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Treatment of American Cutaneous Leishmaniasis with Orally Administered Allopurinol Riboside

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Eighteen patients received 1,250 mg of allopurinol riboside (AR) four times daily for 28 d. Nine of the patients concurrently received 500 mg probenecid (PB) four times daily. Cure was assessed clinically and parasitologically. Patients who had culture-positive and nonhealing lesions 3 mo after therapy received pentavalent antimony. Of the nine patients who received AR alone, four (44%) had clinical improvement at the end of therapy and two (22%) were culture-negative. A third patient became culture negative at 2 mo after therapy. The culture-negative patients were completely healed at 1 mo and remained so at 1 y after therapy. Of the nine patients who received AR plus PB, four had complete healing and two had clinical improvement at the end of therapy; however, all patients remained culture-positive. At 2–3 mo after therapy, six (67%) of the patients were completely healed, and of these, five (56%) were culture-negative. The drug was well-tolerated.

Cutaneous leishmaniasis is a common parasitic disease endemic in many regions of Central and South America and in the Middle East. Current therapies are limited by toxicity, lack of efficacy, or inconvenient dosage schedules. For example, the most commonly used drug in Latin America is meglumine antimoniate, usually in a regimen of 20 mg/kg per day intramuscularly for 20 d [1]. The inconvenience of such a treatment regimen, requiring daily injections, is an important limitation to the use of pentavalent antimony.

Allopurinol riboside (AR), a structural analogue of inosine, is active in vitro against a variety of species of *Leishmania* and *Trypanosoma* [2–6]. Administered orally, this drug has potential for the treatment of American cutaneous leishmaniasis (ACL), judged on the basis of studies of the enzymology and biochemical pathways of purine and nucleic acid metabolism in these parasites and in mammalian cells [2–4, 6] and tolerance and metabolic studies in humans (T. A. Shapiro, personal communication).

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Informed consent was obtained from all patients, and the guidelines for human experimentation of the participating institutions were followed.

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We carried out a pilot study to assess the safety and efficacy of AR alone and in combination with probenecid (PB) in the treatment of acquired ACL in Panama.

Patients and Methods

Patients. Eighteen men aged 18–60 y who had ACL were enrolled in the study. All were natives of Panama. They were otherwise in good health and were within 20% of their ideal body weight. Cutaneous lesions were ulcerative in all cases, with a mean duration before treatment of 5 w (range 3 w to 3 mo). Fifteen patients had single lesions and three had multiple lesions. Patients with >3 lesions or with lesions on the face were not admitted to the study. None of the patients had received prior antileishmanial treatment. Patients with known or suspected allergies or a history of idiosyncratic response to allopurinol, AR, or PB were excluded.

All patients had a complete clinical evaluation prior to the study. Physical parameters were monitored daily during the 28-d treatment period. Additional clinical evaluations were conducted at ~1, 2, 3, 4, 6, and 12 mo after treatment.

Study design. The study was an open, noncomparative clinical trial to evaluate the safety, tolerance, and efficacy of AR alone and in combination with PB in patients with cutaneous leishmaniasis. No control group was deemed necessary or ethical because clinical experience in this region indicated that progressive disease of ≥5 w duration does not resolve spontaneously and because of the low but definite incidence of espundia in people infected with *L. braziliensis panamensis*. Following determination of eligibility, each patient was admitted to the medical service of Hospital Santo Tomas in Panama City, Panama. All patients were hospitalized for baseline testing 1 d before drug administration and for the duration of therapy (28 d). Nine patients received 1,250 mg of AR and nine patients received 1,250 mg of AR plus 500 mg of PB in four doses each day for 28 d. PB was added to the latter regimen in an attempt to increase plasma levels of AR.

Lesions were measured at the initial clinical evaluation. Diagnosis of cutaneous leishmaniasis was confirmed by parasitologic, immunologic, and serologic criteria. The lesion border was scraped for stained smear and a punch biopsy was performed for histologic confirmation of leishmaniasis. Part of the tissue obtained by the biopsy was used to inoculate modified Senekjie's medium for parasite cultivation. An intradermal challenge test of delayed reaction to parasite antigen (Montenegro skin test) was performed on all patients. Serologic analysis was by the indirect fluorescent antibody test. Thirteen isolates from 13 patients were identified by their isoenzyme profile as *L. braziliensis panamensis*.

Lesion size was measured weekly during the treatment period. Laboratory tests were done twice a week during the first 2 w of therapy, weekly thereafter during the therapy period, and at each follow-up evaluation after therapy.

Cure was defined as the complete clinical healing of the lesion with disappearance of edema, induration, or other signs of inflammation; a negative culture for parasites of needle aspirate from the healed lesion; and no relapse after 1 y of follow-up. Failure was defined as clinical nonhealing of the lesion and persistence of parasites. Patients who were not cured were subsequently treated with pentavalent antimony.

