941

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EFFECTIVENESS OF ALLOPURINOL AGAINST *LEISHMANIA BRAZILIENSIS PANAMENSIS* IN *AOTUS TRIVIRGATUS*

BRYCE C. WALTON,* JAMES HARPER III,† AND RALPH A. NEAL

Gorgas Memorial Laboratory, Apartado 6991, Panamá 5, Republic of Panamá, and Protozoology Department, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, United Kingdom

Abstract. Orally administered allopurinol at 50 mg/kg for 21 days showed pronounced antileishmanial activity against experimentally induced lesions of *Leishmania braziliensis panamensis* on the nose of Panamanian *Aotus trivirgatus* monkeys, with complete healing in 4 of 5, although parasitologic cure was achieved in only 2 of 5. The same total daily dose of drug, given in a divided dose twice daily, resulted in complete healing in all 5, and parasitologic cure in 4 of 5 animals. Standard treatment controls receiving 40 mg/kg of antimony stibogluconate intramuscularly for 15 days showed healing in 4 of 5 monkeys. It is not known if a similar level of effectiveness would result with this dose of allopurinol in humans, since *Aotus* may have a different pattern of metabolic conversion of the drug to the more active riboside.

No completely satisfactory treatment is available for American cutaneous/mucocutaneous leishmaniasis. The current drugs of choice,[1] pentavalent antimonials, frequently are not effective. They often show adverse side effects, and have the additional disadvantage of requiring daily injections over a 15- to 21-day period which make it unfeasible to treat patients who live in areas too remote to make daily trips to treatment centers, and who do not have the economic resources for a hospital stay to complete treatment. Thousands of persons suffering from this unpleasant and often incapacitating disease are in this category. An effective orally administered treatment would be of tremendous value in dealing with this difficult situation which represents a pressing public health problem in many countries in Latin America.

In 1974 Pfaller and Marr reported that allopurinol (4-hydroxypyrazolo [3,4-d] pyrimidine) has antileishmanial activity at concentrations which can be obtained in human serum.[2] A series of in vitro experiments confirmed the antileishmanial action of this compound and defined the role of some of its major metabolic products.[3–5] Since allopurinol has been used widely for treatment of hyperuricemia in humans with few toxic side effects, and because its pharmacology is well known, it is an attractive candidate as an oral antileishmanial drug. The knowledge already available would greatly reduce the time and expense necessary to make it generally available for treatment of leishmaniasis.

Prior success in producing cutaneous lesions in *Aotus trivirgatus* by intradermal inoculation of *Leishmania braziliensis braziliensis* (Walton unpublished data), and the presumed similarity of *Aotus* to humans in the metabolic conversion of allopurinol, suggested the use of this model. Inoculation on the tip of the nose for the experimental infection, as developed by Christensen and Vasquez,[6] provides a degree of uniformity in lesion development which is not achieved with other sites, and makes this system feasible for evaluation of drug activity. This study was designed to determine if allopurinol has an in vivo effect against Central American cutaneous leishmaniasis at dosage levels permissible in humans.

MATERIALS AND METHODS

Fifteen adult (average 700–800 g) wild-caught *Aotus trivirgatus* of both sexes from Panamá were used. All had previously been experimentally in-

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* Pan American Health Organization/Adjunct Associate Gorgas Memorial Laboratory. Present address: Trypanosomiases and Leishmaniases, Parasitic Diseases Programme, World Health Organization, 1211 Geneva 27, Switzerland.

† Present address: Infectious Disease Branch, National Institute of Neurologic and Communicative Disorders and Stroke, National Institutes of Health, USDHHS, Bethesda, Maryland 20205.

FIGURE 1. Typical lesion produced by *Leishmania braziliensis panamensis* on the nose of *Aotus trivirgatus* at time of beginning of treatment.

fected with malaria, drug cured, and held in the laboratory for more than a year after release from the malaria program. Animals were weighed and serum was collected and stored. Skin testing with leishmanin antigen, using intradermal inoculation on the eye-lid of 0.1 ml. of antigen at five times the concentration for human use, as well as serologic screening by indirect fluorescent antibody test, was done in an effort to eliminate any animals which might have had a previous natural infection.

