Efficacy of Ketoconazole Against *Leishmania braziliensis panamensis* Cutaneous Leishmaniasis

ROLANDO E. SAENZ, M.D., HECTOR PAZ, M.D., *Panama City, Panama,* JONATHAN D. BERMAN, M.D., Ph.D., *Washington, D.C.*

PURPOSE, PATIENTS, AND METHODS: The classic agent for cutaneous leishmaniasis is pentavalent antimony. However, there are no reports of the efficacy of antimony versus placebo or of the efficacy of any alternative therapy versus either antimony or placebo. In the present report, the oral antifungal agent ketoconazole (600 mg/day for 28 days) was compared to a recommended regimen of intramuscular Pentostam (20 mg antimony/kg, with a maximum of 850 mg antimony/day, for 20 days) in a randomized study of the treatment of Panamanian cutaneous leishmaniasis due to *Leishmania braziliensis panamensis*. A separate group of patients with this disease was administered placebo.

RESULTS: Ketoconazole clinically cured 16 of 21 (76%) patients. The lesions on nine patients healed by 1 month after therapy, and the lesions healed by 3 months after therapy on the other seven patients. Side effects were limited to a 27% incidence of mild, reversible hepatocellular enzyme elevation and an asymptomatic, reversible, approximately 70% decrease in serum testosterone in all patients. Pentostam cured 13 of 19 (68%) patients; the lesions on seven patients healed by the end of therapy, and the lesions on four other patients healed by 1 month after the end of therapy. Side effects were a 47% incidence of mild, reversible hepatocellular enzyme elevation and the morbidity due to 20 intramuscular injections in almost all patients. The placebo group of 11 patients had a 0% cure rate. By 1 month after therapy, all placebo-treated patients demonstrated new lesions or one lesion that was 23% to 875% larger than before therapy.

CONCLUSION: Both ketoconazole and Pentostam were more effective than placebo against *L. braziliensis panamensis* cutaneous leishmaniasis. Oral ketoconazole is comparable in efficacy to this parenteral Pentostam regimen and can be recommended as initial treatment for this disease.

From Gorgas Memorial Institute, Panama City, Republic of Panama, and Walter Reed Army Institute of Research, Washington, D.C.

This study was approved by the Human Use Committee at the Gorgas Memorial Institute and by the Human Subjects Review Board, and by the United States Food and Drug Administration. Informed consent was obtained from all subjects in the study.

Requests for reprints should be addressed to Colonel Jonathan D. Berman, M.D., Ph.D., Walter Reed Research Army Hospital, Office of Plans, Room 1091, Washington, D.C. 20307-5100.

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The leishmaniases are initiated by inoculation of *Leishmania* species into the skin during sandfly bites. Multiplication of organisms at the bite site or at metastatic visceral sites results in cutaneous or visceral disease, respectively. Cutaneous disease typically presents as a papule that enlarges over weeks to months to form a shallow ulcer with raised red margins, and that is thought (without definitive data) to ultimately self-heal with scarring in months to years. In cutaneous disease due to *Leishmania braziliensis* species, the ulcers may weep and the disease may metastasize to the proximal skin or, rarely, to the oronasal mucosa (mucosal leishmaniasis). Although cutaneous leishmaniasis results in morbidity rather than in mortality, such patients are generally treated because of the local morbidity and the possibility of metastasis.

All forms of the disease have been classically treated with pentavalent antimony in the form of Pentostam® (sodium stibogluconate) or Glucantime® (meglumine antimonate) [1]. Antimony treatment failures are generally treated with more antimony, with pentamidine, or with amphotericin B. Because these agents are parenteral, potentially or frankly toxic, and generally administered in a closely supervised or hospitalized setting, there has been recent interest in orally administrable antileishmanial agents. Orally active agents would be particularly appropriate for patients with cutaneous leishmaniasis who at present are being closely supervised and kept from normal duties not because of their disease but because of their treatment.

Ketoconazole is an orally administrable agent developed both as a putative replacement for amphotericin B against deep mycoses and as an agent for superficial mycoses. Amphotericin B intercalates with the major fungal membrane sterol, ergosterol, and thereby alters membrane integrity [2]. Ketoconazole inhibits sterol demethylation that results in the biosynthesis of ergosterol and in this way alters membrane composition [3]. The therapeutic index of ketoconazole results from the fact that ketoconazole is a poor inhibitor of demethylation in the biosynthesis of the major mammalian membrane sterol, cholesterol [4]. However, at an achievable serum level (5 μg/mL), ketoconazole does inhibit the further conversion of cholesterol to androgenic sterols, and one side effect of ketoconazole treatment is decreased testosterone levels [5]. Leishmania demethylated sterols are structurally similar to ergosterol [6,7]. In preclinical *in vitro* studies, achievable serum levels of ketoconazole were shown to inhibit sterol biosynthesis and viability of leishmania [8]. In clinical work, ketoconazole was reported to cure cutaneous leishmaniasis acquired in Nicaragua [9], Israel [10,11], Algeria [12], Saudi Arabia, and Ethiopia [13];

to cure approximately half the cases in Belize [14]; and to cure a low percentage of cases in French Guiana [15]. However, these reports are all preliminary in that there were no positive (antimony) or negative (no-treatment or placebo) controls.

We performed a randomized study to compare oral ketoconazole to intramuscular Pentostam in the treatment of Panamanian cutaneous leishmaniasis due to *Leishmania braziliensis panamensis*. In addition, we separately determined the natural cure rate over 2 months in this disease.

