thirds of cases, and cytopenia may occur. Therefore, the maximum tolerated dose is being administered to patients with leishmaniasis and these regimens will not be tolerated by some patients. For infections that occur in regions where these regimens are ineffective or that occur in patients for whom they are ineffec-

Table 4. Efficacy of pentamidine regimens for the treatment of visceral disease in India.

Dosage, no. of pentamidine injections	Outcome (percentage of patients)
4 mg/kg 3 times per w, 15 injections	Initial cure (77)
27 injections	Initial cure (94), relapse (21)
4 mg/kg 3 times per w, 15 injections	Cure (73)
+ Sb (20 mg/[kg · d]) × 20 d, 27 injections	Cure (84), relapse (16)

NOTE. Study was open-label, nonrandomized. Data are adapted from [98].

tive, the daily dose and duration of therapy cannot be raised significantly, and alternative agents should be used.

Pentamidine

To circumvent the problem of clinical resistance to Sb in India, pentamidine has been tried for the treatment of visceral disease. The pentamidine regimen consisted of 4 mg/kg given three times per week until initial parasitological cure was achieved. Seventy-seven percent of patients were cured after 15 injections had been administered (5 weeks), and 94% were cured after a total of 27 injections had been administered (9 weeks) (table 4) [98]. However, 21% of these patients relapsed. Since a 99% cure rate was seen in this region of endemicity in the early 1980s after 5-week courses of injections were administered [101], clinical resistance to pentamidine had been engendered by the 1990s. The addition of a 3-week course of Sb to a 5-week course of pentamidine did not increase the cure rate above that observed with pentamidine alone [98].

The rationale for using pentamidine to treat cutaneous disease was to try to obviate the toxicity and the duration of treatment associated with the use of Sb. For a regimen of pentamidine to be superior to a regimen of Sb, it would have to be less toxic, involve fewer injections, and be equally effective. The repeated administration of 4 mg/kg would be unattractive; therefore, the administration of 2 mg of pentamidine isethionate/kg every other day for 7 days was studied for the treatment of cutaneous disease in Colombia (table 5). This regimen was 96% effective [99].

To further decrease dosing, the same dose of pentamidine (2 mg/kg) was administered every other day for only 4 days, but the cure rate was 84%. However, a higher dose (3 mg/kg)

administered in four every-other-day injections resulted in a 96% cure rate for 51 evaluable patients [100].

The low-dose, short-course regimens for cutaneous leishmaniasis commonly result in myalgias, pain at the injection site, nausea, and headache and uncommonly result in a metallic taste, a burning sensation, numbness, and hypotension [100]. Reversible hypoglycemia occurred in two (2%) of 116 cases. The incidence and severity of these side effects are higher when the high-dose, long-course regimens are used for treatment of visceral disease; however, it can be difficult to distinguish side effects that are due to pentamidine from those that are due to kala-azar or to Sb if it is administered concomitantly.

Jha et al. [102] reported a 20% incidence of tachycardia and/or hypotension and a 1% incidence of hyperglycemia among patients receiving pentamidine. Thakur et al. [98] reported a 10% incidence of hyperglycemia and an 8% incidence of hypoglycemia, but most of his patients received Sb in addition to pentamidine.

Because the high-dose, long-course regimen of pentamidine used for visceral disease is probably more toxic than are antimonial regimens, pentamidine should be used only when it is likely to be more effective than antimony. Pentamidine might be used in regions where treatment failure with antimony is common and also where pentamidine has not been widely used (i.e., pentamidine resistance is unlikely to have developed) and in individual cases of antimony treatment failure. For cutaneous disease, the high cure rate associated with a low dose of pentamidine given for a short period makes it an attractive alternative to Sb and the treatment of choice in cases of Sb treatment failure.

Amphotericin B and Lipid-Associated Amphotericin B

The use of formulations of amphotericin B for treatment of the leishmaniasis is biochemically rational because the target of amphotericin B is ergosterol-like sterols, which are the major membrane sterols of *Leishmania* species as well as fungi [113]. Before 1990, amphotericin B was administered infrequently because of its known infusion-related side effects (fever, chills, bone pain, and, rarely, cardiac arrest) and delayed side effects (decreased potassium levels and decreased renal function) [104, 114, 115].

Amphotericin B formulations are now being more widely used for visceral leishmaniasis and constitute the major advance

Table 5. Efficacy of pentamidine regimens for the treatment of cutaneous leishmaniasis in Colombia.

Reference	Study design	Dosage, no. of injections	No. of patients cured/total no. of patients (%)
[99]	Open-label, nonrandomized	2 mg/kg q.o.d., 7	23/24 (96)
[100]	Open-label, nonrandomized	2 mg/kg q.o.d., 4	32/38 (84)
		3 mg/kg q.o.d., 4	49/51 (96)

Table 6. Efficacy of regimens of amphotericin B (AmB) and of lipid-associated AmB for visceral leishmaniasis.

