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TREATMENT OF CUTANEOUS LEISHMANIASIS WITH A TOPICAL ANTILEISHMANIAL DRUG (WR279396): PHASE 2 PILOT STUDY

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Abstract. We studied the efficacy of WR279396, a topical formulation of aminoglycosides that cures 100% of cutaneous leishmaniasis lesions in mice. We conducted what is to our knowledge the first controlled study of WR279396 therapy for clinical cutaneous leishmaniasis. A total of 45 Colombian soldiers, all men, were randomly assigned to treatment with WR279396 (33 patients) or placebo (12 patients). Each lesion was treated twice daily for 20 days. Lesions were measured at the end of therapy and at 45, 90, and 180 days after treatment began. A total of 17 (61%) of 28 assessable WR279396-treated patients were cured, and 5 (55%) of 9 assessable placebo-treated patients were cured ($P = 0.9$). For the 36 lesions treated with WR279396 that were cured, cure took a mean of 35 days, whereas for the 6 lesions that were cured in the group of patients receiving placebo, cure time took a mean of 56 days ($P = 0.04$). WR279396 is a nontoxic topical formulation that significantly accelerated cure time in patients with *Leishmania panamensis* cutaneous leishmaniasis.

INTRODUCTION

Cutaneous leishmaniasis can be divided into New World disease, primarily caused by *Leishmania braziliensis* complex and *L. mexicana* complex, and Old World disease, primarily caused by *L. major* and *L. tropica. Leishmania braziliensis* complex organisms cause ulcerative disease that frequently metastasizes to the draining lymph nodes[1,2] and that may rarely metastasize to the oronasal mucosa.[3] The natural cure rate is relatively slow. In Guatemala, only 22% of lesions reepithelialized, with a median time of ~ 3 months.[4] In contrast, the ulcers due to *L. mexicana* complex generally do not metastasize, and they cure relatively rapidly. In Guatemala, 88% reepithelialized at a median time of 3 months.[4] For Old World disease, ~ 70% of lesions due to *L. major* disease typically heal by 4 months,[5,6] but the natural history of *L. tropica* disease has not been well investigated.

Treatment is recommended for New World disease caused by the *L. braziliensis* complex to diminish the time before natural cure of the ulcer and to attempt to prevent metastasis. First-line treatment for such cases is therapy with pentavalent antimony in the form of glucantime or pentostam. Because these agents are parenteral and can be considerably toxic, treatment of New World disease caused by *L. mexicana* and Old World disease caused by *L. major* is problematic. ''No treatment at all'' may be chosen,[7] with intralesional injections of antimony being employed for persistent lesions (Buffet P, unpublished data).

The modest difference between disease morbidity and treatment morbidity for some cases of New World disease and for many cases of Old World disease has generated a 30-year search for nonparenteral, relatively nontoxic antileishmanial agents. Topical agents have been of particular interest. If the active ingredients penetrate the ulcer sufficiently to reach the parasites, they would be effective. The side effects might be modest, and at any rate, they should be local. The first such clinical agent was paromomycin (15%) plus methylbenzethonium chloride (12%) in paraffin, which healed Israeli *L. major* lesions more rapidly than did placebo.[8] Because methylbenzethonium chloride caused local pain, a second clinical formulation was made that contains paromomycin (15%) and urea (10%) in paraffin. In trials against *L. major* in Tunesia[5] and in Iran,[6] lesions treated with this formulation healed at the same rate as did lesions treated with placebo.

To develop a topical antileishmanial formulation that penetrates the skin and kills the parasite yet is easily tolerated, investigators at Walter Reed Army Institute of Research have formulated paromomycin (15%) in a variety of carriers. WR279396 contains paromomycin (15%) plus gentamicin (0.5%) in a complex base sufficiently hydrophilic to solubilize the aminoglycosides, yet is sufficiently hydrophobic to promote penetration of the agents into the ulcer. In rodent tests, by 1 month after therapy, WR279396 healed cutaneous lesions due to *L. major, L. amazonensis,* and *L. panamensis,* whereas paromomycin-methylbenzethonium only healed lesions due to *L. major.*[9]

We here report the first test of WR279396 for efficacy and tolerance in patients with cutaneous leishmaniasis. The population was Colombian soldiers who acquired disease during operation in endemic regions, then were evacuated to Bogota for treatment.