PATIENTS AND METHODS

First Study: Ketoconazole Versus Pentostam

PATIENTS: Panamanians with cutaneous lesions clinically diagnosed as leishmaniasis and who gave informed consent were potentially eligible for the study. Patients were initially examined between March 1986 and March 1988. Patients were then included in the study if leishmania organisms were cultured from lesion material or seen in smears of lesion material. Patients were excluded if they had facial or mucosal lesions, significant concomitant disease of any organ, or abnormalities on subsequent baseline tests (complete blood count; determination of serum levels of glucose, glutamic oxaloacetic transaminase [SGOT], glutamic pyruvic transaminase [SGPT], bilirubin, alkaline phosphatase, urea nitrogen, creatinine, cholesterol, and calcium; electrocardiogram; chest radiograph).

TREATMENT COURSE: After inclusion into the study, patients were admitted to the Santo Tomas Hospital and randomized into two groups: ketoconazole (three 200-mg tablets, a total of 600 mg, before sleep each day for 28 days) or Pentostam (20 mg antimony [Sb]/kg/day with a maximum of 850 mg Sb/day, intramuscularly for 20 days). Randomization was accomplished by card drawing. Since the patients averaged 64 kg in weight, the patients received on the average 13 mg Sb/kg/day. During treatment, patients were asked daily for symptomatic complaints, and blood was drawn weekly for complete blood counts and serum chemistries. In addition, blood was drawn before, during, and after treatment for testosterone levels, and during treatment for ketoconazole levels.

MEASUREMENT OF LESION SIZE: The long and short axes of each lesion were measured to the nearest mm by one observer. The lesion was then assumed to be rectangular, and the area of the lesion was calculated by multiplying the lengths of the two axes.

FOLLOW-UP: Patients were seen in clinic 1, 2, 3, 6, and 12 months after the end of treatment. At these times, any nonhealed lesion was measured.

PARASITOLOGIC DIAGNOSIS: Lesions were examined for the presence of leishmania upon entry into the study, on the last day of treatment, and, if appropriate, at follow-up. Lesions were scraped or underwent biopsy. Scraped and biopsy materials were stained with Giemsa's stain and examined for amastigotes, and also were inoculated onto modified Senekji's [16] medium for culture. Demonstration either of leishmania amastigotes in stained material or of promastigotes in cultured material constituted a diagnosis of leishmaniasis. The technician initially evaluating the specimens was unaware of the clinical status of the patient.

Cultured parasites were typed by isoenzyme analysis [17] to determine the species and subspecies.

DEFINITION OF LESION HEALING, FAILURE, AND RELAPSE: A lesion was defined to be healed if it had undergone complete re-epithelialization. A lesion was definitively healed if it had not clinically relapsed by the 12-month follow-up examination. A lesion was defined to have failed therapy if it had diminished by less than 25% by the 1-month follow-up examination (i.e., was greater than 75% of its original size by 1 month after the end of the approximately 1-month period of treatment). A lesion was defined to have relapsed if it underwent a 100% enlargement after initial diminution or if a new lesion appeared adjacent to the original lesion.

A person was defined to be cured of leishmaniasis if all lesions had undergone definitive healing. Therapy was defined as having failed in a person if any lesion failed to respond to therapy or relapsed.

Although the clinician who initially judged the clinical status of the lesion was aware of the patient's treatment group, photographs of the lesions provided objective evidence of clinical status.

SPECIAL ANALYSES: Serum ketoconazole levels were determined by bioassay against a susceptible fungus by Dr. M. Rinaldi (University of Texas, San Antonio, Texas). Serum testosterone levels were determined by radioimmunoassay by Roche Biomedical Laboratory, Raritan, New Jersey.

Second Study: Placebo

Patients were initially examined between April 1988 and January 1989, whereas patients in the study comparing Pentostam to ketoconazole were first seen between March 1986 and March 1988. Scrapings from lesions clinically diagnosed as leishmaniasis were examined for the presence of leishmanial organisms by Giemsa's stain and by culture. If parasites were found and if the history and physical examination revealed no concomitant medical problems, the patient was entered into the placebo study. Placebo-treated patients were not administered blood or other laboratory tests because their treatment would not require that such parameters be followed. Patients were given placebo tablets simulating ketoconazole (three tablets each night for 28 days). The size of the lesions was measured at the beginning and end of the placebo treatment period, and at follow-up 1 month later.

RESULTS

First Study: Ketoconazole Versus Placebo

PATIENT CHARACTERISTICS: *Number of patients:* Twenty-two patients were entered into the ketoconazole group, and 19 patients were randomized into the Pentostam group. The reason for the discrepancy in patient numbers between the two groups was that Pentostam was transiently unavailable, and three patients (ketoconazole-treated Patients 8, 9, and 10) could not be randomized. All patients were male.

Patient ages and weights: For the ketoconazole group, the age range was 16 to 48 years; the mean age was 25 years and 18 of 22 (82%) were between 16 and 30 years of age. For the Pentostam group, the age range was 17 to 67 years; the mean age was 34 years and 11 of 19 (58%) patients were 16 to 30 years of age. The weight range for the ketoconazole group was 36 to 74 kg (mean ± standard deviation (SD), 59 ± 7.5 kg). The weight range for the Pentostam group was 41 to 77 kg (64 ± 8.5 kg).