Reference	Study site (leishmanial resistance)	Study design	Drug, regimen	No. of patients cured/ total no. of patients (%)
[103]	India (Sb and pentamidine)	Open label, nonrandomized	AmB, 1 mg/k q.o.d. \times 20 injections	298/300 (99)
[104]	India	Open-label, nonrandomized	AmB, 1 mg/(kg \cdot d) \times 20 d	40/40 (100)
[105]	India	Open-label, randomized	AmB, 0.5 mg/kg q.o.d. \times 14 injections;	40/40 (100)
[106]	India (Sb)	Open-label, randomized	Sb, 20 mg/(kg \cdot d) \times 40 d*	25/40 (62)
			AmB, 0.5 mg/kg q.o.d. \times 14 injections; pentamidine, 4 mg/kg q.o.d. \times 20 injections*	59/60 (98) 46/60 (77)
[107]	Europe	Open-label, nonrandomized	L-AmB, 1 mg/(kg \cdot d) \times 21 d	... [†]
[33]	Europe	Open-label, nonrandomized	L-AmB, 1 mg/(kg \cdot d) \times 21 d;	10/10 (100)
[108]	Europe	Open-label, nonrandomized	3 mg/(kg \cdot d) \times 10 d	10/10 (100)
			L-AmB, 4 mg/kg on d 1–5, 10;	13/13 (100)
[109]	Sudan	Open-label, nonrandomized	3 mg/kg on d 1–5, 10; 3 mg/kg on d 1–4, 10	41/42 (98) 29/32 (91)
			L-AmB, 3–5 mg/kg on d 1, 4, 11; 3–5 mg/kg on d 1, 4, 7, 9, 11, 14	8/16 (50) 14/16 (88)
[110]	India	Open-label, nonrandomized	L-AmB, 2 mg/kg on d 1, 5, 10	10/10 (100)
[33]	Europe [‡]	Open-label, nonrandomized	L-AmB, 1.5 mg/(kg \cdot d) \times 21 d	3/11 (27)
[6]	Brazil	Open-label, nonrandomized	ABCD, 2 mg/(kg \cdot d) \times 10 d;	10/10 (100)
[111]	India (Sb)	Open-label nonrandomized	2 mg/(kg \cdot d) \times 7 d;	9/9 (100)
2 mg/(kg \cdot d) \times 5 d			9/10 (90)	
[112]			ABL, 3 mg/kg q.o.d. \times 5 injections; 3 mg/(kg \cdot d) \times 5 d	21/21 (100) 4/4 (100)

NOTE. ABCD = amphotericin B colloidal dispersion; ABL = amphotericin B lipid complex; L-AmB = liposomal amphotericin B; AmB = amphotericin B; NA = not available; Sb = antimony.

* Comparison regimen.

[†] One patient was cured.

[‡] Study of HIV-infected patients.

in antileishmanial chemotherapy during the last 10 years. The reasons for the increased use of amphotericin B are a greater demand due to the rise of kala-azar that is clinically resistant to antimony and to pentamidine, coupled with a better supply in that new, less toxic amphotericin B formulations are available.

Amphotericin B (deoxycholate) has been given to large numbers of patients with kala-azar that is clinically resistant to Pentostam and pentamidine (table 6). In Bihar, 99% of patients were cured with the standard regimen of 20 injections (1 mg/kg given every other day) (table 6) [103]. Further studies showed that the duration of therapy could be diminished by administering the drug daily rather than every other day (table 6) [104] and that in this patient population, a dose

of 1 mg/(kg \cdot d) could be administered initially rather than incrementally [116].

Mishra et al. [105, 106] found that the daily dose could be diminished from 1 mg/kg to 0.5 mg/kg; all patients who had not received Sb or who had Sb-resistant Kala-azar and were given amphotericin B at a dosage of 0.5 mg/kg every other day for 14 days (total dose, 7 mg/kg) were cured (table 6). The 62% cure rate for the comparison group given Pentostam indicates that in the middle 1990s, the incidence of primary stibogluconate resistance in Bihar was \sim 40%. Thakur et al. [117] found that amphotericin B could also be administered to children (1 mg/kg every other day, for a total of 20 injections); the success rate was 100% for 50 patients. In addition, these

authors found that amphotericin B was effective at a dosage of 1 mg/kg for 20 days, without apparent harm to the fetus, in five pregnant women [118].

The need to develop less toxic, perhaps more effective formulations of amphotericin B for systemic mycoses has led to three new clinical formulations of amphotericin B in which deoxycholate has been replaced by other lipids; these formulations are liposomal amphotericin B (L-AmB [AmBisome], Nexstar, San Dimas, CA), amphotericin B colloidal dispersion (ABCD [Amphocil]; Sequus, Menlo Park, CA), and amphotericin B lipid complex (ABLC [Abelcet]; Liposome Co., Princeton, NJ). All three formulations are available for clinical use in western Europe; ABLC has been marketed in the United States.

In general, these formulations are well taken up by the reticuloendothelial system, where leishmania reside, and are poorly taken up by the kidney, the major target of organ toxicity [119]. Therefore, these formulations would be predicted to be effective against visceral leishmaniasis, to have minimal renal toxicity, and, if amphotericin B remains bound to the lipid particles, to have minimal infusion-related toxicity. Indeed, since leishmania multiply only in phagocytic cells and are almost exclusively found in these cells (in contrast to other amphotericin B-susceptible pathogens that reside both in phagocytes and elsewhere), visceral leishmaniasis may be the disease for which the new lipid-associated amphotericin B formulations have the best therapeutic index.

