



HAL
open science

Healing of old world cutaneous leishmaniasis in travelers treated with fluconazole: Drug effect or spontaneous evolution?

Gloria Morizot, Pascal Delgiudice, Eric Caumes, Emmanuel Laffitte, Pierre Marty, Alain Dupuy, Claudine Sarfati, Smain Hadj-Rabia, Herve Darie, Anne-Sophie Le Guern, et al.

► To cite this version:

Gloria Morizot, Pascal Delgiudice, Eric Caumes, Emmanuel Laffitte, Pierre Marty, et al.. Healing of old world cutaneous leishmaniasis in travelers treated with fluconazole: Drug effect or spontaneous evolution?. *American Journal of Tropical Medicine and Hygiene*, 2007, 76 (1), pp.48-52. 10.4269/ajtmh.2007.76.48 . pasteur-01375238

HAL Id: pasteur-01375238

<https://riip.hal.science/pasteur-01375238v1>

Submitted on 25 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

HEALING OF OLD WORLD CUTANEOUS LEISHMANIASIS IN TRAVELERS TREATED WITH FLUCONAZOLE: DRUG EFFECT OR SPONTANEOUS EVOLUTION?

GLORIA MORIZOT, PASCAL DELGIUDICE, ERIC CAUMES, EMMANUEL LAFFITTE, PIERRE MARTY,
ALAIN DUPUY, CLAUDINE SARFATI, SMAIN HADJ-RABIA, HERVE DARIE, ANNE-SOPHIE LE GUERN,
AFIF BEN SALAH, FRANCINE PRATLONG, JEAN-PIERRE DEDET, MAX GRÖGL, AND PIERRE A. BUFFET*

Pôle de Recherche Biomédicale Centre Médical, Institut Pasteur de Paris, Paris, France; Centre Hospitalier de Fréjus, Fréjus, France; Service des Maladies Infectieuses et Tropicales, Centre Hospitalier Universitaire Pitié-Salpêtrière, Paris, France; Service de Dermatologie et Vénérologie, Centre Hospitalier Universitaire Vaudois, Lausanne Switzerland, Service de Parasitologie, Centre Hospitalier Universitaire de Nice, Nice, France; Service de Dermatologie Centre Hospitalier Universitaire Necker, Paris, France; Service de Dermatologie et Service de Parasitologie, Centre Hospitalier Universitaire Saint Louis, Paris, France; Institut Pasteur, Tunis, Tunisia; Tripler Army Medical Center, Honolulu, Hawaii; Centre National de Référence des Leishmania, Montpellier, France

Abstract. The efficacy of fluconazole was evaluated in 35 travelers with parasitologically proven imported Old World cutaneous leishmaniasis (CL). *Leishmania major* (mainly MON-25) was identified in 15 patients and strongly suspected given the transmission area in 12 of these patients. Daily oral fluconazole (200 mg/day for adults and 2.5 mg/kg/day for children) was prescribed for six weeks. Outcome definition was based on re-epithelialization rate at day 50. Of the 27 *L. major*-infected patients, 12 (44.4%) were cured. This cure rate is similar to the placebo cure rate from trials in *L. major* CL in which, as in the present report, the definition of outcome relied exclusively on re-epithelialization. These data question the assumption that oral fluconazole is consistently effective for treatment of CL caused by *L. major*.

INTRODUCTION

A sharp increase in the worldwide incidence of cutaneous leishmaniasis (CL) has been observed over the last 10 years (from 1 million to 1.5 million new cases per year),¹ which has likely increased the number of imported CL cases throughout Europe. For example, 123 cases of imported or autochthonous CL were reported to the French National Reference Center of *Leishmania* in 2004, which was four times higher than the mean of 30–31 cases per year that had been reported over a two-year period in 1986–1987.² *Leishmania major* from North Africa and south Saharan Africa is more frequently involved with CL than *L. infantum* and *L. tropica* (500/C/ Banque Leishmania/BLR rapport Activité 2001). Most Old World CL lesions heal spontaneously over months to years, and mean time to healing is typically shorter in those caused by *L. major* than those caused by *L. infantum* or *L. tropica*.³