MATERIALS AND METHODS

Colombian soldiers acquired infection in the provinces of Uraba and Magdalena Medio. Most patients were evacuated to the Central Military Hospital in Bogota for diagnosis and treatment. Some patients were diagnosed and treated at the Instituto Colombiano de Medicina Tropical in Uraba. Diagnosis was based on visualization of organisms via Giemsa smears or monoclonal antibody staining in lesion material obtained by aspiration or scraping.[10] Because this was the first clinical trial of this formulation, inclusion criteria were designed to admit patients with mild to moderate but not severe disease, and with ulcerative but not papular or nodular lesions.

Diagnosed patients were admitted to the protocol if the total ulcer lesion size was < 2000 mm^2, lymphadenopathy

TABLE 1
Salient patient, efficacy, and toxicity data

Characteristic	Parameter*	WR279396	Placebo	*P* value
Presenting characteristics	No. patients	33	12	
	Age (yr), mean ± SD	23 ± 2.6	26 ± 9	
	No. who failed to respond to previous therapy (%)	9 (27%)	3 (25%)	
	Total number of lesions	56	17	
	Pretherapy lesion size (mm^2), mean ± SD	155 ± 153	203 ± 259	0.4†
Efficacy per patient	Cured patients			
	No. patients (no. with previous therapy)	17 (5)	5 (0)	
	Lesion size (mm^2), mean ± SD	158 ± 171	109 ± 169	
	Failed patients			
	No. patients (no. with previous therapy)	11 (3)	4 (2)	
	Lesion size (mm^2), mean ± SD	139 ± 106	135 ± 123	
	Nonassessable patients			
	No. patients (no. with previous therapy)	5 (1)	3 (1)	
	Lesion size (mm^2), mean ± SD	190 ± 154	398 ± 380	
	Percentage cured [no. cured/(no. cured + no. failed)]	61%	55%	0.9‡
Efficacy per lesion	No. cured lesions	36	6	
	Cure time (days), mean ± SD	35 ± 21	56 ± 28	0.04†
	No. failed lesions	15	6	
	Nonassessable lesions	5	5	
	Percentage cured [no. cured/(no. cured + no. failed)]	71%	50%	0.3‡
Side effects	No. patients	33	12	
	No. patients with side effects (%)	18 (55%)	4 (33%)	0.3‡
	Total no. of days of side effects (days/symptomatic patient)	65 (3.6 per patient)	10 (2.5 per patient)	

* SD = standard deviation.
† *t*-test.
‡ Chi-square test or Fisher's exact test.

was < 1 cm in diameter, and there was no disease of the oronasal mucosa. In addition, screening laboratory values (such as serum levels of creatinine) had to be within normal limits and the patients had to be without concomitant medical problems. Admitted patients were randomly assigned to treatment with WR279396 or placebo (the base used in WR279396) in a 2:1 allocation. The base for WR279396 contains isopropyl palmitate, lactic acid, methyl paraben, propyl paraben, propylene glycol, sodium lauryl sulfate, sorbitol, stearyl alcohol, urea, water, and white petrolatum. For this study, WR279396 contained approximately 37% water.

Each ulcerative lesion was treated twice a day for 20 days with 0.0005 mL/mm^2 of WR279396 or placebo. Ulcer size was measured in 2 perpendicular directions in millimeters with a Digimatic caliper (VWRbrand Digital Calipers, Bridgeport, NJ) before therapy, at the end of the 20 days of therapy, and at 1.5, 3, and 6 months after the beginning of therapy. On each of the 4 posttherapy examination days, any lesion that had not 100% reepithelialized was investigated for the presence of parasites as before therapy.

Lesion cure was defined as 100% reepithelialization of the lesion without relapse by the 6-month follow-up. Lesion failure was defined as lack of 100% reepithelialization by 6 months or doubling of the lesion size at a previous examination period, at which point the patient was removed from the protocol and treated with meglumine antimonate. Determination of lesion cure and failure was made by a clinician blinded as to the treatment group of the patient. For a patient to be cured, all of his lesions had to resolve. The protocol's end point was cure of the patient.