Davidson et al. [107] reported the first clinical use of lipid-associated amphotericin B formulations for leishmaniasis; they gave L-AmB to a patient whose disease was clinically resistant to Sb and to pentamidine. The patient was cured with 50 mg of drug (~ 1 mg/kg) given daily for 21 days (table 6). The desire to shorten the duration of therapy with this parenteral agent led to trials of a larger dose for a shorter period of time (i.e., 3 mg/kg for 10 days), and then to intermittent administration of the drug. The regimen needed to assure a high rate of cure was found to be 3 mg/kg of the drug on days 1–5 and on day 10 (total dose, 18 mg/kg) (table 6) [108]. This regimen resulted in a small change in renal function: mean values of blood urea nitrogen increased by 25% from day 0 to day 10 [108]. However, the officially recommended regimen in the United Kingdom reflects the desire to administer a total dose of ~ 22 mg/kg so as to provide a margin of safety in terms of efficacy.

Other populations have also been treated with L-AmB. Ten Indian patients with kala-azar were cured with a dose of 2 mg/kg given on days 1, 5, and 10 (total dose, 6 mg/kg) [110]. In a study of the treatment of kala-azar under field conditions in Sudan, a regimen of 3–5 mg/kg given in each of three injections was much less successful than was a regimen of 3–5 mg/kg given in each of six injections (table 6) [109]. This study showed that at least under field conditions, a total dose of ~ 12 mg/kg is not effective.

Two infants aged 8 months were successfully treated with L-AmB at a dosage of 3 mg/(kg \cdot d) for 10 days, which shows

that L-AmB is well tolerated by children [120]. However, the success rate for the HIV-infected patients with kala-azar has been as poor with this drug as with other agents. Although patients are initially cured, at least one-half relapse, generally within 6 months [121]. Two Sudanese patients with PKDL and a patient with a severe case of mucosal disease were cured with huge total doses of L-AmB (~ 90 mg/kg [122, 123]).

Although ABCD was the subject of the first reported series of patients treated with these new formulations, few patients have received this formulation. The reason that ABCD is rarely administered is that although a relatively low dose (2 mg/(kg \cdot d)) given for a short period (7 days) was 100% successful (table 6), administration of this formulation resulted in a high incidence of infusion-related side effects (chills, fever, and increased respiratory rate) in children [6, 111]. A high incidence of infusion-related side effects was also reported in a phase 1 study of patients infected with systemic mycoses [124].

ABLC is the most recent formulation studied for the treatment of kala-azar. A dose of 3 mg/kg administered every other day for 5 injections was 100% successful in patients with antimony-resistant kala-azar, one-third of whom were children, as was a dose of 3 mg/(kg \cdot d) for 5 consecutive days in a smaller number of patients (table 6) [112]. Infusion-related toxicity occurred in these patients; 95% had chills and fever during the first infusion, and 50% had these symptoms during the last infusion. There was no change in mean parameters of renal function. Work is ongoing to determine the minimum therapeutic dose of ABLC when it is given daily for 5 days; a dose of 2 mg/(kg \cdot d) given for 5 days appears to cure 95% of patients (S. Sundar, personal communication).

Lipid-associated amphotericin B formulations are parenteral agents that distribute to the visceral reticuloendothelial system. Their primary use is for visceral leishmaniasis; the huge dosages used so far for treatment of severe mucocutaneous disease suggest that these drugs will be used infrequently for nonvisceral disease. Lipid-associated amphotericin B or amphotericin B should be used instead of antimony when leishmaniasis has been contracted in India or another region known for a high incidence of antimony treatment failure, when patients have predisposing conditions that make antimonial toxicity likely, or for rescue in cases of antimony treatment failure.

In spite of the fact that lipid-associated amphotericin B formulations are designed to replace amphotericin B, in the absence of comparative studies, it is not yet clear whether lipid-associated amphotericin B should actually replace amphotericin B for the treatment of visceral leishmaniasis. Mishra et al. [105] found that amphotericin B at a total dose of 7 mg/kg was highly effective in Indian patients with visceral leishmaniasis. Since the recommended total dose of L-AmB is ~ 22 mg/kg in the United Kingdom, an effective dose of L-AmB in India is 6 mg/kg, the minimal effective dose of ABLC in India is ~ 10 mg/kg, and the minimal effective dose of ABCD in Brazil is ~ 10 mg/kg, it is not likely that the new formulations will be more effective than amphotericin B itself.

Table 7. Efficacy of parenteral paromomycin regimens for the treatment of visceral or cutaneous leishmaniasis.