Abstention is a theoretically attractive option; however, it is more easily adopted when writing recommendations than when facing a patient. Many clinicians would favor therapy because lesions are often disfiguring, may affect daily occupation and/or social life when located on the hands, feet, or face, and frequently give rise to atrophic scars. However, reference therapeutic options such as pentavalent antimonial drugs may be more harmful than the disease.⁴ Severe adverse events rarely occur in young patients in clinical trials,^{5,6} but these events may be more frequent in older patients treated without follow-up of laboratory parameters and electrocardiograms.⁷ Thus, search for safe and easily administered therapies is a priority.

In this context, oral fluconazole proved more efficient than placebo in an *L. major* focus in Saudi Arabia.⁸ Because comparative trials in CL caused by *L. major* had previously shown the inefficiency of either intramuscular⁹ or intralesional¹⁰ pentavalent antimonial drugs, oral fluconazole became the best substantiated therapeutic option.¹¹ A standardized fol-

low-up procedure is applied to all patients with CL in our clinics. This provided the framework to assess the efficacy of oral fluconazole in patients returning from different Old World areas with one or several CL lesions.

MATERIALS AND METHODS

Patients. Treatment and follow-up was performed in 2003–2005 at the Infectious Disease Clinic of the Pasteur Institute in Paris and at reference clinics in France and Switzerland. Diagnosis was based on *Leishmania* amastigotes on Diff-Quik (American Scientific Products, McGraw, IL)–stained smears of lesion material obtained by scraping, promastigotes in culture of material obtained by aspiration, or positive polymerase chain reaction (PCR) results. Patients were not treated with fluconazole if there was any evidence of serious underlying disease (cardiac, renal, or pulmonary), acquired immunodeficiency syndrome, disease of the oronasal mucosa, or ongoing or planned pregnancy. Screening laboratory values had to be within normal limits.

Patients received fluconazole at a dosage of 200 mg/day for six weeks for adults and 2.5 mg/kg/day for six weeks for children. Patients of all ages and independent of the number or location of their lesions were prescribed fluconazole. The data of these patients were analyzed whenever they fulfilled the following criteria: CL parasitologically confirmed by smear and/or culture and/or PCR, with objective measurement of the lesions at day 1 and direct objective measurement of the lesions or measurement on dated photographs at day 50 ± 8, and if clinical follow-up over the telephone was performed later than day 90.

The infecting species was either identified or strongly suspected on the basis of epidemiologic criteria. For example, strong suspicion of *L. major* infection meant that patients traveled exclusively to a *L. major* focus according to reference tables (World Health Organization Technical Report on Leishmaniasis Control, 1990) and maps (<http://www.Edisan.fr>). Most patients were born in northern and Sahelian Africa, had lived in Europe, and had spent weeks to months in their city of origin during the *Leishmania* transmission season. Be-

* Address correspondence to Pierre A. Buffet, Institut Pasteur Medical Center, 28 Rue du Docteur Roux, 75724 Paris CEDEX 15, France. E-mail: pabuffet@pasteur.fr

TABLE 1

Characteristics of patients with imported Old World cutaneous leishmaniasis with proven or strongly suspected *Leishmania major* infection