A recent human isolate of *Leishmania braziliensis panamensis*, isolated from a cutaneous lesion acquired in Panamá, was used to produce the experimental infections. This stock, designated GML 335-A, was identified as *L. b. panamensis* by the characteristic electrophoretic pattern of isoenzymes,[7] as well as on the basis of growth characteristics in blood-agar medium and the pattern of pathogenesis in hamsters.[8] Promastigotes from an early in vitro subpassage were cryopreserved in liquid nitrogen until being used to inoculate flasks of blood-agar medium[9] to produce promastigotes for the infections. Cultures were harvested at 6–9 days by washing the surface of the agar with TC-199 tissue culture medium. Promastigotes were concentrated by centrifugation, numbers estimated by microscopic examination, and 0.1 ml containing $\pm 3 \times 10^6$ organisms was inoculated intradermally in the tip of the nose by tuberculin syringe and 27-gauge needle. The experimental infections were confirmed by presence of amastigotes in smears from scrapings and by growth in blood agar medium inoculated with needle aspirates taken from the margin of the lesions.

The monkeys were separated into three groups of five animals: A, untreated controls; B, allopurinol treatment; and C, standard antimony treatment control.

Experiment 1

At 71 days post infection, when lesions had evolved into characteristic open ulcers (Fig. 1), treatment was commenced for groups B and C. Allopurinol, supplied by Wellcome Foundation, Great Britain as pure powder, was suspended in 0.3% methyl cellulose and 50 mg/kg was administered by gastric intubation once daily for 21 days. The standard treatment control was sodium stibogluconate (Pentostam) in 100 mg Sb/ml aqueous solution, administered intramuscularly 40 mg/kg $\times$ 15 days. Antileishmanial effect was determined by weekly measurements of entire lesion size, to include all visible evidence of involvement, i.e., depigmentation, swelling, etc. (total lesion), and measurement of only the de-epithelialized portion of the lesion (open ulcer). Two measurements were taken at right angles and expressed as the mean.

Evidence of persistence of viable parasites was obtained by blood agar cultures[9] inoculated from needle aspirates from the margin of lesion or scar at 15, 30 and 60 days post-treatment.

Experiment 2

After the initial post-treatment observation period, the five untreated control animals of group A still had open lesions. These were then designated group D and were treated with the same amount of allopurinol in a divided dose, i.e., 25 mg/kg twice daily for 21 days, and evaluated in a like manner.

RESULTS

Allopurinol, as administered, exhibited a pronounced antileishmanial effect judged by the clin-

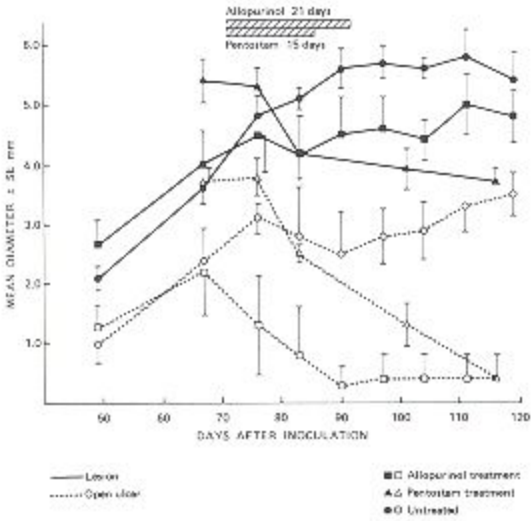

FIGURE 2. Effect of allopurinol and sodium stibogluconate (Pentostam) on lesions due to *Leishmania braziliensis panamensis* in *Aotus trivirgatus*. Experiment 1: Allopurinol was given orally once a day for 21 days at a dose level of 50 mg/kg; sodium stibogluconate was given intramuscularly at 40 mg SB^V/kg for 15 days.