Reference	Type of disease, study site	Regimen	No. of patients cured/ total no. of patients (%)
[125]	Visceral, Kenya [†]	15 mg/(kg · d) × 19 d 15 mg/(kg · d) × 14 d + Sb (20 mg/[kg · d]) × 19 d*	15/19 (79) 20/23 (87)
[126]	Visceral, India	17 mg/(kg · d) × 20 d + Sb (20 mg/[kg · d]) × 20 d	18/22 (82)
[127]	Visceral, Sudan [†]	15 mg/(kg · d) × 17 d + Sb (20 mg/[kg · d]) × 17 d Sb (20 mg/[kg · d]) × 30 d*	60/67 (90) 62/67 (93)
[128]	Cutaneous, Colombia	22.5 mg/(kg · d) × 14 d	15/30 (50)
[129]	Cutaneous, Belize [†]	14 mg/(kg · d) × 20 d Sb (20 mg/[kg · d]) × 20 d*	10/17 (59) 15/17 (88)

* Comparison regimen.

[†] Open-label, randomized study.

The advantage of the new formulations is that they are less toxic than amphotericin B; therefore, the total doses can be administered over a brief interval of 5–10 days. For situations in which toxicity and duration of therapy are the major considerations, the new formulations will be attractive. For situations in which cost is a major concern, amphotericin B may be preferred to the new and relatively expensive formulations.

The absence of comparative studies also makes it difficult to determine which formulation should be used when the decision is made to use lipid-associated amphotericin B. On the basis of infusion-related side effects, ABCD seems to be the most toxic formulation, and L-AmB seems to be the least toxic formulation. Findings on comparison of the minimum effective doses of L-AmB in Europe and in India suggest that efficacy data from one geographic area may not pertain to another geographic area; thus, the relative efficacies of the three formulations can be determined only from studies performed in the same geographic area.

Paromomycin (Aminosidine)

Paromomycin is an aminoglycoside licensed in Europe for the parenteral treatment of bacterial diseases for which aminoglycosides are customarily used. The recommended dose is 15 mg/(kg · d), given for 10 days. Although paromomycin differs from neomycin B only in the substitution of CH₂OH for CH₂NH₂ on one of the three sugar groups of neomycin B, paromomycin has broad antiparasitic activity not shared by the neomycins or other aminoglycosides, and oral paromomycin is recommended for the clinical treatment of intestinal amebic infections and tapeworm infections.

Injectable paromomycin has been used as monotherapy for visceral leishmaniasis. In a study in Kenya, paromomycin sulfate (14–16 mg/[kg · d]) given for a mean of 19 days, cured 79% of patients (table 7) [125]. Attempts have been made to augment the efficacy of paromomycin by administering it in combination with stibogluconate. The combination of paromomycin plus Pentostam, given for 20 days, cured 82% of Indian

patients (table 7) [126], a percentage comparable to that observed when Pentostam is administered alone for twice as long (40 days). Similarly, for disease in Sudan, paromomycin and Pentostam, administered at full daily doses for approximately half the usual duration of Pentostam therapy, were equal in efficacy to Pentostam administered for the full time (table 7) [127]. The use of half as much paromomycin or stibogluconate in the combination resulted in less efficacy (~70%) [130].

Injectable paromomycin has been used less successfully for the treatment of cutaneous leishmaniasis. Even a high daily dose (22.5 mg/kg), given for 14 days, cured only 50% of patients in Colombia [128], and similar results were seen in Belize [129] (table 7). The combination of paromomycin and stibogluconate has also been used as therapy for the more serious syndrome of diffuse cutaneous disease [131].

Although effective for visceral disease, paromomycin monotherapy is probably not as effective as antimonial therapy in regions where leishmania are susceptible to antimony, and paromomycin monotherapy is less effective than formulations of amphotericin B. The combination of paromomycin with Sb generally results in a regimen that is highly efficacious for visceral disease; this results in two parenteral agents being administered for half the time that one parenteral agent (antimony) must be administered.

Because paromomycin is an aminoglycoside, it has the potential for renal toxicity and eighth cranial nerve toxicity. Although these side effects are rarely seen if paromomycin is used as monotherapy at the recommended dosage, administration of paromomycin for longer periods of time or in combination with other agents may result in these side effects [132].

Cytokines

Badaro et al. [133] first reported the use of human recombinant interferon- γ as an adjunct to antimony therapy for visceral leishmaniasis; these investigators found that seven of nine cases of Sb-resistant kala-azar could be cured with the combination of interferon- γ (~100 μ g/m² per day) and antimony (20 mg/[kg · d]),

given for 28 days. A subsequent trial showed that interferon- γ was only partially effective by itself; four of nine patients in India who had not received previous treatment showed no response to interferon- γ therapy, and only a partial elimination of parasites was observed for five of these patients [134].

It was found that interferon- γ , in combination with Sb, could speed the elimination of parasites in previously untreated patients: both Kenyans who received interferon- γ (100 $\mu\text{g}/\text{m}^2$ every day) plus Pentostam (20 mg/[kg \cdot d]) [135] and Indians who received interferon- γ (100 $\mu\text{g}/\text{m}^2$ per day) plus Pentostam (20 mg/[kg \cdot d]) [136] experienced more rapid elimination of parasites when treated with the combination for 28 days than with antimony alone. The increase in the number of parasites eliminated by week 3 was statistically significant in India ($P = .006$): 14 (93%) of 15 patients who received combination therapy had been cured by week 3, whereas only 6 (40%) of 15 who received Pentostam had been cured. However, by week 4, 11 (73%) of the latter 15 patients had been cured.