Patient	Age (years)/sex	Country (area) of contamination	No. of lesions	Duration of lesions (months)	Identified species and zymodeme*	Suspected species*	Positive parasitologic test results	Outcome†
1	1/F	Algeria (Msila)	7	3.5	–	<i>L. major</i>	Smear/culture/PCR	CCR
2	1/M	Algeria (Biskra)	10	3.5	<i>L. major</i> MON-25	–	Culture/PCR	Failure
3	5/M	Algeria (Biskra)	1	3.5	<i>L. major</i> MON-25	–	Smear/culture	CCR
4	8/F	Senegal	1	3	–	<i>L. major</i>	Smear	Failure
5	9/F	Mauritania	1	5	–	<i>L. major</i>	Smear	Failure
6	10/F	Mauritania	2	5	<i>L. major</i> MON-74	–	Smear/culture	CCR
7	24/M	Burkina Faso	1	3	–	<i>L. major</i>	Smear	Failure
8	28/M	Morocco (Ouarzazate)	3	5	<i>L. major</i>	–	PCR	CCR
9	30/F	Algeria (Biskra)	1	3.5	<i>L. major</i> MON-25	–	Culture/PCR	Failure
10	34/M	Morocco (Ouarzazate)	2	5	–	<i>L. major</i>	Smear	CCR
11	36/M	Tunisia (Tozeur)	2	6	<i>L. major</i> MON-25	–	Smear/culture	Failure
12	41/F	Tunisia (Bouajza)	4	3	<i>L. major</i> MON-25	–	Smear/culture	CCR
13	48/F	Morocco (Ouarzazate)	20	2	<i>L. major</i> MON-25	–	Smear/culture/PCR	Failure
14	48/F	Burkina Faso	4	4	<i>L. major</i> MON-74	–	Smear/culture	Failure
15	50/M	Tunisia (Kebizi)	4	3	<i>L. major</i> MON-25	–	Smear/culture	CCR
16	52/M	Burkina Faso	7	6	–	<i>L. major</i>	Histopathologic examination	Failure
17	52/M	Burkina Faso	1	3.5	<i>L. major</i> MON-74	–	Smear/culture	CCR
18	53/M	Senegal	2	2	–	<i>L. major</i>	Smear	Failure
19	54/F	Algeria (Msila)	7	7	<i>L. major</i> MON-25	–	Smear/culture	Failure
20	57/M	Algeria (Biskra)	6	2.5	<i>L. major</i> MON-25	–	Smear/culture	CCR
21	60/M	Mali	14	3	–	<i>L. major</i>	Smear/culture	CCR
22	61/F	Algeria (Biskra)	2	2.5	<i>L. major</i> MON-25	–	Smear/culture	Failure
23	62/M	Senegal	7	3	–	<i>L. major</i>	Smear	Failure
24	67/F	Algeria (Msila)	6	3	<i>L. major</i> MON-25	–	Smear/culture/PCR	Failure
25	69/F	Algeria (Batna)	3	5	–	<i>L. major</i>	Smear/culture	CCR
26	69/M	Senegal	1	1	–	<i>L. major</i>	Smear	CCR
27	70/F	Algeria (Biskra)	1	3	–	<i>L. major</i>	Smear	Failure‡

* Results are expressed as species name plus zymodeme (MON-#) for isolates identified by isoenzyme electrophoresis, or as species name only for isolates identified by polymerase chain reaction (PCR) or suspected given the area of transmission.

† CCR = complete clinical response.

‡ This patient received fluconazole for four weeks.

cause *L. major*, *L. infantum*, and *L. tropica* foci rarely overlap in Africa, we suspected more than one potential infecting species only in a few patients (Tables 1 and 2). Outcome was defined as 1) complete clinical response: 100% re-epithelialization of all lesions at day 50 ± 8 or ≥ 50% re-epithelialization of all lesions at day 50 ± 8 with 100% re-epithelialization of all lesions at day 90 ± 8, or 2) clinical failure: < 50% re-epithelialization of at least one lesion at day

50 ± 8 or < 100% re-epithelialization of at least one lesion at day 90 ± 8.