TABLE I

Response to Therapy in Patients Receiving Ketoconazole

Patient Number	Lesion Number	Site	Duration (weeks before therapy)	Size Before Therapy (mm^2)	Size After Therapy (% pretherapy size)	Size 1M After Therapy (% pretherapy size)	Size 2M After Therapy (% pretherapy size)	Results of Therapy*
Cured								
1	1	Right hand	8	315	165	11	0	Heal 2M
2	1	Left leg	4	1,764	48	31	4	Heal 3M
3	1	Right arm	10	380	63	17	0	Heal 2M
	2	Right arm		160	0	0	0	
	3	Left knee		150	0	0	0	
4	1	Right wrist	10	594	100	26	14	Heal 3M
5	1	Right leg	8	340	0	0	0	Heal Rx
	2	Left arm		2	0	0	0	
6	1	Right back	8	140	0	0	0	Heal Rx
7	1	Abdomen	8	414	0	0	0	Heal 2M
	2	Left leg		480	17	21	0	
11	1	Left leg	7	425	43	6	0	Heal 2M
12	1	Right arm	8	400	8	0	0	Heal 1M
	2	Right arm		63	0	0	0	
14	1	Right hand	8	108	222	0	0	Heal 1M
	2			160	19	0	0	
15	1	Right arm	10	130	100	0	0	Heal 1M
16	1	Left leg	12	375	6	0	0	Heal 1M
17	1	Right thigh	6	345	0	NA	0	Heal Rx
20	1	Left arm	7	510	0	0	0	Heal Rx
21	1	Right leg	4	252	16	40	1	Heal 3M
	2	Left elbow		20	0	0	0	
22	1	Left leg	20	1,000	0	0	0	Heal Rx
Therapy failed								
8	1	Right arm	8	391	53	0		Relapse 2M
	2	Right arm		323	124	0		
9	1	Right arm	12	150	187	181		Fail 1M
	2	Right arm		570	43	100		
10	1	Right arm	4	42	320	750		Fail 1M
	2	Right arm		260	192	290		
13	1	Right wrist	6	153	78	105		Fail 1M
18	1	Right arm	4	165	33	30		Relapse 2M
	2	Right arm		126	0	0		
19	1	Left forearm	8	480	78	78		Fail 1M
	2	Right forearm		252	71	71		
	3	Right forearm		72	0	0		

M = month(s); NA = not available.
* Results of therapy signify whether the lesion healed at the end of therapy (heal Rx) or at a specified month after therapy, or failed to heal or relapsed at a specified month.

LESION SITES, DURATION, AND SIZE PRIOR TO THERAPY: The lesion sites for the ketoconazole and Pentostam groups are listed in **Tables I** and **II.** There was a mean of 2.1 lesions per ketoconazole-treated patient, and 23 of the 35 lesions (66%) were on the upper extremities. There was a mean of 2.6 lesions per Pentostam-treated patient, with 25 of 49 lesions (51%) being on the upper extremities. Multiple (greater than 2) lesions frequently consisted of a group of lesions in the same anatomic area.

The duration of at least one lesion as stated by the patient is also shown in Tables I and II. For the ketoconazole group, there was a mean ± SD duration of disease of 8.2 ± 3.5 weeks; for the Pentostam group, there was a duration of 12.5 ± 3.0 weeks.

The mean ± SD area of the lesions on ketoconazole-treated patients was 333 ± 319 mm^2. The size of the lesions on Pentostam-treated patients was 350 ± 470 mm^2.

PARASITOLOGIC DIAGNOSIS: Scrapings and biopsies were performed on at least one lesion prior to treatment. The results of culturing the scraping and the biopsy specimen, of Giemsa-staining the scraping, and of Giemsa-staining the biopsy specimen are shown in **Tables III** and **IV.** For the ketoconazole group, all three methods of diagnosis were positive for leishmania in 13 of the 22 patients (59%); two tests were positive in four patients (18%); and one test was positive in five cases (23%). For the 19 Pentostam-treated patients, all three tests were positive in 13 patients (68%); two tests were positive in three cases (16%); one test was positive in two cases (10%); and in one case (5%) no test was positive. This patient (10) was nevertheless treated on clinical grounds and rapidly improved after chemotherapy, and is therefore included in the analyses as a patient who had cutaneous leishmaniasis.

When parasites cultured from ketoconazole-treated patients were speciated, 16 isolates were *L. braziliensis panamensis* and one isolate (from Patient 8) was *Leishmania mexicana.* All 14 isolates cultured from Pentostam-treated patients were *L. braziliensis panamensis.*

CLINICAL AND PARASITOLOGIC RESPONSE TO ANTILEISHMANIAL THERAPY: *Ketoconazole group:* The clinical response of the lesions to ketoconazole therapy is listed in Table I. For each patient (column 1) and lesion (column 2), the area of the lesion prior to treatment (column 5), the area of the lesion expressed as a percent of the original lesion after the approximately 1-month period of therapy (column 6), and the area of