The combination of interferon- γ and antimony was not as effective against Brazilian Sb-resistant disease as originally thought (six of 14 subsequent patients were cured [137]), but the combination did cure nine (69%) of 13 patients with antimony-resistant visceral disease in India [138]. However, two patients died of drug toxicity, perhaps because of the cumulative effect of previous therapy plus that of the combination therapy [138].

Because interferon- γ is an injectable agent with noticeable side effects (see below), intralesional rather than systemic treatment has generally been tried for cutaneous lesions. In one study of cutaneous leishmaniasis due to *L. braziliensis guyanensis*, 12 of 13 lesions became smaller after injection of interferon- γ , but 6 of 13 control (saline-injected) lesions also became smaller [139]. Interferon- γ was also compared to antimony for the intralesional treatment of cutaneous disease due to *L. tropica*. Lesions were treated with one or the other agent by infiltration of four opposing sites around the lesions once a week for 5 weeks. At 10 weeks, 38 (100%) of 38 antimony-treated lesions vs. 14 (38%) of 37 interferon- γ -treated lesions had partially or completely healed [140].

The combination of antimony and systemic (i.e., intramuscular) interferon- γ has been tried for the treatment of antimony-resistant mucocutaneous disease. Four patients with cutaneous disease and nine patients with mucosal disease, all of whom had received a 28-day course of Sb (20 mg/[kg \cdot d]) that did not result in cure, received a lower dose of antimony (10 mg/[kg \cdot d]) in combination with interferon- γ daily for 30–60 days. All but one of the patients were cured [141].

Although these patients essentially received a full course of antimony (half the standard antimony dose for up to twice the standard time), they had not been cured with previous antimonial therapy; thus, the high rate of cure suggests that interferon- γ contributed to the therapeutic effect. Recombinant IL-2 has been used to treat a few patients with disseminated cutaneous leishmaniasis [142].

The side effects that are probably attributable to interferon- γ in the combination of systemic antimony and interferon- γ are fever alone, which occurs frequently; a flu-like illness consisting of fever, chills, fatigue, myalgias, and headache, which occurred in 5 (31%) of 16 patients in one study; and, rarely, leukopenia [136].

Systemic interferon- γ is not sufficiently active to be used by itself and has side effects, but interferon- γ does augment the efficacy of antimony. The therapeutic role of systemic interferon- γ is in combination with known antileishmanials for the treatment of difficult cases (primarily visceral leishmaniasis).

Oral Agents

The attractiveness of oral treatment—particularly for cutaneous leishmaniasis, which can be treated on an outpatient basis—has led to clinical trials of orally administrable agents for which there is biochemical evidence of antileishmanial activity. Imidazoles such as ketoconazole and triazoles such as itraconazole inhibit ergosterol biosynthesis; therefore, their use is biochemically rational for the treatment of infections due to leishmania.

Ketoconazole, the oldest clinical agent in these classes of compounds, was used in the initial studies [143]. For cutaneous disease in Guatemala, ketoconazole (600 mg per day for 4 weeks) was effective against rapidly self-curing disease due to *L. mexicana mexicana* (cure rate, eight [89%] of nine cases) but less effective against slowly self-curing disease due to *L. braziliensis* (cure rate, seven [30%] of 23 cases) [89]. Ketoconazole was ineffective for disease due to *L. tropica*, which is probably slowly self-curing: of 14 patients who received 200 mg of the drug twice a day for 10 weeks, none was cured [144].

Attempts to treat visceral leishmaniasis with ketoconazole have been the subject of clinical notes. In a Kenyan study, ketoconazole (15 mg/[kg \cdot d] for 21 days) decreased the splenic parasite load in seven patients by 1 log but did not result in a change in spleen size or other clinical parameters of disease [145]. At one Indian center, ketoconazole (200 mg three times a day for 4 weeks) cured four of five patients [146] and seven of nine patients with Sb- and/or pentamidine-resistant kala-azar [147]; however, an average drug dosage of 300 mg twice daily for 30 days failed to reduce the parasite load in any of six patients described in another note [148].

Itraconazole is more easily tolerated than ketoconazole. In essentially uncontrolled studies, itraconazole (200 mg per day [adult dosage] for 4–8 weeks) cured 15 (79%) of 19 patients from the Old World with cutaneous leishmaniasis [149] and 10 (66%) of 15 patients from India who had cutaneous leishmaniasis [150]. Akuffo et al. [151] pointed out the requirement for controlled studies of Old World cutaneous leishmaniasis; these investigators found that itraconazole (200 mg per day for 4 weeks) was slightly less active (improvement was noted for

two of four patients) than placebo (improvement was noted for four of five patients) in a small double-blinded study.

Although fluconazole is inactive *in vitro*, it has been administered for treatment of kala-azar in India at a dose of 6 mg/(kg · d) for 30 days to adults or 12 mg/(kg · d) for 30 days to patients <18 years of age. Approximately half of 10 patients had apparent clinical and parasitological cure but relapsed within 2 months [152].