Determination of drug toxicity. At the beginning (day 0), middle (days 14–21), and end of treatment (days 42–55), we checked for symptoms suggesting possible drug side effects, i.e., gastrointestinal, neurologic, cutaneous, and any unusual symptom experienced by the patient since the last consultation. Laboratory tests to monitor liver enzymes (aspartate

TABLE 2

Patients with imported Old World cutaneous leishmaniasis due to *Leishmania infantum*, *L. tropica*, or undetermined species

Patient	Age (years)/sex	Country (area) of contamination	No. of lesions	Duration of lesions (months)	Identified species and zymodeme*	Suspected species*	Positive parasitologic test results	Outcome†
1‡	29/F	Afghanistan	2	16	<i>L. tropica</i>	–	PCR	CCR
2	72/F	Israel	1	5	<i>L. tropica</i> MON-137	–	Smear/culture	Failure
3	2/F	Algeria	1	9	<i>L. infantum</i> MON-24	–	Smear/culture	Failure§
4	34/M	Algeria	3	9	<i>L. infantum</i> MON-24	–	Smear/culture	Failure§
5	48/F	Tunisia	1	6	<i>L. infantum</i> MON-24	–	Smear/culture	Failure§
6	80/F	Algeria	1	5	–	<i>L. infantum</i>	Smear/PCR	Failure
7	25/M	Morocco (several areas)	12	2	–	<i>L. tropica</i> ? <i>L. infantum</i> ? <i>L. major</i> ?	Smear	CCR
8	46/F	Algeria (several areas)	1	5	–	<i>L. major</i> ? <i>L. infantum</i> ?	Smear	CCR§

* Results are expressed as species name plus zymodeme (MON-#) for isolates identified by isoenzyme electrophoresis, or as species name only for isolates identified by polymerase chain reaction (PCR) or suspected given the area of transmission.

† CCR = complete clinical response.

‡ Case report previously reported.¹²

§ Incomplete treatment: 4 weeks (patients 3 and 5), 3 weeks (patient 8), and 10 days (patient 4).

aminotransferase and alanine aminotransferase) were performed prior to and during the third week of therapy.

RESULTS

In 2003–2005, 45 patients were prescribed fluconazole, 24 at the Institut Pasteur in Paris and 21 at other centers. Data from 35 patients were analyzed (Tables 1 and 2). Data from 10 patients were not included in the analysis for the following reasons: one had a purely nodular lesion, one had received previous antimonial drug therapy less than 10 days before taking fluconazole, one did not purchase fluconazole because of cost, and seven did not return for follow-up and/or did have photographs taken at day 50 ± 8, which made objective measurement impossible.

Except for two patients infected with *L. tropica*, four with *L. infantum*, and two for which more than one species could be responsible for the lesion (Table 2), all patient isolates were identified or strongly suspected to be *L. major* (as defined in Materials and Methods). Culture was positive in 18 patients included in the analysis. Twenty isolates could be identified either by isoenzyme typing (18 isolates) or PCR (2 isolates). Fifteen isolates were identified as *L. major* (11 MON 25, 3 MON 74, and 1 unknown; Table 1), three as *L. infantum* MON 24, and two as *L. tropica* (Table 2). Efforts to contact patients who did not comply with the usual follow-up schedule were unsuccessful.

Of the 27 *L. major*-infected patients, 12 (44.4%) were cured (Table 1 and Figure 1). Of eight patients infected with non-*L. major* or undetermined species (two *L. tropica*, four *L. infantum*, and two undetermined), three were cured. However, only four patients in this second group had completed a full course of therapy (Table 2). Two patients complained of nausea and abdominal pain and one patient reported a mild and clinically insignificant increase in the level of aspartate aminotransferase (59 U/L, normal = 14–50 U/L). In one patient, treatment was interrupted because of toxicity (severe nausea). This patient was lost for follow-up and was not included in the efficacy analysis.

DISCUSSION

Oral fluconazole administered to Saudi patients infected with *L. major* MON-26⁸ was associated with a cure rate of 36% at seven weeks (day 9) compared with 10% in placebo-treated patients. At 13 weeks (day 91), results of fluconazole were still superior to those of placebo (88% versus 66%, percentages from Figure 1 in the report by Alraghi and others⁸). Compared with previous reports, those cure rates are low. The placebo cure rate in this Saudi trial (6% at day 42 and 10% at day 49)⁸ is the lowest reported for treatment of CL caused by *L. major*.^{8,9,13–18} Other studies performed in *L. major* foci reported cure rates in the placebo group at days 45–50 of 17%,¹⁸ 18–32%,¹³ 22.8%,¹⁴ 44%,¹⁵ 44.3%,¹⁶ 53%,¹⁷ and 55%.⁹ We recently conducted a phase II study in *L. major* (mainly MON-25)-infected patients that included a placebo group, in which the day 50 cure rate was 71%.