Sensitivity testing of organisms. Organisms obtained by biopsy were grown in modified Senekjie's medium. Once established, each culture (24 total) was sent by airfreight to Denver, Colorado, for testing for sensitivities to three pyrazolopyrimidines. Promastigotes were subcultured into HOSMEM [7] twice and then grown in the same medium in the presence of allopurinol riboside (4-hydroxypyrazolo-[3,4-*d*]pyrimidine-1-riboside), thiopurinol riboside (4-thiopyrazolo[3,4-*d*]pyrimidine-1-riboside), or 9-deazainosine. Titration curves were constructed to obtain a median effective dose (ED_{50}) for each isolate. When isolates were obtained from more than one specimen from a patient, all were tested. Organisms were grown to late logarithimic phase, ~5 d, counted on a Coulter Model ZBI (Coulter Electronics, Hialeah, Fla) and expressed as organisms per milliliter. Each titration curve was compared with a control grown without the antibiotic and titration curves were expressed as a percentage of control.

Results

Pretreatment findings. The lesions of 16 of the 18 patients were positive for parasites by stained smear, culture, or biopsy before therapy. These patients were also immunologically positive. The two remaining patients were negative at the initial clinical evaluation for parasites but were positive by immunologic, serologic, and clinical criteria; they were

Table 1. Response of patients with American cutaneous leishmaniasis to treatment with allopurinol riboside with and without probenecid: results at end of therapy.

Patient no.	AR group		AR + PB group	
	% Reepithelialization of lesion	Culture results	% Reepithelialization of lesion	Culture results
1	>75	No growth	<25	Growth
2	<25	Growth	100	Growth
3	0	No growth	0	Growth
4	<25	Growth	>50	Growth
5	>25	Growth	100	Growth
6	<25	Growth	100	Growth
7	<25	Growth	100	Growth
8	>25	Growth	<25	Growth
9	100	Growth	>50	Growth

NOTE. AR group received 1,250 mg of allopurinol riboside per day; AR + PB group received 500 mg of probenecid in addition to allopurinol riboside.

culture positive at the end of the treatment period. Fifteen patients had one lesion, two patients had two lesions, and one patient had three lesions. All patients were otherwise in good health.

Efficacy assessment. A comparison of the response of the two groups at the end of therapy is shown in table 1 and the 12-mo follow-up results and final assessment is shown in table 2. Of the nine patients who received AR alone, four (44%) had clinical improvement (>25% healed) and two (22%) were

Table 2. Response of patients with American cutaneous leishmaniasis to treatment with allopurinol riboside with and without probenecid: follow-up results at 12 mo.

Group, patient no.	% Reepithelialization of lesion	Culture results	Final assessment	Month of cure or failure*
AR				
1	100	No growth	Cure	1
2	<25	Growth	Failure	1
3	100	No growth	Cure	1
4	<25	Growth	Failure	1
5	<25	Growth	Failure	1
6	<25	Growth	Failure	3
7	>25	Growth	Failure	1
8	>25	Growth	Failure	1
9	100	No growth	Cure	2
AR + PB				
1	100	No growth	Cure	4
2	100	No growth	Cure	2
3	<25	Growth	Failure	1
4	100	Growth	Failure	3
5	100	No growth	Cure	4
6	100	No growth	Cure	4
7	0	Growth	Failure	3
8	>25	Growth	Failure	1
9	100	No growth	Cure	2

NOTE. AR group received 1,250 mg of allopurinol riboside per day; AR + PB group received 500 mg of probenecid in addition to allopurinol riboside.

* Month in which culture was negative, indicating cure, or month patient was retreated with pentavalent antimony based on clinical and cultural findings that indicated progressive worsening of the lesion.

culture-negative at the end of therapy. One of the culture-negative patients did not show clinical improvement until 1 mo after therapy. A third patient became culture-negative at 2 mo after therapy. All these patients remained culture-negative and were completely healed at 1 mo and at 1 y after therapy. Of the nine patients who received AR + PB, six (67%) had marked clinical improvement at the end of therapy. All nine patients remained culture-positive for ≤2 mo after therapy. Five (56%) were culture-negative and completely healed at 2–4 mo and 1 y after therapy. Thus, three patients in the AR group (33%) and five in the AR + PB group (56%) were cured (clinically and parasitologically). No relapses were observed in the eight cured patients after 1 y of follow-up.

There was no correlation between clinical improvement at the end of therapy and parasitologic cure. Four patients showed clinical improvement at the end of therapy but remained culture-positive throughout the study period. Two patients showed no clinical improvement at the end of therapy but became culture-negative and completely healed 1 and 4 mo after therapy.

All patients who were not cured with AR were successfully treated with one course of pentavalent antimony.

Patient tolerance. The drug was well tolerated by all patients. One patient had an unexplained transient episode of acute urinary retention on day 25 of therapy; therapy was discontinued and the retention resolved within 1 d. Its relationship to the drug is unclear. Three patients experienced transient headache and myalgias, and one patient developed transient abdominal pain and minor pruritis. There was no evidence of significant clinical adverse effects nor changes in laboratory values of hematologic, liver, or kidney function. There were no EKG abnormalities.

