Activity of Oral Atovaquone Alone and in Combination with Antimony in Experimental Visceral Leishmaniasis

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BALB/c mice with established visceral infection caused by the intracellular protozoan *Leishmania donovani* were treated with oral atovaquone for 7 days. Treatment with 100 mg/kg of body weight per day was optimal and halted parasite replication in the liver. In mice treated with atovaquone, the effect of a suboptimal dose of pentavalent antimony was converted from partially leishmanistatic to leishmanicidal. These results demonstrate the *in vivo* antileishmanial effect of atovaquone and suggest a potential role for this oral agent in visceral leishmaniasis as an adjunct to conventional antimony treatment.

Conventional therapy for visceral leishmaniasis (kala-azar), a disseminated intracellular protozoal infection, consists of daily injections of pentavalent antimony given for a prolonged period of ≥ 28 days (4). Thus, it is not surprising that identification of an antileishmanial agent which can be administered orally and that can induce activity in patients with kala-azar represents a high treatment priority in this worldwide disease. Among several oral antiprotozoal agents tested during the past decade (4), at least one, an 8-aminoquinoline (designated WR6026), appears to have some promise, as judged by recent results from an initial field trial in Kenya (14).

Atovaquone, an hydroxynaphthoquinone now in clinical use in both oral and parenteral form in patients with toxoplasmosis and *Pneumocystis carinii* infection, is also active experimentally against other protozoa as well (1, 3, 5, 6, 12). Toward *Leishmania donovani*, an agent of visceral leishmaniasis, for example, Jernigan and colleagues (7) reported that clinically achievable levels of atovaquone exert a direct microbicidal effect against the extracellular promastigote form. Although the latter effect was not observed by Croft and coworkers (3), *in vitro* activity was demonstrated for atovaquone in macrophages parasitized by the more relevant intracellular amastigote form of *L. donovani*. In addition, those investigators also found that in infected mice, atovaquone administered subcutaneously (but not orally) resulted in moderate inhibition of visceral *L. donovani* replication (3).

Given this background and in view of the interest in identifying an active oral agent, we elected to retest atovaquone *in vivo* and to extend our experimental analysis of treatment for visceral leishmaniasis (9, 10) to atovaquone in combination with conventional pentavalent antimony.

MATERIALS AND METHODS

Visceral infection. Female BALB/c mice (weight, 20 to 30 g; Charles River Laboratories, Wilmington, Mass.) were infected via the tail vein with 10^7 *L. donovani* amastigotes (1 Sudan strain) obtained from infected hamster spleen homogenates (9). The course of visceral infection was assessed microscopically by using stained liver imprints. Liver parasite burdens, expressed as Leishman-Donovan units (LDU), were calculated as the number of amastigotes per 1,000 liver cell nuclei $\times$ liver weight (in grams) (9).

Treatment. Two weeks after challenge (day 0), liver burdens were determined, and groups of three mice each received no further treatment or began once-daily treatment by gavage with 0.1 ml of commercial vegetable oil alone or oil con-

taining atovaquone. The drug was generously provided by Michael Rogers (Glaxo Wellcome Co., Research Triangle Park, N.C.) in powder form and was prepared daily in vegetable oil to deliver 50, 100, or 200 mg/kg of body weight per dose. In a previous study in rats (6), treatment by gavage with 50 and 100 mg of atovaquone per kg yielded mean peak levels in plasma of 71 and 74 $\mu\text{g/ml}$, respectively. Studies in mice have demonstrated steady-state concentrations in plasma of 22 ± 8 and 53 ± 7 $\mu\text{g/ml}$ (mean $\pm$ standard deviation) after 6 days of once-daily oral treatment with 50 and 200 mg/kg, respectively (19).

All mice were sacrificed after 1 week of daily treatment (day 7). Liver parasite burdens in treated mice on day 7 were compared with the burdens in controls on day 0 to determine parasite killing; day 7 burdens in treated mice were compared with the day 7 burdens in controls to determine the inhibition of parasite replication (9, 10). A two-tailed Student's *t* test was used to analyze differences in liver parasite burdens.

In separate experiments designed to test the effect of combining atovaquone with antimony, we used a well-described, convenient model in which animals receive a single injection of pentavalent antimony (9, 10). (Conventional treatment for human visceral leishmaniasis consists of daily injections of pentavalent antimony, usually for 4 weeks [4].) In these experiments, groups of mice infected for 2 weeks were treated starting on day 0 with (i) atovaquone alone (100 mg/kg/day for 7 days), (ii) a suboptimal dose (10 mg/kg) of pentavalent antimony alone (sodium stibogluconate; Pentostam; Burroughs Wellcome) given on day 0 as a single intraperitoneal injection (9), or (iii) atovaquone plus antimony by the preceding regimens. Controls consisted of untreated mice and animals treated with a suboptimal dose of antimony plus vegetable oil. An additional group of mice was treated on day 0 with a single injection of 50 mg of pentavalent antimony per kg alone (9). Liver parasite burdens were determined in all mice on day 7.