In our group of 27 fluconazole-treated patients with proven or suspected *L. major* MON-25 or MON-74 CL acquired in northern or Sahelian Africa, and with an evaluable outcome,

the cure rate at day 50 was 44.4% (Table 1 and Figure 1). Although this cure rate is similar to the fluconazole cure rate of 36% at day 49 in Saudi Arabia, it is similar to the 44% and 44.3% placebo cure rates at days 45–50 observed in trials using a re-epithelialization-based criterion. We find it more relevant to compare our results with those analyzed according to an equivalent criterion.^{15,16}

Among factors that may explain the wide range of spontaneous cure rates in the previously published trials, the criterion used to determine lesion outcome is probably an important one. Objective criteria based exclusively on ulceration size (or its antonym, the percentage of re-epithelialization),^{15,16} were associated with higher cure rates than subjective criteria taking in account more than one characteristic of lesions,^{8,18} an observation consistent with the fact that the disappearance of the induration or inflammation of a lesion usually occurs several weeks after its re-epithelialization.¹⁶ In one trial, induration size was explicitly included in the outcome definition.¹⁸ The placebo cure rate was 17%.¹⁸ Cure in the Saudi trial was defined as complete healing of all lesions.⁸ Complete healing was not further defined but likely took into account not only re-epithelialization but also lesion induration, color, or both. Thus, a lesion that would have been reported as still active during the trial performed in Saudi Arabia might have been considered cured in other studies, including the present report. If interpreted following the criterion used in Saudi Arabia, the cure rate in our fluconazole-treated patients group would have been lower (because outcome was not objectively defined by Alrajhi and others⁸ we could not use their criterion). Seven patients were excluded from the analysis because they were lost to follow-up. If we assume that all seven excluded patients were cured, the cure rate is 55.9%; if all seven patients were not cured, the cure rate is 35.3%. Both values are still close to placebo cure rates from several previous trials. Our patients presented later in the evolution than patients in Saudi Arabia (3.7 versus 1.9 months, Table 1 and Alrajhi and others⁸), thus increasing the likelihood of spontaneous healing in our cohort. These observations favor the hypothesis that spontaneous evolution was the predominant factor of lesion healing in the present report.

The *L. major* species is not homogenous. At least 16 *L. major* zymodemes (MON 3, 4, 5, 6, 7, 21, 23, 25, 26, 39, 64, 65, 66, 67, 68, and 74) with different geographic distributions have been isolated from humans (500/C/BanqueLeishmania/BLRappportActivité2001).^{19,20} The MON-25 zymodeme is the only *L. major* zymodeme reported in north African countries (Morocco, Algeria, Tunisia, and Libya). Most if not all patients included in the Saudi trial were infected with *L. major* MON-26,⁸ the only *L. major* zymodeme reported from the Middle East. In sub-Saharan Africa the most frequent zymodeme is MON-74, but MON-117, MON-196, and MON-26 have also been reported. Both *L. major* MON-74 and MON-26 circulate in Egypt. Zymodemes might be associated with distinctive and unique natural evolutions. Also, in CL, the infecting species has a proven influence on therapeutic outcome.²¹ No report on the influence of zymodemes is available, but discrepancies on the efficacy of meglumine antimoniate and allopurinol on CL caused by *L. panamensis* in Colombia have been reported.^{22–24} Although these data are from New World foci, they support the hypothesis that zymodeme-dependent drug efficacy might induce cure rate dis-