TABLE II

Response to Therapy in Patients Receiving Pentostam

Patient Number	Lesion Number	Site	Duration (weeks before therapy)	Size Before Therapy (mm^2)	Size After Therapy (% pretherapy size)	Size 1M After Therapy (% pretherapy size)	Results of Therapy*
Cured							
1	1	Upper back	10	420	0	0	Heal Rx
4	1	Lower back	24	2	0	0	Heal Rx
	2	Lower back		96	0	0	
	3	Lower back		24	0	0	
6	1	Left elbow	6	234	0	0	Heal 1M
	2	Right ankle		304	43	0	
	3	Left hand		42	0	0	
	4	Neck		12	0	0	
8	1	Thorax	8	15	0	0	Heal Rx
	2	Jaw		12	0	0	
	3	Wrist		70	0	0	
	4	Clavicle		49	0	0	
	5	Axilla		20	0	0	
9	1	Left arm	6	500	0	0	Heal Rx
	2	Left arm		357	0	0	
	3	Left arm		525	0	0	
	4	Left arm		94	0	0	
10	1	Left arm	20	600	0	0	Heal Rx
	2	Left arm		4	0	0	
	3	Left thorax		168	0	0	
	4	Left finger		18	0	0	
11	1	Left arm	40	506	0	0	Heal Rx
12	1	Right arm	12	6	0	0	
	2	Right arm		12	0	0	
	3	Right arm		980	41	6	Heal 4M
13	1	Right arm	8	609	0	0	Heal 1M
	2	Right arm		99	35	0	
14	1	Left shoulder	20	357	0	0	Heal Rx
15	1	Right hand	12	1,140	4	0	Heal 1M
16	1	Left arm	6	54	55	0	Heal 1M
	2	Right arm		204	0	0	
18	1	Left leg	12	1,900	17	11	Heal 2M
Therapy failed							
2	1	Left leg	20	1,944	49	0	Relapse 3M
3	1–6	Left leg	4	Each ~48	4	0	Relapse 2M
5	1	Right arm	4	315	0	0	Relapse 2M
	2	Right arm		504	0	0	
	3	Right arm		308	0	0	
	4	Right arm		440	0	0	
7	1	Back	12	1,575	0	0	Relapse 8M
	2	Back		30	0	0	
	3	Back		42	0	0	
	4	Back		6	0	0	
17	1	Right arm	6	968	0	0	Relapse 2M
19	1	Right arm	8	500	22	0	Relapse 3M

M = month(s).

* Results of therapy signify whether the lesion healed at the end of therapy (heal Rx) or at a specified month after therapy, or relapsed at a specified month.

the lesion as a percent of original area at the 1-month and 2-month posttreatment follow-up periods (columns 7 and 8) are shown. The final result of therapy is also shown (column 9).

For the ketoconazole group, 16 of 22 patients (73%) were cured in the sense that all lesions underwent complete re-epithelialization by 3 months after the end of therapy and none relapsed by 12 months after the end of therapy. Clinical healing of all lesions by the end of the 1-month period of therapy occurred in only five of the 16 ultimately cured patients (31%). Lesions on four patients (25%) completely re-epithelialized at 1 month after the end of therapy; lesions on four patients (25%) healed by 2 months after the end of therapy; lesions on three patients (17%) first showed clinical healing 3 months after therapy.

There were six patients (27%) in the ketoconazole group in whom therapy was judged to have failed. (One of these six was the only patient infected with *L. mexicana*, Patient 8). Four of the ketoconazole failures (in Patients 9, 10, 13, and 19) were treatment failures by the criterion of less than 25% decrease in the size of the lesion by 1 month after the end of therapy. One lesion in each of these four patients was 181%, 750%, 105%, and 78%, respectively, of the pretherapy size. For two other patients (8 and 18), lesions that healed after therapy relapsed in the sense that multiple 1- to 4-mm diameter nodules were found to surround the scar of the previous ulcer 2 months after the end of therapy.

Change in the size of lesions at the end of therapy was not well correlated with eventual healing. The lesions on Patients 1, 4, 14, and 15 were as large or larger at the end of therapy than prior to therapy. By 1

TABLE III

Adverse Reactions and Parasitologic Data in Ketoconazole-Treated Patients

Patient Number	Adverse Reactions*	Parasitologic Data† Before Therapy (cult/sp/bx)	Parasitologic Data† After Therapy (cult/sp/bx)
Cured			
1	SGOT = 48 (W4) SGOT = 35 (M1)	−/+/−	−/−/−
2	None	−/−/+	+/−/−
3	SGPT = 42 (W3) SGPT = 16 (M1)	+/+/+	−/−/−
4	None	−/+/+	−/−/−
5	SGOT = 72 (W3) SGOT = 20 (W4)	+/+/−	−/−/−
6	SGOT = 55 (W3) SGOT = 34 (W4)	+/+/+	−/−/ND
7	SGOT = 57 (W4) SGOT = 19 (M2)	+/+/+	ND/−/−
11	None	+/−/−	+/−/+
12	None	+/−/−	−/−/−
14	None	+/+/+	−/−/−
15	None	+/+/+	−/+/−
16	None	+/+/+	−/−/ND
17	None	+/+/+	−/+/ND
20	None	+/+/+	−/+/ND
21	None	+/+/−	+/+/ND
22	SGPT = 77 (W1) SGPT = 9 (W2)	+/−/−	−/+/ND
Therapy failed			
8	None	+/+/+	+/+/+
9	None	+/+/+	+/+/+
10	None	+/+/+	+/+/−
13	None	+/+/+	+/+/+
18	None	+/+/+	+/+/ND
19	None	−/+/+	−/+/+

* For adverse reactions, the abnormal SGOT or SGPT value (in IU/L) and the week (W) at which the abnormality occurred are shown, followed by a subsequent normal value and the week or follow-up month (M) at which value normalized. None = no abnormal laboratory value.
† Parasitologic data refer to presence (+) or absence (−) of leishmanial organisms in culture (cult) of either lesion scraping or biopsy specimen, in stained scraping (sp), or in stained biopsy specimen (bx). ND = not determined.

month after therapy, however, these lesions were 11%, 26%, 0%, and 0%, respectively, of the pretreatment size, and all these lesions later demonstrated definitive healing. On the other hand, the lesions on Patients 13 and 19 were smaller at the end of therapy than prior to therapy, yet these lesions did not continue to diminish in size and became therapeutic failures.