RESULTS AND DISCUSSION

Effect of atovaquone alone. Mice were left undisturbed for the first 2 weeks after challenge and then were treated with atovaquone once daily for 7 consecutive days. During this 7-day period, liver parasite burdens doubled in untreated and oil-treated controls (Table 1). As also shown in Table 1, while atovaquone at 50 mg/kg, the lowest dose used, induced some antileishmanial effect, treatment with 100 mg/kg arrested the intracellular hepatic replication of *L. donovani*. Doubling the dose to 200 mg/kg yielded no additional effect. These results differ from those reported by Croft and colleagues (3), who found that in a similar model of *L. donovani* infection, atovaquone (100 mg/kg/day for 5 days) had moderate inhibitory activity when it was administered subcutaneously but had no effect when it was administered in oral form. A separate strain of *L. donovani* and a different formulation and vehicle for atovaquone were used in our study and may account for these contrasting results.

Effect in combination with antimony. In contrast to the killing of visceral *L. donovani* induced by pentavalent antimony treatment in this model (9, 10), atovaquone's effect was leishmanistatic (Table 1). To test whether we could nevertheless

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TABLE 1. Antileishmanial effect of atovaquone^a

Treatment (dose [mg/kg])	Liver parasite burden (LDU) on day 7	% Killing
A^b		
None	2,138 ± 96	0
Oil	2,215 ± 212	
Atovaquone		
50	1,753 ± 102	
100	1,134 ± 62 ^c	0
200	1,205 ± 86 ^c	0
B^d		
None	2,396 ± 130	0
Atovaquone	1,358 ± 88 ^c	
Antimony (10)	1,717 ± 100	0
Antimony + atovaquone	837 ± 59 ^e	31
Antimony + oil	1,846 ± 98	0
Antimony (50)	731 ± 60 ^e	40

^a Two weeks after *L. donovani* challenge (day 0), mice receiving treatment A were treated once daily for 7 days with the indicated concentration of drug. For mice receiving treatment B, starting on day 0, mice were treated for 7 days with atovaquone alone (100 mg/kg/day), a single injection of 10 mg of pentavalent antimony per kg alone or pentavalent antimony and then 7 days of daily atovaquone or vegetable oil, or a single injection of 50 mg of antimony per kg given on day 0. Results are means ± standard errors of the means for six mice per group from two experiments.

^b The liver parasite burden on day 0 for mice in treatment group A was 1,093 ± 58 LDU.

^c *P* < 0.05 versus the result on day 7 for untreated controls.

^d The liver parasite burden on day 0 for mice in treatment group B was 1,210 ± 66 LDU.

^e *P* < 0.05 versus the results for untreated controls on both day 0 and day 7.

take advantage of such an effect, mice infected for 2 weeks were treated with a suboptimal dose of antimony (9) plus full-dose atovaquone (100 mg/kg). The results in Table 1 indicate that in mice treated with atovaquone, the effect of 10 mg of pentavalent antimony per kg was converted from partially leishmanistatic to leishmanicidal. The latter effect was comparable to that induced by treatment with fivefold more antimony (50 mg/kg) used alone (Table 1).

In addition to documenting the fact that orally administered atovaquone has measurable in vivo antileishmanial activity, these results also raise the possibility that atovaquone may have a future role in visceral leishmaniasis as an adjunct to conventional antimony treatment. This strategy, combining antimony with a second injected agent including an additional antibiotic (2, 13, 18) or a pro-host defense cytokine such as gamma interferon (15, 16), has been developed in part as an effort to offset the declining efficacy of pentavalent antimony in kala-azar (11). The notion that a well-tolerated oral agent such as atovaquone (5) may (i) enhance the effect of or permit a reduction in the dose or duration of initial antimony treatment or (ii) perhaps be of use in retreatment regimens in patients who fail to respond to or relapse after antimony therapy has obvious clinical appeal. The appeal of such a combination regimen would be particularly high in areas of the world such as India, where, despite recommendations that initial treatment consist of daily injections of pentavalent antimony for 30

to 40 days (4, 17), failure rates of 30 to 40% are now being encountered in patients with kala-azar (8, 16).

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