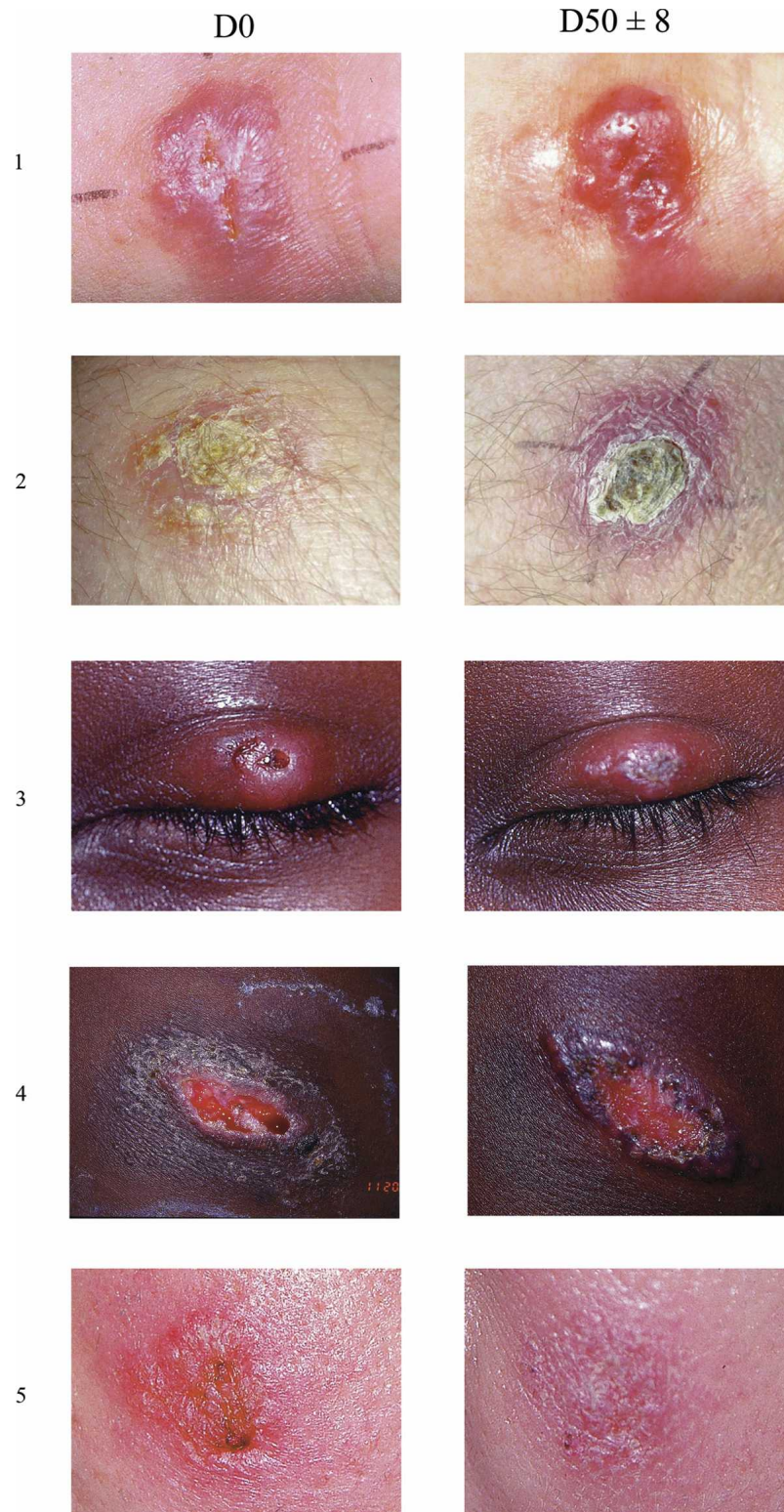


FIGURE 1. Representative samples of failure (1–4) and complete clinical response (5) as comparatively determined from lesion aspect at baseline (D0) and at day 50 ± 8 days (D50 ± 8, i.e., 30 ± 8 days after the end of a 20-day treatment course with oral fluconazole, 2.5 mg/kg/day for six weeks). This figure appears in color at www.ajtmh.org.

crepancies between patients infected with the same species but who were infected in different geographic areas (e.g., Middle East versus Africa). Clinical trials including a large proportion of *L. major* MON 25-infected patients are necessary.