The pretreatment size of lesions that healed was slightly larger (371 ± 376 mm^2) than that of lesions that failed to heal (249 ± 164 mm^2), but the difference was not significant ($p > 0.5$) because of the wide range of lesion size. The pretreatment duration of lesions that healed was 8.6 ± 3.7 weeks, a number slightly but insignificantly ($p > 0.3$) greater than the pretreatment duration of lesions that failed to heal (7 ± 3.0 weeks).

The parasitologic response of the lesions at the end of ketoconazole therapy is shown in Table III. For the 16 patients who were cured by the end of therapy or who were eventually cured, lesions were parasitologically sterile in all attempted tests for only nine (56%) patients at the end of therapy. The presence of one or two positive diagnostic test results out of three at the end of therapy thus did not predict that a therapeutic failure would ensue. On the other hand, results of all attempted tests for parasites were positive at the end of therapy for four of the six patients in whom therapy failed. In our study, a therapeutic failure ultimately resulted in four of the five times when all post-ketoconazole parasitologic tests were positive.

The highest serum ketoconazole level in the patients who were cured (7.9 ± 3.1 μg/mL) was insignificantly greater ($p > 0.4$) than the level in patients who were not cured (6.7 ± 2.1 μg/mL).

Pentostam group: The clinical response to Pentostam therapy is shown in Table II. Thirteen of 19 patients (68%) were cured. Of the 13 cures, seven demonstrated complete re-epithelialization of lesions by the end of the approximately 1 month of therapy, and the size of the unhealed lesions in the other six patients was 4% to 55% (mean = 30%) of the initial lesion size. There were no lesions in which therapy failed. Instead, the six patients who were not cured had relapses of lesions that had clinically healed by the end of therapy (Patients 5, 7, and 17) or were much improved by the end of therapy (Patients 2, 3, and 19). Relapses occurred by 2 to 3 months after the end of therapy, except for Patient 7, and were characterized by 1- to 4-mm diameter nodules surrounding the scar of the previous ulcer.

The mean pretreatment size of those lesions that healed (295 ± 413 mm^2) was smaller than the mean pretreatment size of lesions that relapsed (702 ± 700 mm^2), but because of the large standard deviations the difference was statistically significant only with $0.1 > p < 0.2$. The pretreatment duration of lesions that healed (14 ± 9.7 weeks) was insignificantly larger (p

TABLE IV

Adverse Reactions and Parasitologic Data in Pentostam-Treated Patients

Patient Number	Adverse Reactions*	Parasitologic Data† Before Therapy (cult/sp/bx)	Parasitologic Data† After Therapy (cult/sp/bx)
Cured			
1	SGPT = 105 (W1) SGPT = 45 (W2)	+/+/+	−/−/−
4	None	+/+/−	−/+/ND
6	None	+/+/+	−/−/−
8	None	+/+/+	−/+/ND
9	SGOT = 52 (W3) SGOT = 27 (M1)	+/+/+	−/−/−
10	None	−/−/−	−/−/ND
11	None	+/+/+	−/−/ND
12	SGOT = 120 (W2) SGOT = 18 (W3)	+/+/+	−/−/ND
13	SGOT = 54 (W3)	+/+/+	−/+/−
14	None	+/−/+	−/−/ND
15	None	−/+/−	−/+/ND
16	SGPT = 55 (W1) SGPT = 33 (W2)	+/+/+	−/−/ND
18	SGOT = 61 (W2) SGOT = 50 (W3)	−/+/−	−/−/ND
Therapy failed			
2	None	+/+/+	−/−/−
3	None	+/+/+	−/+/−
5	None	+/+/+	+/−/−
7	SGOT = 86 (W3)	+/+/−	−/−/ND
17	SGOT = 61 (W1) SGOT = 28 (W3)	+/+/+	+/−/ND
19	SGOT = 58 (W3)	+/+/+	−/+/ND

* For adverse reactions, the abnormal SGOT or SGPT value (in IU/L) and the week (W) at which the abnormality occurred are shown, followed by a subsequent normal value and the week or follow-up month (M) at which value normalized. None = no abnormal laboratory value.
† Parasitologic data refer to presence (+) or absence (−) of leishmanial organisms in culture (cult) of either lesion scraping or biopsy specimen, in stained scraping (sp), or in stained biopsy specimen (bx). ND = not determined.