The hypoxanthine analog allopurinol inhibits purine catabolism in mammalian cells and purine anabolism in leishmania [153]. Although allopurinol has been used to treat leishmaniasis for ~15 years, the first controlled trial has just been completed. A large, randomized, placebo-controlled, double-blinded study conducted in Colombia showed that allopurinol (20 mg/[kg · d] for 28 days) was no better than placebo when the drug was given primarily to patients with *L. panamensis* cutaneous leishmaniasis (18 [33%] of 55 patients vs. 17 [37%] of 46 patients, respectively, were cured) [41a]. The conclusion drawn was that allopurinol monotherapy is ineffective against Colombian cutaneous disease and therefore is unlikely to be effective against cutaneous disease due to other organisms.

Whether allopurinol in combination with other agents has a role in the treatment of leishmaniasis is difficult to decide. An agent with no efficacy by itself would be theoretically unlikely to be effective. On the other hand, other *Leishmania* species may be more susceptible to allopurinol than Colombian *L. panamensis* was, and there have been a large number of uncontrolled studies in which allopurinol was successfully used in combination therapy.

Reports in which allopurinol was administered in combination with standard antileishmanials for visceral leishmaniasis have been summarized previously [154]. In one study of cutaneous leishmaniasis, 24 of 25 Iranian patients who failed to respond to conventional therapy were successfully treated with a combination of allopurinol (20 mg/[kg · d] for 30 days) and Sb (~20 mg/[kg · d] for 15 days) [155], and allopurinol plus Sb given for 15 days at the same dosages was much more effective (26 [74%] of 35 patients were cured) than Sb alone (12 [36%] of 33 patients were cured) in a randomized study [156]. However, the cure rate with Sb alone in this study was noted to be surprisingly low [157].

Allopurinol and ketoconazole have been used together or in sequence for the treatment of leishmaniasis. A renal transplant recipient with visceral leishmaniasis was successfully treated with a combination of allopurinol (300 mg daily) and ketoconazole (200 mg twice a day for 6 weeks) [158], and a case of New World cutaneous leishmaniasis failed to respond to treatment with ketoconazole but was cured after allopurinol (300 mg three times a day for 5 weeks) was administered [159].

WR 6026 is an oral agent whose use has no biochemical rationale. WR 6026 is a primaquine analog that was extremely active against visceral leishmaniasis (but not against cutaneous leishmaniasis) in hamsters and is in trial for treatment of kala-azar. In the first study in which a low dose (1 mg/[kg · d] for

28 days) was used, four (50%) of eight cases of Kenyan visceral disease were cured [160].

Oral agents have not yet demonstrated appreciable efficacy in the treatment of leishmaniasis. Modestly effective oral drugs may be useful in combination therapy. Compared with other potential adjunctive agents, the oral agents are the least effective, but they are also the least toxic. Combination therapy with oral agents could be tried when both efficacy and minimal toxicity are important. Examples of such situations are rescue therapy for patients with organ dysfunction due to other systemic diseases and long-term maintenance therapy for patients who are expected to relapse. The oral agents might also be tried as monotherapy for fast-healing cutaneous leishmaniasis due to organisms such as *L. mexicana mexicana* and *L. major*. In these latter cases, the oral agents could be considered to be minimally toxic adjuncts to the primary curative agent, natural immunity.

Local Agents

Local treatment of cutaneous leishmaniasis is attractive because the ease of drug administration is appropriate for outpatients and because absorption of drug into the circulation and systemic toxicity should be minor.

Intralesional administration of antileishmanial agents is a method of treatment that has been used for decades; in two recent reports [139, 161] (one containing >1,000 cases), investigators reiterate that there is a cure rate of ~75% with intralesional Sb therapy (table 8). This technique is effective; however, the injections are administered intermittently over ~1 month, each lesion has to be injected individually, and there are no data on large series from the New World.

Major emphasis has been placed on topical application of paromomycin-containing formulations. El-On and associates [165] showed that in Israel, *L. major* lesions treated with 15% paromomycin plus 12% methylbenzethonium chloride in soft white paraffin twice a day for 10 days cleared more rapidly (cure rate, 100% at 21–30 days) than did untreated lesions on the same patients (cure rate, 100% at 51–60 days), and this formulation has now been marketed in Israel.

With respect to the New World, this formulation was reported to be highly effective in Ecuador, where 90% of 52 patients were cured 100 days after therapy [166]; however, the trial was uncontrolled, and the natural cure rate in Ecuador can be 75% [154]. In Colombia, investigators studied the combination of topical paromomycin (15%) and methylbenzethonium chloride (5%) plus a short course of parenteral Glucantime because it was believed that the topical agent by itself was unlikely to be effective and that the systemic agent might clear parasites that had already disseminated from the cutaneous site. The cure rate was low for patients given the topical agent for 10 days plus Glucantime for 3 days (8 [42%] of 19 patients were cured), but the rate was high for patients given the topical

Table 8. Efficacy of local regimens for the treatment of cutaneous leishmaniasis.