Although our data were not generated by a formal clinical trial, they deserve clinical attention. These data question the widespread assumption, due to the prominence of the previous report from Saudi Arabia,⁸ that oral fluconazole is consistently effective for treatment of CL caused by *L. major*.

Even for patients in northern countries, a six-week course of fluconazole is expensive.²⁵ Almost 15% of our patients could not afford a full course of fluconazole because of the high cost (at least 600 Euros for a full course in France). Definitive evidence is required before recommending fluconazole in the treatment of imported Old World CL.

Received February 3, 2006. Accepted for publication August 23, 2006.

Authors' addresses: Gloria Morizot, Herve Darie, Anne-Sophie Le Guern, and Pierre A. Buffet, Pôle de Recherche Biomédicale Centre Médical, Institut Pasteur de Paris, Paris, France. Pascal Delgiudice, Centre Hospitalier de Fréjus, Fréjus, France. Eric Caumes, Alain Dupuy, Claudine Sarfati, and Smain Hadj-Rabia, Assistance-Publique Hôpitaux de Paris, Paris, France. Emmanuel Laffitte, Service de Dermatologie et Vénérologie, Centre Hospitalier Universitaire Vaudois, Lausanne Switzerland. Pierre Marty, Service de Parasitologie; Centre Hospitalier Universitaire de Nice, Nice, France. Afif Ben Salah, Institut Pasteur, Tunis, Tunisia. Francine Pratlong and Jean-Pierre Dedet, Centre National de Référence des *Leishmania*, Montpellier, France. Max Grögl, Tripler Army Medical Center, Honolulu, HI 96859.