TABLE V

Response to Therapy in Placebo-Treated Patients

Patient Number	Lesion Number	Site	Duration (weeks before therapy)	Size Before Therapy (mm^2)	Size After Therapy (% pretherapy size)	Size 1M After Therapy (% pretherapy size)	Parasitologic Diagnostic Testing (cult/sp)†	Results of Therapy‡
1	1	Neck	6	275	50	173	+/+	Fail 1M
	2	Left arm		238	13	42		
	3	Left arm		90	47	80		
	4	Left forearm		70	69	40		
2	1	Left finger	8	108	428	268	+/+	Fail 1M
3	1	Right shoulder	5	2	600	975	+/+	Fail 1M
	2	Left forearm		2	750	1,200		
	3	Left forearm		2	100	0		
4	1	Right forearm	8	81	67	123	+/+	Fail 1M
	2	Left forearm		6	66	100		
5	1	Right leg	6	140	200	303	+/+	Fail 1M
	2	Right leg		81	100	160		
6	1	Left leg	6	150	360	406	+/+	Fail 1M
7	1	Right forearm	10	140	45*	NA	+/+	Fail Rx
	2	Right forearm		100	81*	NA		
	3	Right forearm		224	5*	NA		
	4	Right forearm		42	238*	NA		
8	1	Left leg	10	140	180	225	+/+	Fail 1M
	2	Left leg		1	15,400	23,800		
	3	Left leg		45	33	78		
9	1	Left finger	12	70	114	137	+/+	Fail 1M
10	1	Right forearm	6	63	127	159	+/+	Fail 1M
11	1	Right thigh	4	121	235	165	+/+	Fail 1M

M = month; NA = not applicable.

* Seven new lesions seen at the end of therapy. Patient was removed from protocol and re-treated.

† Results in terms of culture (cult) of lesion scraping and stain of scraping (sp) are given.

‡ Therapeutic results list date of failure. Rx = end of therapy; 1M = end of the 1-month follow-up period.

>0.9) than the mean pretreatment duration of lesions that failed to heal (9 ± 6.2 weeks).

The parasitologic response of the lesions by the end of Pentostam therapy is listed in Table IV. Four of the 13 patients who were cured had a positive diagnostic test result for leishmanial organisms at the end of therapy. Two of the six patients who relapsed were parasitologically negative at the end of therapy.

ADVERSE REACTIONS IN THE KETOCONAZOLE AND PENTOSTAM GROUPS: Adverse reactions in patients administered ketoconazole or Pentostam are shown in Tables III and IV. All ketoconazole-treated patients experienced a decrease in their serum testosterone levels to a mean nadir of 123 ng/mL, which was 29% of the mean pretreatment level of 430 ng/mL. No patient noticed diminution of libido or of beard growth during therapy. Recovery of testosterone levels to within the normal range occurred in all patients after therapy. Sixteen of the 22 patients receiving ketoconazole (73%) had no other laboratory abnormalities, symptoms, or signs during therapy. The laboratory abnormalities recorded in the other six patients were mild elevations of liver transaminase values that normalized during or after therapy (Table III). Subjective complaints consisted of headache (four patients), abdominal pain (two), fever (two), nausea (one), and malaise (one).

There were no laboratory abnormalities in 10 of the 19 patients receiving Pentostam (53%). Laboratory abnormalities in the other nine patients consisted of mild elevations of liver enzymes, which partially or completely resolved despite continued therapy in five patients. There were considerable subjective complaints, however. Sixteen of the 19 patients (84%) complained of pain at the intramuscular injection site. In addition, 11 of 19 (58%) complained of myalgia, four patients had headache or arthralgia, and two patients had nausea or fever.

Second Study: Placebo

The placebo-treated patients were between 16 and 43 years of age. The mean age was 31 years and six patients were less than 31 years old.

Data on the 11 placebo-treated patients are summarized in **Table V.** Sixty-one percent of the lesions were on the upper extremities, and there were an average of 2.1 lesions per patient. The pretreatment duration of the lesions was 7.4 ± 2.5 weeks. The lesions had a size of 95 ± 77 mm^2 prior to placebo therapy. Cultures were obtained on lesions from seven of the 11 patients. All isolates were *L. braziliensis panamensis.*

At the end of the approximately 1-month period of placebo therapy, lesions were 178 ± 195% of their pretreatment size, with a range of 5% to 750% (lesion 2 on Patient 8, which enlarged 15,400%, is excluded from these calculations). At the end of the 1-month follow-up period, lesions were 257 ± 320% of the pretreatment size, with a range of 0% to 1,200% (excluding lesion 2 on Patient 8). At the end of the treatment period, therapy was considered to have failed in Patient 7 because of the appearance of seven new nodular lesions. At the end of the 1-month period of follow-up, therapy was judged to have failed in Patients 1 to 6 and 8 to 11 because each patient had a lesion that was 123% to 975% of the pretreatment size.

Although therapy failed in all placebo-treated patients, not all lesions failed to heal. For Patient 3, lesion 3 healed while lesions 1 and 2 enlarged by five- to six-fold. For Patient 7, lesion 3 virtually healed while lesion 4 enlarged 2.4-fold and seven new lesions

developed. Table V also indicates that placebo-treated lesions could undergo diminution in size before enlargement (Patients 1, 4, and 8). Some lesions rapidly enlarged. For example, lesion 2 on Patient 8 grew from 1 mm × 1 mm to 17 mm × 14 mm in 2 months.

Retreatment of Patients in Whom Ketoconazole, Pentostam, or Placebo Therapy Failed

Patients in whom ketoconazole, Pentostam, or placebo therapy failed were retreated with the local standard of care, pentavalent antimony in the form of Glucantime or Pentostam (20 mg Sb/kg, with a maximum of 850 mg Sb/day, intramuscularly for 12 days), and all were cured.

COMMENTS

Treatment of cutaneous leishmaniasis is recommended to decrease the cure time and perhaps to increase the cure rate compared to that occurring naturally, and in an attempt to prevent subsequent mucosal disease. We have seen 40 cases of mucosal leishmaniasis in Panama, a region previously thought to be free of mucosal disease, from 1985 to the present. However, because cutaneous leishmaniasis is a moderate outpatient problem rather than a severe clinical one, therapy should not only be effective but should have merely modest adverse effects. The therapeutic regimen should be less onerous than the disease.