ical improvement of the lesions. As shown in Figure 2, after the 1st week of treatment with 50 mg/kg once daily, both the total lesion diameter and open ulcer diameter in group B, were less than those of the untreated control group, although the differences were not significant ($P = 0.7316$ and 0.369, respectively). At the end of the 21-day treatment period the ulcers had completely re-epithelialized in 4 of the 5 animals, while the ulcers of all of the untreated controls continued to enlarge. The ulcer in the one treated animal (No. 8626) which did not heal had regressed from an open 3 × 4 mm ulcer to a dry 1 × 3 mm scab, but with the borders remaining slightly elevated. However, as shown in Table 1, parasitologic cure was achieved in only 2 of 5 animals; needle aspirate cultures from the raised margin of the scabbed lesion (No. 8626) taken 15 days and 29 days post-treatment were positive, as were cultures taken from a healed lesion at 15 days (monkey No. 8458) and at 60 days from a healed lesion in which small rugose elevated areas remained at the margins (No. 9165). The antileishmanial effect of allopurinol is also demonstrated by means of the very rough quantitation afforded by needle aspirate cultures as shown in Table 2. Nine of 10 tubes inoculated from the untreated animals were positive upon the first examination on day 4, all were positive on day 8, with peak growth being achieved in 9 of 10 tubes. In contrast, the number

TABLE 1

Effect of allopurinol treatment on cultures of material from cutaneous leishmanial lesions in Aotus *monkeys*

Group*	Monkey no.	Culture results—days post-treatment		
		15	29	60
	Experiment 1			
A	8653	+	+	+
	8743	+	+	+
	8797	+	+	+
	9028	+	+	+
	9064	+	+	+
B	8558	+	–	–
	8626	+	+	–
	8740	–	–	–
	8762	–	–	–
	9165	–	–	+
C	8447	–	–	–
	8744	–	–	–
	8802	–	+	–
	9149	–	–	–
	9150	–	–	–
	Experiment 2			
D†	8653	–	–	–
	8743	–	–	–
	8797	–	–	+
	9028	–	–	–
	9064	–	–	–

* Group A, untreated; B, treated with allopurinol, 50 mg/kg for 21 days; C, sodium stibogluconate-treated; D, treated with allopurinol 25 mg/kg twice a day for 21 days.
† Same animals as in Group A in Experiment 1.

of promastigotes in the two positive cultures from the allopurinol treated animals remained very low, most likely indicating that a very small number of parasites was inoculated, and that possibly allopurinol affected the ability to transform and grow as promastigotes.

Regardless of the absence of untreated controls in Experiment 2, it is clear from the rapid healing observed that treatment with the same amount of drug administered twice daily also resulted in rapid clinical improvement of the lesions in group D. Complete healing was achieved in all five monkeys, although in one animal (No. 8797) in which two 1 × 1 mm satellite papules had developed at the borders of the 5 × 7 mm open lesion, the satellites remained raised with a tendency to scab. Needle aspirates taken at 60 days post treatment yielded a positive culture, although aspirates taken at 15 and 29 days were negative (Table 1).

Treatment with sodium stibogluconate in group C resulted in complete healing of lesions in 4 of 5 monkeys. In the animal with incomplete healing (No. 8802), raised borders and a dry scaly scab

TABLE 2

Comparison of cultures of material from cutaneous leishmanial lesions in Aotus *monkeys taken 15 days after treatment with allopurinol with cultures from untreated animals*

Monkey no.	Tube	No. of days in culture* 4	8	12
Untreated animals				
8653	A	−	++	+++
	B	+	++	+++
8743	A	+	++	+++
	B	−	+	++
8797	A	+	++	+++
	B	+	+−	+++
9028	A	+	++	+++
	B	+	−+	−−−
9064	A	+	++	+++
	B	+	++	+++
Allopurinol-treated animals				
8558	*A*	−	−	+
	B	−	−	−
8626	*A*	+	+	++
	B	−	−	−
8740	*A*	−	−	−
	B	−	−	−
8762	*A*	−	−	−
	B	−	−	−
9165	*A*	−	−	−
	B	−	−	−

* −, negative culture; +, <5 organisms per low power field (lpf); ++, 5–25 organisms per lpf; +++, >25 organisms per lpf.