Patients were interviewed daily during therapy for dermatological side effects (e.g., pain, erythema, and edema). If side effects were mentioned, they were graded 1 (mild) if barely perceptible, 2 (moderate) if well defined, and 3 (severe) if impairing normal duties. High- and low-tone hearing tests and a Romberg test were performed to determine eighth nerve function. To determine if the aminoglycosides in WR279396 were sufficiently absorbed to cause nephrotoxicity, serum creatinine level was measured at the end of therapy.

Continuous data were compared by Student's *t*-test. Nominal data were compared by the chi-square test or by Fisher's exact test when there were < 5 members per cell.

Written informed consent was obtained from all subjects, and the ethical guidelines for the Hospital Militar Central, the Walter Reed Army Institute of Research, and the Human Subject Review Board, Office of the Surgeon General, Department of the Army, were followed. The study was performed under current Good Clinical Practices (cGCP).

RESULTS

Patient characteristics. Of 45 patients, 33 were randomized to the WR279396 group (active group) and 12 were randomized to the placebo group. The reason for the lack of exact 2:1 assignment was that randomization was performed for a possible total of 60 patients to allow for drop-outs, and a relatively large number of active treatments were randomized to the first 45 patients. All patients were men aged ~ 25 years (Table 1). The pretherapy lesion sizes were a mean of 166 mm^2. There was no statistical difference in pretreatment lesion sizes between the active and the placebo group ($P = 0.4$, *t*-test). The mean number of lesions was 1.6 per patient. There were 12 patients (27%) who had failed to respond to previous treatment with glucantime. This high percentage of patients who failed to respond to previous

therapy occurred because patients had to be released from routine duties and evacuated to Bogota for this study, and commanders were particularly willing to release medically difficult patients such as those who had failed to respond to standard antimonial therapy.

Determination of *Leishmania* species by culture was successful in only 5 of the 45 smear-positive patients. All 5 isolates were *L. panamensis.*

Efficacy. In a topical treatment study, efficacy can be evaluated on a per-patient basis, or because each lesion was individually treated, on a per-lesion basis.

Patients cured. In the active group, 17 (61%) of 28 assessable patients cured. The 11 failures to respond were due to doubling of the lesion size in 8 patients (6 of the enlarged lesions were parasitologically positive) and enlargement after marked diminution in 3 patients (2 of the relapsed lesions were parasitologically positive). In the placebo group, 5 (55%) of 9 assessable patients were cured ($P = 0.9$ in comparison to the active group). All of the placebo cures were chemotherapeutically naive patients who had not previously received glucantime. All 4 failures were due to doubling of the lesion; 2 were parasitologically positive.

The 5 nonassessable patients in the active group were so designated because they absconded after treatment ($n = 1$), they self-administered glucantime after treatment ($n = 1$), they had initial disease of the lip and were admitted via a protocol violation ($n = 1$), they demonstrated one cured lesion and one lesion that failed to cure ($n = 1$), and the lesion size diminished by 90% but not by 100% ($n = 1$). The 3 nonassessable patients in the placebo group all had lesions that at the end of therapy were relatively unchanged but were parasitologically positive. These patients were removed from the protocol and treated with glucantime at the patients' request. If the criteria of failure had been based on parasitological as well as clinical data, all nonassessable patients in the placebo group would have been declared as treatment failures, and the placebo failure rate would have been 5 (42%) of 12. In comparison, one of the patients in the active group who ultimately clinically cured would have been declared a parasitological failure, and the cure rate in the active group would have been 16 (57%) of 28 ($P = 0.60$).

Lesions cured. In the active group, 36 (71%) of 51 assessable lesions were cured and 5 lesions were not assessable. In the placebo group, 6 of 12 assessable lesions were cured (50%, $P = 0.3$) and 5 lesions were not assessable. There was no statistical difference between the pretreatment sizes for cured versus uncured lesions in patients treated with WR279396 ($P = 0.7$) or in patients treated with placebo ($P = 0.8$).