Reference	Study site (design)	Regimen	Results*
[139]	Syria (open label, nonrandomized)	Intralesional Sb weekly \times 5 injections	29/38 (76)
[161]	Saudi Arabia (open-label, nonrandomized)	Intralesional Sb q.o.d. \times 8–24 injections	756/1,050 (72)
[162]	Colombia (open-label, nonrandomized)	Topical paromomycin/MBCL b.i.d. \times 10 d + Glucantime, 20 mg/(kg \cdot d) \times 3 d	8/19 (42)
		Topical paromomycin/MBCL b.i.d. \times 10 d + glucantime, 20 mg/(kg \cdot d) \times 7 d	18/20 (90)
[163]	Tunisia (blinded, randomized)	Topical paromomycin/urea b.i.d. \times 14 d;	4.5 w (22)
		placebo b.i.d. \times 14 d [†]	13 w (70)
			4.5 w (20)
[164]	Iran (blinded, randomized)	Topical paromomycin/urea b.i.d. \times 14 d;	13 w (70)
		placebo b.i.d. \times 14 d [†]	4.5 w (47)
			13 w (68)
			4.5 w (44)
			18 w (68)

NOTE. MBLC = methylbenzethonium chloride; Sb = antimony.

* Data are no. of patients cured/total no. of patients (%) or duration of treatment (% of patients cured).

[†] Comparison regimen.

agent for 10 days plus Glucantime for 7 days (18 [90%] of 20 patients were cured) (table 8) [162].

These results showed that the topical agent by itself would not be appreciably effective in Colombia but that the combination of the topical agent and a 1-week course of meglumine was as effective as had been found historically for a standard 20-day course of Glucantime. Nevertheless, the usefulness of a topical agent plus a short course of Sb remains to be confirmed in a controlled trial.

Because there is an appreciable rate of local reactions to the paromomycin/methylbenzethonium chloride formulation (25% of patients develop a burning sensation and pruritis and 15% of patients develop vesicles) [162], another formulation has been developed in which the methylbenzethonium chloride has been replaced by 10% urea. Although 10% paromomycin/10% urea administered for \leq 12 weeks cured 23 (85%) of 27 patients with Old World leishmaniasis in an open study [167], this formulation, administered for 2 weeks, was no more effective than placebo in controlled trials for *L. major* disease. In Tunisia [163] and in Iran [164], the percentage of treated patients who were cured at 4.5 weeks and at 13 weeks after treatment was virtually identical to the percentage who were cured in the placebo group (table 8).

The results of these controlled trials show that the paromomycin/urea ointment is ineffective when administered for 14 days and that the natural cure rate for Old World cutaneous leishmaniasis can be high 15 weeks after medical attention has been sought. It is possible that the paromomycin/urea formulation might be effective if it is administered for longer than 2 weeks. However, a longer treatment period will make it more

difficult to show a cure rate that is different from that for placebo and will make the regimen less attractive.

The use of commercially available topical imidazole creams is financially attractive and is a biochemically rational approach. Topical miconazole (2%) and topical clotrimazole (1%) were administered twice a day for 30 days to patients in Saudi Arabia. However, 1 month after therapy only 16% of clotrimazole-treated lesions had fully healed, and none of the miconazole-treated lesions had fully healed [168].

The fundamental problem with local treatment for cutaneous leishmaniasis is that this disease is not a superficial problem as are infections due to the dermatophytes. *Leishmania* amastigotes reside deep in the dermis and also disseminate to the lymphatic system and mucosal membranes. For successful treatment of cutaneous lesions with local injections, the drug has to deeply infiltrate the lesion, which is difficult to achieve in a standardized manner, particularly for Western clinicians who rarely treat this disease. Even when topical agents are effective in vitro, they also must penetrate deeply to be effective against cutaneous lesions.

Even a huge concentration of agent and a vehicle designed to aid penetration may not be sufficient to achieve the necessary penetration. For example, the concentration of paromomycin in the paromomycin/urea formulation is 15,000 μ g/mL, whereas a 100% lethal dose of paromomycin in vitro is 10 μ g/mL [169]; although urea is added as a aid to penetration, the paromomycin/urea formulation has so far been ineffective in controlled trials. An additional major concern about the use of local therapy is that it should not cure lymph node infection or protect against mucosal disease if metastasis has already started.

Table 9. Regimens for the treatment of leishmaniasis.

Type of disease	Regimen	Region or <i>Leishmania</i> species	Approximate cure rate (%)
Visceral	Sb, 20 mg/(kg · d) × 28 d	Africa	85–90
		Brazil, Europe	~90
	Lipid-associated AmB	India	>95
	AmB, 0.5 mg/kg q.o.d. × 14 injections or 1.0 mg/(kg · d) × 20 injections	India	>95
Visceral (secondary regimens)	Lipid-associated AmB	Africa, Brazil, Europe	>95
	Pentamidine, 4 mg/kg 3 times per week × 15–25 injections	India	75
	Paromomycin, 15 mg/(kg · d) × 20 d	Kenya, India	80
	Interferon, 100 µg/m ² q.o.d. + Sb, 20 mg/(kg · d) × 20–28 d	India, Kenya	90
Cutaneous	Sb, 20 mg/(kg · d) × 20 d	All regions, species	90
	Pentamidine, 2 mg/kg q.o.d. × 7 injections or 3 mg/kg q.o.d. × 4 injections	<i>L. panamensis</i>	90
	Topical (paromomycin/MBLC) b.i.d. × 10 d	<i>L. major</i>	>90
	Intralesional Sb weekly × 5 injections	<i>L. major</i>	75
Mucosal	Sb, 20 mg/(kg · d) × 28 d	All regions, species	60
	AmB, 1 mg/kg q.o.d. × 20–30 injections	All regions, species	>75

NOTE. AmB = amphotericin B; MBLC = methylbenzethonium chloride; Sb = antimony.