REFERENCES

- Blum J, Desjeux P, Schwartz E, Beck B, Hatz C, 2004. Treatment of cutaneous leishmaniasis among travellers. *J Antimicrob Chemother* 53: 158–166.
- Jeannel D, Tuppin P, Brucker G, Danis M, Gentilini M, 1989. Leishmaniasis in France. *Lancet* 2: 804.
- Bryceson A, 1987. Therapy in man. Peters W, Killick-Kendrick R, eds. *The Leishmaniasis*. London: Elsevier, 847–869.
- Masmoudi A, Maalej N, Mseddi M, Souissi A, Turki H, Boudaya S, Bouassida S, Zahaf A, 2005. Glucantime injection: benefit versus toxicity. *Med Mal Infect* 35: 42–45.
- Herwaldt BL, Berman JD, 1992. Recommendations for treating leishmaniasis with sodium stibogluconate (Pentostam) and review of pertinent clinical studies. *Am J Trop Med Hyg* 46: 296–306.
- Berman JD, 1997. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. *Clin Infect Dis* 24: 684–703.
- Sampaio RN, Martins Netto E, Faria E, Sampaio J, de Freitas L, Marsden P, 1988. Sudden death caused by glucantime. *An Bras Dermatol* 63: 35–37.
- Alrajhi AA, Ibrahim EA, De Vol EB, Khairat M, Faris RM, Maguire JH, 2002. Fluconazole for the treatment of cutaneous leishmaniasis caused by *Leishmania major*. *N Engl J Med* 346: 891–895.
- Belazzoug S, Neal RA, 1986. Failure of meglumine antimoniate to cure cutaneous lesions due to *Leishmania major* in Algeria. *Trans R Soc Trop Med Hyg* 80: 670–671.
- Chahed MK, Ben Salah A, Louzir H, Marrakchi H, Zaatour A, Ftaiti A, Ben Chaabane B, Sidhom M, Dellagi K, Ben Ismail R, 1999. Efficacy of intra-lesional glucantime in the treatment of zoonotic cutaneous leishmaniasis in basic health care conditions. *Arch Inst Pasteur Tunis* 76: 13–18.
- Buffet PA, Morizot G, 2003. Cutaneous leishmaniasis in France: towards the end of injectable therapy? *Bull Soc Pathol Exot* 96: 383–388.
- Laffitte E, Genton B, Panizzon RG, 2005. Cutaneous leishmaniasis caused by *Leishmania tropica*: treatment with oral fluconazole. *Dermatology* 210: 249–251.
- Ben Salah A, Zakraoui H, Zaatour A, Ftaiti A, Zaafouri B, Garraoui A, Olliaro PL, Dellagi K, Ben Ismail R, 1995. A randomized, placebo-controlled trial in Tunisia treating cutaneous leishmaniasis with paromomycin ointment. *Am J Trop Med Hyg* 53: 162–166.
- Momeni AZ, Aminjavaheri M, Omidghaemi MR, 2003. Treatment of cutaneous leishmaniasis with ketoconazole cream. *J Dermatolog Treat* 14: 26–29.
- Asilian A, Jalayer T, Whitworth JA, Ghasemi RL, Nilforooshzadeh M, Olliaro P, 1995. A randomized, placebo-controlled trial of a two-week regimen of aminosidine (paromomycin) ointment for treatment of cutaneous leishmaniasis in Iran. *Am J Trop Med Hyg* 53: 648–651.
- Momeni AZ, Jalayer T, Emamjomeh M, Bashardost N, Ghassemi RL, Meghdadi M, Javadi A, Aminjavaheri M, 1996. Treatment of cutaneous leishmaniasis with itraconazole. Randomized double-blind study. *Arch Dermatol* 132: 784–786.
- el-Safi SH, Murphy AG, Bryceson AD, Neal RA, 1990. A double-blind clinical trial of the treatment of cutaneous leishmaniasis with paromomycin ointment. *Trans R Soc Trop Med Hyg* 84: 690–691.
- Iraji F, Sadeghinia A, 2005. Efficacy of paromomycin ointment in the treatment of cutaneous leishmaniasis: results of a double-blind, randomized trial in Isfahan, Iran. *Ann Trop Med Parasitol* 99: 3–9.
- Lanotte G, Rioux JA, Maazoun R, Pasteur N, Pratlong F, Lepart J, 1981. Significance of enzymatic polymorphism in *Leishmania*: on three heterozygous stocks of *Leishmania infantum* Nicolle, 1908, *Leishmania cf. tarentolae* Wenyon, 1921 and *Leishmania aethiopica* Bray, Ashford and Bray, 1973. *Ann Parasitol Hum Comp* 56: 575–591.
- Lanotte G, Rioux JA, Lepart J, Maazoun R, Pasteur N, Pratlong F, 1984. Numerical cladistics of the phylogeny of the genus *Leishmania* Ross, 1903 (Kinetoplastida-Trypanosomatidae). Use of enzyme characteristics. *C R Acad Sci III* 299: 769–772.
- Navin TR, Arana BA, Arana FE, Berman JD, Chajon JF, 1992. Placebo-controlled clinical trial of sodium stibogluconate (Pentostam) versus ketoconazole for treating cutaneous leishmaniasis in Guatemala. *J Infect Dis* 165: 528–534.
- Martinez S, Marr JJ, 1992. Allopurinol in the treatment of American cutaneous leishmaniasis. *N Engl J Med* 326: 741–744.
- Velez I, Agudelo S, Hendrickx E, Puerta J, Grogl M, Modabber F, Berman J, 1997. Inefficacy of allopurinol as monotherapy for Colombian cutaneous leishmaniasis. A randomized, controlled trial. *Ann Intern Med* 126: 232–236.
- Martinez S, Gonzalez M, Vernaza ME, 1997. Treatment of cutaneous leishmaniasis with allopurinol and stibogluconate. *Clin Infect Dis* 24: 165–169.
- Zvulunov A, Klaus S, Vardy D, 2002. Fluconazole for the treatment of cutaneous leishmaniasis. *N Engl J Med* 347: 370–371.