The 36 lesions that were treated with WR279396 were recognized as cured at the end of therapy on Day 20 (20 lesions), at the first follow-up on Day 45 (13 lesions), and on Day 90 (3 lesions). The 6 placebo lesions that were cured were recognized on Day 20 (1 lesion), Day 45 (3 lesions), and Day 90 (2 lesions). The mean cure time of 35 days for lesions cured with WR279396 was statistically different ($P = 0.04$) from the mean cure time for lesions cured by placebo (56 days).

An additional consideration for cutaneous diseases is the appearance of the site after healing. The lesions healed by WR279396 appear in this small study to be cosmetically acceptable (Figure 1).

Adverse reactions. In the active group, 18 (55%) of 33 patients experienced local reactions, all of which were reported as having a pain grade of 1 and lasting a mean of 3.6 days, except for one patient, who had Grade 2 erythema for 1 day. Four (33%) of 12 placebo patients reported a pain grade of 1 for a mean of 2.5 days each. The percentage of patients with side effects did not differ ($P = 0.3$) between the active and placebo groups.

No patient demonstrated an increase in creatinine values to higher than normal values (1.5 mg/dL) by the end of therapy. No patient had other than incidental changes in eighth nerve function, as assessed by hearing and Romberg tests.

DISCUSSION

In this Phase 2 pilot clinical study of the topical antileishmanial agent WR279396, treatment with WR279396 was compared with treatment with topical placebo (the cream base of WR279396). On the basis of clinical criteria used in this study, 61% of patients treated with WR279396 were cured, and 55% of patients treated with placebo were cured. If parasitological criteria as well as clinical criteria for failure had been used in this study, the cure rate in the active group would have been 57% and the cure rate in the placebo group would have been 42%.

The low cure rate for WR279396, compared with that expected from the blinded mouse studies [9], could be due to the composition and thinness of rodent skin compared with the thickness of human skin. In spite of a complex cream carrier, the hydrophilic aminoglycosides that are the active ingredients of WR279396 apparently only partially penetrated the parasites located in human dermal tissue. Another reason could be the total of ~44% water in the mouse studies, but approximately 37% water in the present clinical work. Preparation of the larger amount of clinical material necessitated a reduction in the water content, but the lower content may have diminished solubility of the cationic aminoglycosides. Finally, the possibility exists that the apparent lack of a significant effect on the cure rate was an artifact of the small study size. The placebo cure rate was high but nevertheless not far from the figure of 37% recently recorded for *L. panamensis* in Colombia.[3]

Because topical agents only treat the lesion to which they are applied, cure can also be evaluated on a per-lesion basis. The lesion cure rate did not statistically differ, but the lesion cure time did statistically differ: 35 days for lesions treated with WR279396 that cured compared with 56 days for lesions treated with placebo that cured ($P = 0.04$).

Side effects in both groups consisted almost entirely of Grade 1 local pain that lasted a mean of 4 days in 55% of patients in the active treatment group and 3 days in 33% of patients in the placebo group.

WR279396 is therefore a nontoxic topical formulation that significantly diminished lesion cure time in this study of *L. panamensis* disease. In comparison, standard treatment of Colombian *L. panamensis* with parenterally administered glucantime has a higher cure rate of ~ 90%[3,11] in patients presenting with similar characteristics (1.2 lesions per pa-