In spite of these problems, it is possible to conceive of strategies by which virtually all cutaneous disease could be topically treated. An effective topical agent would be the treatment of choice for *L. major* and *L. mexicana mexicana* infections, which generally do not disseminate. Topical treatment would also be appropriate for cutaneous disease that relapses after systemic therapy is administered, since nascent metastasis would probably be eliminated by the systemic agent. A topical agent in combination with short-course systemic therapy might be appropriate for cutaneous disease that has or is likely to have already disseminated.

Although virtually all cutaneous disease could therefore conceivably be treated with a topical agent or a topical agent in combination with short-course systemic therapy, proving that such a regimen is effective will require careful clinical studies. A high cure rate for infections due to *L. major* and *L. mexicana mexicana* will be simple to demonstrate in an uncontrolled experiment but difficult to differentiate from rapid natural cure rates. A high cure rate for disease due to *L. braziliensis* complex will be difficult to achieve.

Conclusion

Classic visceral and cutaneous leishmaniasis remains endemic in the developing world. The occurrence of coinfection with *Leishmania* species and HIV reminds us that southern Europe is part of the region of endemicity surrounding the Mediterranean. The epidemic of kala-azar in Sudan shows

that an explosive increase in the incidence of leishmaniasis can occur in these regions. The mild visceral infections observed in Operation Desert Storm veterans shows that travelers to areas where leishmaniasis are endemic are at risk for these diseases for which no chemoprophylaxis or immunoprophylaxis is available.

The diagnosis of leishmaniasis is still made with use of the classic parasitological methods of visualizing or culturing organisms from infected tissue. These methods are highly sensitive for detecting visceral disease: the organisms are both visualized and cultured from the spleen in >90% of cases; however, these methods are only moderately sensitive for detecting cutaneous disease.

The new methods most likely to be clinically useful are the skin test for cutaneous and mucosal disease and an ELISA based on a recombinant antigen, such as r39, for visceral leishmaniasis.

For the treatment of visceral leishmaniasis, Sb remains effective in Africa and, probably, in Brazil. The virtual 100% efficacy of amphotericin B regimens (both amphotericin B deoxycholate and lipid-associated amphotericin B) makes them competitive with Sb as first-line therapy for kala-azar from any region. These drugs should be used for disease in India, which is likely to be resistant to Sb. Lipid-associated amphotericin B should be chosen over amphotericin B deoxycholate if diminution of the duration of therapy and side effects is of paramount importance.

Because the taxonomy of *Leishmania* species is based on parasite factors that are unrelated to survival or to drug suscep-

tibilities, species that infect the same tissue may or may not share susceptibility to a drug. The efficacy of a drug regimen against a species that causes visceral disease in one region does not automatically signify that such a regimen will be effective against another species in the same region or in another region.

The amphotericin B and lipid-associated amphotericin B regimens listed in table 4 are effective for the species found in regions where these drugs have been tested but may not be effective for disease in other regions. For example, the large dosage of L-AmB recommended for European kala-azar may not be necessary and may unnecessarily increase the cost of therapy, the duration of therapy, and toxicity for patients with diseases from other regions. The lower dosages of amphotericin B, ABCD, and ABLC that have been reported to be effective in India and Brazil may not be effective for European disease. Therefore, patients should be treated with an amphotericin B formulation and regimen that have been reported to cure disease from the region where the infection was acquired.

Secondary regimens are listed in table 9. The amphotericin B-containing formulations are listed as secondary regimens for regions where Sb could be used as primary therapy. The other secondary regimens—pentamidine monotherapy, paromomycin monotherapy, and interferon- γ combined with antimony—are all likely to be less effective than amphotericin B-containing formulations.

Increasing the duration of Sb therapy to 20 days has resulted in a high rate of cure for cutaneous leishmaniasis from all regions reported. Decreasing the dosage of pentamidine has resulted in decreased toxicity and a regimen that is competitive with antimony, at least for infections due to *L. panamensis*. As in the case of the amphotericin B regimens for visceral disease, the advent of a new regimen for cutaneous disease creates the practical problem of testing the regimen for efficacy against multiple species and in multiple regions before the regimen is deemed generally effective.

The disadvantage of both of these parenteral, moderately toxic regimens is that they may result in overtreatment of lesions due to *L. major* and *L. mexicana mexicana*, which generally heal rapidly and do not disseminate. In the future these diseases should be treatable with a topical formulation. However, at present the only topical agent with demonstrable efficacy is paromomycin/methylbenzethonium chloride for *L. major* infection. Another choice for treatment of nondisseminated cutaneous leishmaniasis is intralesional injections of Sb. As yet, there is no proven secondary therapy for disseminating cutaneous leishmaniasis.

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