Heretofore, the reason that agents have been recommended for cutaneous leishmaniasis is that they cure visceral disease, a characteristically non-self-curing and fatal disease. Thus, organic pentavalent antimonials (Pentostam and Glucantime) have become the treatment of choice for all forms of leishmaniasis, including cutaneous leishmaniasis, because they generally cure kala-azar with acceptable, although poorly delineated, side effects. Intuition as to how data from visceral disease might be applicable to cutaneous disease led to the 1984 World Health Organization's recommendation for cutaneous leishmaniasis of "10 to 20 mg Sb/kg/day until clinical and parasitologic cure is achieved" [18]. In addition, since a maximum daily dosage of 850 mg Sb seemed appropriate for visceral leishmaniasis, a standard of 10 to 20 mg Sb/kg/day with a maximum daily dosage of 850 mg Sb has generally been followed for cutaneous leishmaniasis.

Since 1984, only two published studies on cutaneous leishmaniasis have compared the World Health Organizations regimen of pentavalent antimonials to some other therapeutic regimen in attempts to authenticate the recommendations. In a study of Algerian cutaneous leishmaniasis presumably due to *Leishmania major*, 55% of untreated lesions cured in 2 months, compared to 48% of lesions in patients given 17 mg Sb (in the form of Glucantime)/kg/day for 15 days [19]. In a recent study in patients with *L. braziliensis panamensis* infection treated at Walter Reed, there was a 100% cure rate in patients given 20 mg Sb (in the form of Pentostam)/kg/day (with no upper limit on daily dose) for 20 days, compared to a 76% cure rate in patients given 10 mg Sb/kg/day for 20 days ($p = 0.03$) [20]. Thus, the only report in which pentavalent antimony was compared to no treatment for cutaneous leishmaniasis showed the drug to be no more effective than no treatment, and the only demonstration of Sb efficacy is that a high dose (20 mg/kg/day) is more effective than a low dose (10 mg/kg/day) against one organism. Given this lack of controlled data demonstrating either efficacy or modest side effects for the standard agent, it is understandable that there are no controlled data for any of several putative alternative treatments for cutaneous leishmaniasis.

Placebo Study

Our study comparing ketoconazole to Pentostam in the treatment of *L. braziliensis panamensis* cutaneous leishmaniasis originated when the importance of placebo controls was not fully appreciated. A group in which patients were treated with placebo for approximately 1 month was therefore investigated after the treatment phase of the ketoconazole versus Pentostam study was completed. The placebo study showed that therapy failed in 100% of patients with cutaneous leishmaniasis caused by *L. braziliensis panamensis*. The variable natural course of this disease in the short term is suggested by the fact that some lesions diminished in size or healed while other lesions on the same patients enlarged. Nevertheless, at least one lesion on each patient failed to heal or relapsed by the criteria used for the ketoconazole- and Pentostam-treated patients (less than 25% decrease in initial size by the end of a 1-month period of follow-up or appearance of new lesions). In fact, placebo therapy failed by more than the minimal criteria in patients: one lesion on each patient was 23% to 875% larger at the end of the 1-month follow-up than at the beginning of treatment.

For ethical reasons, patients were treated with pentavalent antimony when the previous therapy had failed, and we have no information on how much time longer than 2 months after initiation of therapy is required for cure of this disease. The question of the time for natural cure of New World cutaneous leishmaniasis has not been well addressed in published studies. Eleven cases of disease presumably due to *L. mexicana* lasted for 1 to greater than 18 months before self-cure [21], and approximately half of a series of Brazilian cases were reported in a brief note to self-cure in 6 months [22].

Pentostam Group

In the main study comparing Pentostam to ketoconazole in a randomized manner, the 19 patients receiving Pentostam were administered 20 mg Sb/kg/day with a maximum of 850 mg/day intramuscularly for 20 days. Each of the approximately 64-kg patients therefore received approximately 13 mg Sb/kg/day for 20 days. All lesions were smaller at the end of therapy. Ten patients had complete healing of all lesions by the end of therapy and 18 of the 19 patients demonstrated complete re-epithelialization by 1 month after the end of therapy. Nevertheless, six patients—including three of those who had apparent healing of all lesions by the end of therapy—ultimately relapsed and their therapy was judged to be a clinical failure. A cure rate of only 13 of 19 (68%) was achieved. This cure rate is in accord with our previous experience with this patient population, in which 850 mg Sb (Glucantime)/day for 20 days cured 21 of 28 (75%) patients, and 850 mg Sb (Pentostam)/day for 20 days cured 14 of 22 (64%) patients [23].

The Pentostam group was similar to the placebo group in the important fact that all speciated organisms in both groups were *L. braziliensis panamensis*. Nevertheless, the Pentostam group was not random-

ized versus the placebo group and the groups were not comparable in several other ways. For example, the mean size of the placebo-treated lesions (95 mm^2) was much smaller than the mean size of the Pentostam-treated lesions (350 mm^2), and this difference is statistically significant ($0.02 > p < 0.05$). (In addition, the mean duration prior to therapy of the placebo-treated lesions [7.4 weeks] was less than the mean duration of the Pentostam-treated lesions [12.5 weeks], but the difference was not significant [$p > 0.7$]). For the Pentostam group, the mean size of lesions that relapsed was 2.4 times the mean size of lesions that healed, although the difference was significant only with $0.1 > p < 0.2$. The Pentostam data therefore suggest that if a smaller pretherapy lesion size has any effect on drug-induced cure, the effect would be to augment the cure rate. Thus, the difference between the pretreatment lesion size in the placebo and Pentostam groups would, if anything, lead to an artifactual increase in the placebo cure rate. The 0% placebo cure rate is therefore not artifactually low, and this study indicates that Pentostam is more effective than placebo against *L. braziliensis panamensis* cutaneous leishmaniasis.