Sensitivity of promastigotes in vitro. All organisms isolated from patients were sensitive to all three inosine analogues in vitro. The ED_{50} of *L. braziliensis panamensis* to allopurinol riboside ranged from 0.3 to 6.3 μg/ml (median 2.2; SD 1.54); for thiopurinol riboside, 0.6–5.8 μg/ml (median 2.0; SD 1.68); for 9-deazainosine, 0.03–0.37 μg/ml (median 0.16; SD 0.11). The molecular weights of all three agents are similar and the values can be compared directly.

Discussion

This is the first clinical study to assess the efficacy of AR in the treatment of ACL. However, there are clinical data on the use of the parent compound, allopurinol, to treat visceral leishmaniasis. A small number of patients with visceral leishmaniasis received doses of allopurinol of 10.7–35.8 mg/kg per day for 2 or 3 w [8].

An antileishmanial effect of varying degree was reported, but the "clinical cures" were sometimes followed by relapse. Patients on the highest dose had a better clinical response than those on lower doses. Urine specimens from many of these patients were analyzed for drug levels and showed a conversion of allopurinol to AR ranging from none to 30% of the dose.

Kager et al. [9] treated 10 patients with kala-azar with 16–24 mg/kg of allopurinol daily. Four of six patients who had previously failed to respond satisfactorily to stibogluconate sodium were designated as cured. In one of these, stibogluconate sodium was added to allopurinol therapy. In another, relapse occurred after an apparent "cure," but response to further allopurinol therapy was observed. Four patients who had received no previous treatment had less satisfactory results with allopurinol therapy.

Chunge et al. [10] treated five patients with visceral leishmaniasis who were unresponsive to stibogluconate sodium with a combination of allopurinol and stibogluconate sodium. The patients were treated for 14–54 d with 20 mg of allopurinol/kg body weight per day in three doses and 20 mg of antimony/kg body weight once or twice daily. Parasitologic cures were achieved in all patients within 19 d and there were no relapses during 1 y of follow-up.

The success of AR as an antileishmanial agent will probably depend on the ability to maintain high blood levels. Phase I studies indicated that the absorption of AR is incomplete (T. A. Shapiro, personal communication). At steady state, 41% of the administered dose could be accounted for in the urine. At 1,500 mg four times daily, plasma levels reached 5–10 μg/ml, the expected therapeutic range, but the half-life is short (3.50 ± 1.09 h) and plasma levels were thought to be too low to assure adequate therapy.

The observation that the renal clearance of AR exceeded the glomerular filtration rate led to the use

of PB in efficacy studies because it has been shown to block the renal tubular transport of a similar organic acid [11]. Plasma concentrations of AR are about two to three times higher when PB is given concurrently (D. Nelson, personal communication). Our data suggest that concurrent administration of PB enhances the therapeutic effect, but the sample size is too small to conclude that the difference between the two groups is significant. The AR + PB group in this study had a cure rate (clinical and parasitologic) of 56% versus 33% in the group given AR alone (not statistically significant).

Although the cure rate was low, an encouraging result of this study was the complete absence of relapses in cured patients. In a recent study of the efficacy of pentavalent antimony in patients with ACL [12], the cure rate was 59% and relapses were common (22%). This result can be compared to the rate for the AR+PB group (56%) in the present study because the patient population was the same, the nature and severity of the lesions was similar, and there was no prior chemotherapy.

The sensitivity of the patient isolates to the two pyrazolopyrimidines and 9-deazainosine indicates that inhibition of leishmania by certain inosine analogues is not simply a laboratory phenomenon. Previous studies have shown that certain inosine analogues have broad antiprotozoan activity [6], but our study is the first to test this observation against organisms recovered immediately from patients. The fact that these fresh isolates have the same range of sensitivities as those studied previously in the laboratory [2–4, 6, 13] suggests that this antiprotozoan activity can be safely explored clinically. Although these sensitivity tests were done with the promastigote form of the organism, the data can be extrapolated to the amastigote: the metabolism of these compounds in the amastigotes is identical to that in the promastigote and the level of sensitivity is the same [3, 14–17]. The tenfold increase in sensitivity of the organisms to 9-deazainosine compared to the two pyrazolopyrimidines is also consonant with previous investigations in vitro and in tissue culture [6, 15]. Sensitivity of leishmania to pyrazolopyrimidines has been shown, in a tissue culture system, to be highly synergistic with pentavalent antimonials [17]. This raises the possibility that pyrazolopyrimidines can be combined with pentavalent antimony in a clinical setting to achieve an improved cure rate while decreasing the toxicity of antimony. A large clinical trial to test this has been organized by one of us (J. J. M.) and is now in progress in Colombia, Peru, and Bolivia under the auspices of the World Health Organization.

Although the cure rate was low, our present results suggest that AR may be a promising agent for the treatment of ACL. Adjustments in dosage or treatment schedule or combination with other agents may be needed to improve efficacy. A controlled study using a higher dose of AR alone and a study of the combination of AR and pentavalent antimony are in progress to assess fully the clinical usefulness of AR.

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