Suppression of Posttreatment Recurrence of Experimental Visceral Leishmaniasis in T-Cell-Deficient Mice by Oral Miltefosine

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T-cell-deficient nude mice infected with Leishmania donovani were treated with miltefosine and then given either no treatment or intermittent miltefosine. Intracellular visceral infection recurred in untreated mice but was suppressed by once- or twice-weekly oral administration of miltefosine. Miltefosine may be useful as oral maintenance therapy for T-cell-deficient patients with visceral leishmaniasis.

In an experimental model of visceral leishmaniasis caused by the intracellular protozoan Leishmania donovani, the activities of a new oral antileishmanial agent, hexadecylphosphocholine (miltefosine) (6, 17, 18), in T-cell-deficient athymic (nude) and T-cell-intact euthymic mice proved comparable (12). This result raised the possibility that miltefosine may have a role as an initial oral treatment approach to the growing problem of AIDS-associated visceral leishmaniasis (kala-azar) in CD4 cell-depleted patients (1, 4, 11).

A second, related therapeutic issue in this clinical setting is relapse of infection once any initially effective treatment is discontinued (1, 2, 7, 8, 11, 13, 15; R. N. Davidson and R. Russo, Letter, Clin. Infect. Dis. 91:560, 1994). Since successful host defense against this disease, including the prevention of posttreatment relapse, is T-cell dependent and driven by CD4 (Th1) cell-derived cytokines (9, 11, 13, 14), it is not surprising that recurrence of AIDS-related kala-azar is predictable if therapy is stopped (1, 2, 7, 8, 11, 13, 15; Davidson and Russo, Letter, Clin. Infect. Dis., 1994). This study extended the analysis of miltefosine by testing whether it may also be useful as a long-term treatment to prevent recrudescence of visceral infection in the T-cell-deficient host.

Materials and Methods. (i) Animals and visceral infection. Athymic (nude) BALB/c mice (20 to 30 g) (Charles Rivers Laboratories, Wilmington, Mass.) were injected via the tail vein with 1.5 × 10⁷ hamster spleen-derived L. donovani amastigotes (one Sudan strain) (12). Visceral infection was monitored microscopically using Giemsa-stained liver imprints, and liver parasite burdens were measured by calculating, in a blinded fashion, the number of amastigotes per 500 cell nuclei multiplied by the liver weight (in milligrams) (Leishman-Donovan units [LDU]) (12). The histologic reaction in the liver was assessed using formalin-fixed, stained tissue sections (12).

(ii) Initial and maintenance treatment. Two weeks after L. donovani challenge, liver parasite burdens were determined for 4 of 28 infected mice, and then the animals received either no treatment (n = 4) or oral miltefosine by gavage (n = 20). Miltefosine, generously provided as a powder by ASTA Medica AG (Frankfurt, Germany), was dissolved in tap water and administered once daily at 25 mg/kg of body weight in a volume of 0.3 ml for five consecutive days (12). Two days after treatment ended, four untreated and four treated mice were sacrificed. LDU at this point (week 3) were compared to initial LDU (at week 2) to determine the extent of initial parasite killing (12). The remaining 16 treated mice were randomly divided into four groups to receive, for the next 9 weeks, either no further treatment or single doses of miltefosine (25 mg/kg) given twice weekly, once weekly, or every 2 weeks. Twelve weeks after infection, liver parasite burdens were determined for all animals.

Results and discussion. Between week 2 and week 3, the period during which miltefosine was initially administered, liver parasite burdens increased in untreated nude mice from 2,124 ± 269 (Fig. 1) to 2,994 ± 212 LDU (mean ± the standard error of the mean [SEM]) (n = 8, data not shown). In mice treated for 5 days, LDU (mean ± SEM) at week 3 were reduced to 496 ± 87, indicating 77% initial killing 1 week after therapy began (Fig. 1). Treated mice were then given either no additional miltefosine or single doses twice per week, once per week, or every second week for the next 9 weeks. As shown in Fig. 1, parasite replication resumed in treated mice that were given no additional drug, and liver burdens at week 12 were 3.5-fold higher than those at week 3. While miltefosine admin-

FIG. 1. Initial effect of miltefosine treatment in nude BALB/c mice and prevention of subsequent relapse. Two weeks after infection, nude mice were initially treated with drug for 5 days. Two days later (end of week 3), treated mice either were given no further drug or received single doses every second week, once weekly, or twice weekly for the next 9 weeks. Results are from two experiments and are means ± SEMs for seven to eight mice per group.
stered biweekly did not prevent recurrence of visceral replication, treatment given once or twice weekly was clearly active in suppressing infection for the duration of the experiment. Histologic examination of the livers at week 12 confirmed in suppressing infection (15); however, monthly

pentamidine injections (7) and daily oral allopurinol use appears to have little effect (15).

While it still needs to be tested in patients coinfected with human immunodeficiency virus, miltefosine is remarkably active in kala-azar (6, 17, 18) and has additional advantages: (i) a long circulating half-life (~8 days) (17) and (ii) experimental antileishmanial efficacy which is direct and does not require a host T-cell-dependent response for full expression (12). The results reported here suggest that miltefosine has promise as a convenient oral treatment to suppress or prevent relapse of visceral infection in the T-cell-deficient host.

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REFERENCES