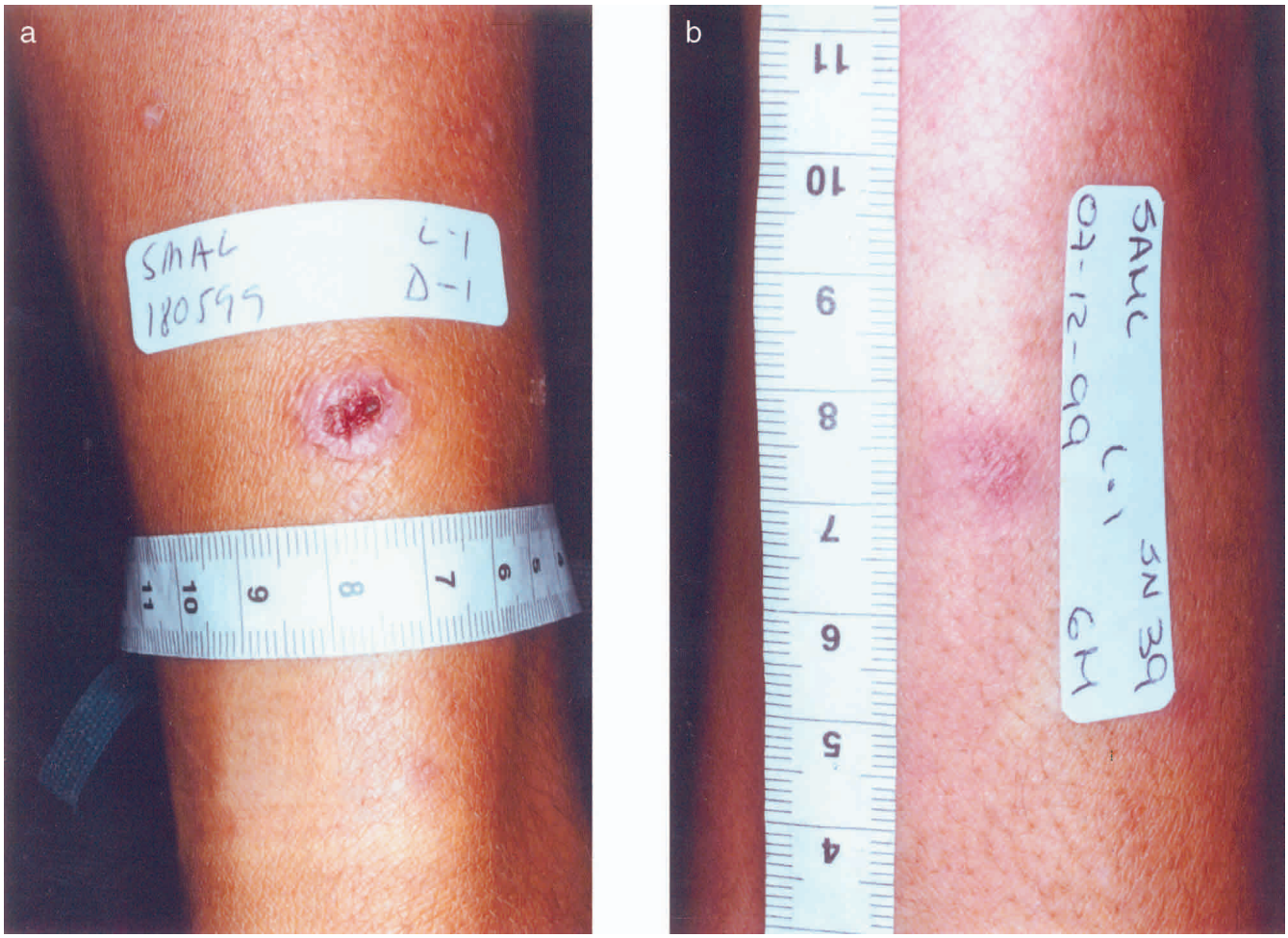

FIGURE 1. Cutaneous leishmaniasis lesion treated with WR279396 **(a)** before and **(b)** 6 months after treatment.

tient, mean lesion size of 267 mm^2),[3] but also a clinically significant incidence of adverse effects to the heart, liver, pancreas, and musculoskeletal system.[12,13] For *L. braziliensis* complex lesions, the need to rapidly cure essentially all lesions justifies the use of poorly tolerated parenteral therapy. Disease caused by *L. mexicana* in the New World and disease caused by *L. major* in the Old World, however, largely self-cure within 6 months.

The therapeutic goal in treatment of *L. mexicana* and *L. major* is to speed up cure without generation of drug toxicity and to promote a cosmetically attractive result. The encouraging data in this study on cure time suggests that WR279396 may accelerate healing of cutaneous leishmaniasis ulcers. The next clinical test for WR279396 is for *L. major* cutaneous leishmaniasis under carefully controlled circumstances. It is hoped that lesions treated with WR279396 will cure more rapidly than lesions treated with placebo and that a place for topical therapy of cutaneous leishmaniasis can be established.

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