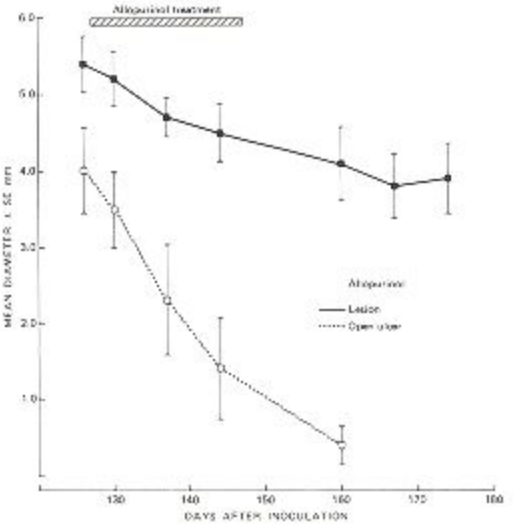

FIGURE 3. Results of daily treatment with allopurinol 50 mg/kg for 21 days given orally in two doses of 25 mg/kg. Experiment 2. The treated monkeys were from the untreated group of the previous experiment (see Fig. 2).

remained after treatment and a positive culture was obtained from an aspirate from the border taken at 29 days. In two other animals positive cultures were obtained at 60 days, although the ulcers remained completely closed and the margins appeared only minimally raised (Table 1).

Although the total lesion size was smaller than in the controls (Experiment 1) or diminished after treatment (Experiment 2) this measurement was not generally statistically significant (Fig. 3). The changes were slight since the measurement included the zone of scarring and depigmentation which remained and was evident in even parasitologically cured animals, so this parameter is not very useful in evaluating drug effect. However, it is clear that allopurinol resulted in significant reduction of diameter of the open ulcers, as did the standard treatment control ($P = 0.0067$).

DISCUSSION

Many antileishmanial drug trials against cutaneous infections in animals have relied upon clinical healing as the only criterion for evaluating drug action. Had the results been similarly judged in this study, the effect of both allopurinol and the standard antimony treatment control would have appeared to be much more favorable. The use of needle aspirate cultures provided the most definitive criteria by detecting viable residual parasites in cases where complete clinical healing is achieved. However, the inconsistency of results between culture attempts at 15, 29 and 60 days, in some cases, indicates that this technic is not absolutely dependable for detecting very small numbers of surviving amastigotes. More accurate quantitation of the number of residual viable parasites could be achieved by the use of standard size biopsies instead of needle aspirates. However, the resulting alteration of the very small lesion could affect the course of development of the lesion and would introduce another variable factor which it is considered would offset the value of the procedure. These difficulties highlight the difficulties involved in antileishmanial drug testing.

These data should not be interpreted as a comparison of allopurinol and sodium stibogluconate, due to the small number of observations. The value of these observations is to show that the *L. b. panamensis* infections in *Aotus trivirgatus* do respond to the antimonial compound. Sera were collected pre- and post infection, and serially pre- and post-treatment on all monkeys for IFA testing

to determine if monitoring of changes in antibody level might be of use in evaluating treatment.[10] However, as the majority of the experimental infections elicited only minimal level titers and erratic responses were encountered in testing the post-treatment series, this procedure proved to be of no value in assessing treatment response. It is considered that the poor quality of the anti-monkey conjugate used contributed in large measure to the difficulties experienced with the IFA testing.

In the in vitro studies,[2–5] it was determined that the metabolic conversion by the parasite of allopurinol to allopurinol riboside is a key step, since this intracellular product exerts the toxic effect on the parasite. In the human host there is also some conversion to riboside, although the major metabolic breakdown is to oxipurinol, a compound with little or no antileishmanial activity. So far there have been no reports of significant antileishmanial activity of allopurinol in the usual rodent models. Possibly this is due to the almost total breakdown of allopurinol to the inactive oxipurinol by the rodent. It is possible that conversion to riboside by the host, as well as that converted by the parasite itself, enhances the antileishmanial action of allopurinol, and perhaps is necessary to achieve a practical therapeutic effect. The activity of allopurinol in *Aotus trivirgatus* may reflect a different pattern of metabolism of the drug by the simian host. If this is true, the same doseage of allopurinol in man may not produce the same level of antileishmanial activity in man. It remains to be established how similar the drug metabolism of *Aotus* is to the pattern in man.

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