The Walter Reed study [20] demonstrated that 20 mg Sb/kg/day, which cured 100% of patients, was more effective than 10 mg Sb/kg/day (76% cure rate) against disease due to *L. braziliensis panamensis*. The present study utilized 13 mg Sb/kg/day and demonstrated a cure rate of 68% against the same organism. Together, these studies indicate that a maximum daily Sb dose of 850 mg limits adult males to far less than 20 mg Sb/kg/day, and results in an inadequate cure rate in this form of cutaneous leishmaniasis, probably because larger lesions require more Sb. The regimen of 13 mg Sb/kg/day for 20 days employed here resulted in only mild laboratory abnormalities of liver function (although subjective complaints due to intramuscular injections and to myalgias were common). The toxicity of 20 mg Sb/kg/day for 20 days is currently being evaluated in ongoing studies. If objective toxicity is modest, the regimen of 20 mg Sb/kg/day with no upper limit on daily dose should be recommended for *L. braziliensis panamensis* cutaneous leishmaniasis and for cutaneous leishmaniasis due to other organisms in which a low cure rate is achieved with less than 20 mg Sb/kg/day.

Ketoconazole Group

The main purpose of this study was to compare ketoconazole treatment to treatment with Pentostam and with placebo. Ketoconazole cured 16 of 21 (76%) patients with *L. braziliensis panamensis* disease, and 16 of 22 (73%) patients overall. This number is slightly higher than the value of 68% for the Pentostam group with which the ketoconazole group was randomized. In reporting the data, we overlooked certain study irregularities so that all data could be reported. If patients for whom the irregularities occurred were omitted, the difference between the ketoconazole and Pentostam cure rates would be even higher. For example, ketoconazole-treated Patients 8, 9, and 10—in all of whom therapy failed—were not randomized because Pentostam was transiently unavailable. Ketoconazole-treated Patient 8 was the only patient with disease due to *L. mexicana*. Pentostam-treated Patient 10 was entered into the study despite negative parasitologic diagnostic tests. If data from only randomized patients with parasitologically proven disease due to *L. braziliensis panamensis* were reported, there would be three less ketoconazole failures and one less Pentostam cure.

In contrast to Pentostam-induced healing, most lesion healing due to ketoconazole treatment occurred after the end of therapy. Only five ketoconazole-treated patients had complete healing of all lesions by the end of therapy, whereas lesions in four patients completely healed by the 1-month follow-up examination, and seven patients first demonstrated complete healing at the 3-month follow-up. Since some lesions enlarged before re-epithelialization, change of lesion size at the end of therapy was poorly correlated with eventual healing. In terms of the six treatment failures, two were because of relapse and four were due to failure of the lesion to heal (less than 25% diminution in lesion size by 1 month after the end of therapy).

The placebo group was not randomized versus the ketoconazole group, and placebo-treated lesions prior to therapy were smaller than ketoconazole-treated lesions prior to therapy ($0.05 > p < 0.1$). (There was a large standard deviation in lesion diameter for all three groups. Had the deviations been less, the difference in pretherapy size between ketoconazole- and placebo-treated lesions might have been significant at $p < 0.05$.) Nevertheless, there was little correlation of pretreatment size with ketoconazole-induced cure in the ketoconazole group, and the difference between the mean lesion size in the placebo group compared to the mean lesion size in the ketoconazole group should not have led to artifacts in the comparative cure rate. Because the duration of lesions in the placebo group was virtually the same as the duration in the ketoconazole group, a discrepancy in disease duration could not also influence the comparative cure rate. Thus, although the placebo group was not randomized with the ketoconazole group, these studies indicate that ketoconazole is more effective than placebo against *L. braziliensis panamensis* cutaneous leishmaniasis.

For both the Pentostam and ketoconazole groups, the presence of parasites at the end of therapy did not correlate with eventual cure. Four Pentostam-treated patients and seven ketoconazole-treated patients who were eventually cured had at least one positive parasitologic test result at the end of therapy. Two Pentostam-treated patients who eventually relapsed were parasitologically negative at the end of therapy. However, for patients receiving ketoconazole in our study, parasite positivity in a large number of tests at the end of therapy did correlate with eventual therapeutic failure. Treatment was eventually deemed to have failed in four of the five patients who demonstrated parasites in all attempted tests at the end of therapy.

Adverse effects due to this regimen of ketoconazole, 600 mg at night for 28 days, were limited to infrequent, reversible, mild abnormalities on hepatocellular function tests, and to a reversible, approximately 70% diminution of serum testosterone values in all patients. Since oral ketoconazole is absorbed in 1 to 2 hours and has an initial serum half-life of 2 hours [24], and since ketoconazole itself inhibits the enzymatic conversion of cholesterol to androgenic steroids, testosterone biosynthesis recovers to approximately 50% of its initial value by 18 hours after dosing [5]. Administration of the drug at night was designed to minimize the waking hours during which testosterone levels would be low.