Putative Anticryptosporidial Agents Tested with an Immunodeficient Mouse Model

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Lasalocid, sinefungin, and dehydroepiandrosterone were tested for anticryptosporidial activity with an immunodeficient mouse model at doses that have been reported effective when tested with immunosuppressed rodent models. Small but significant reductions in oocyst excretion were only observed under some conditions with lasalocid and dehydroepiandrosterone, but sinefungin had no effect.

Cryptosporidiosis is an enteric protozoan parasitic disease of animals and humans that is self-limiting in the immunocompetent host, but it may cause persistent diarrhea and severe malabsorption in the immunodeficient host (5, 17). Chronic cryptosporidiosis makes a contribution to the morbidity and mortality of a significant number of AIDS patients (10), with the result that finding effective anticryptosporidial chemotherapeutic agents has become a major priority. In spite of a number of promising preliminary clinical reports (1, 12), no effective agent has been found (17).

Several immunodeficient and immunosuppressed animal models have been employed to study the pathophysiology of the Cryptosporidium infection and to evaluate the efficacies of potential antiparasitic agents (3, 4, 8, 9, 11, 13–16, 18, 19). Steroid-immunosuppressed rats and mice have been extensively used in drug evaluation studies, and several agents have been found to be effective with such animal models (3, 4, 8, 9, 13–16). Such studies have been undertaken with short-term infections (3 weeks or less), with the agents often being administered a few hours before or after the inoculation of infecting oocysts or prior to the time of maximum oocyst excretion. The present study compares the anticryptosporidial effects of such agents by using a chronic immunodeficient mouse model rather than an immunosuppressed rodent model.

**Immunodeficient mouse model.** Young adult intact or splenectomized male athymic nude mice were purchased from Harlan Sprague-Dawley Inc. (Indianapolis, Ind.). Cryptosporidium parvum, originally of calf origin, has been carried in a colony of these animals for over 2 years. Oocysts were isolated from the feces of infected mice (2) and administered per os. Oocyst excretion was monitored by collecting five fresh fecal pellets, macerating them in 2 ml of neutral formalin, and counting the number of oocysts visualized in a 10-μl sample of this homogenate by a commercially available immunofluorescent assay (Meridian Diagnostics Inc., Cincinnati, Ohio). The following oocyst excretion score was developed: based on the oocyst counts of the 10-μl samples, 0, no oocysts; 1, <10 oocysts; 2, 11 to 50 oocysts; 3, 51 to 100 oocysts; 4, >100 oocysts.

When a histological study was performed with chronically (>3 months) infected nude mice, linear relationships were found between the oocyst excretion score (y) and the percentage of infected ileal villus cells (x_r), described by the equation

\[ y = 0.76 + 0.092x_r \quad (r = 0.888) \]

and between the oocyst excretion score and the percentage of infected ileal crypt cells (x_c), described by the equation

\[ y = 0.06 + 0.06x_c \quad (r = 0.926) \]

An inverse linear relationship was observed between the oocyst excretion score (y) and the villus-to-crypt ratio (x_v), described by the equation \[ y = 8.58 - 3.93x_v \quad (r = 0.886) \].

The most severe histological changes were associated with the excretion of large numbers of oocysts and a heavy parasite load. A correlation has also been observed between oocyst excretion and the degree of intestinal infection in AIDS patients (6). This nude mouse model of cryptosporidiosis was characterized by a inoculum-oocyst-number-dependent oocyst excretion that began within 1 week with large inocula (>10^5 oocysts) but was not detected for 4 weeks with small inocula (e.g., 10^2 oocysts). Oocyst excretion increased with time and stabilized at a value that was relatively constant for that animal. In contrast, with immunosuppressed rodent models oocyst excretion began in a few days and peaked within 2 to 3 weeks (9). Immunodeficient animals fell into a low (0 to 2) or a high (4) oocyst excretion score group. In addition to reflecting the level of small-intestinal infection, this scoring system tended to blunt the day-to-day variation in numbers of excreted oocysts observed with all animals. Oocyst excretion scores remained relatively constant after 3 months postinoculation. Infected animals remained alive for up to 15 months. Death was usually associated with biliary tree infection and precipitous weight loss. Splenectomized animals could be infected with a smaller number of oocysts and generally had a higher oocyst excretion score than did intact animals. Splenectomy has been used to exacerbate parasitic infections (7) and in tumor transplantation studies to reduce residual immunity in nude mice (20).

In the present study, agents were used that had been demonstrated to possess anticryptosporidial activity with immunosuppressed rat and mouse models (3, 4, 8, 9, 13, 14). Three agents were tested here, the ionophore lasalocid, the steroid hormone dehydroepiandrosterone (DHEA), and the natural nucleoside antibiotic sinefungin.

Lasalocid was administered orally twice daily (120 mg/kg/day) to animals for 3 days, beginning 18 h after the administration of 10^5 oocysts. This treatment prevented the establishment of infection in prednisalone-immunosuppressed mice (8) but only delayed oocyst excretion in the nude mouse model (Fig. 1a). These data were analyzed by a two-way analysis of variance followed by post-hoc Tukey's protected t tests. The lasalocid-treated and untreated groups only differed significantly on the first day the oocyst excretion score was measured (day 6). When the same lasalocid treatment regimen was used...
FIG. 1. (a) Effects of lasalocid treatment for 3 days on oocyst excretion scores following oral inoculation with $10^7$ oocysts. The asterisk indicates the difference between untreated and lasalocid-treated groups ($P < 0.05$). (b) Effects of lasalocid treatment (120 mg/kg/day) for 3 days on the oocyst excretion scores of intact and splenectomized nude mice infected with *C. parvum* for more than 3 months. The asterisk indicates the difference between the mean score on that day and the score on day 0 ($P < 0.05$). (c) Effects of lasalocid and sinefungin treatments for 8 days on the oocyst excretion scores of animals infected with *C. parvum* for more than 3 months. For all panels, error bars indicate standard deviations.

in intact animals and splenectomized animals that had been infected more than 3 months previously, there was a significant decrease in the oocyst excretion score in both animal groups towards the end of the 3-day drug treatment (Fig. 1b). In this experiment, animals acted as their own controls and data were analyzed by an analysis of variance of repeated measures followed by post-hoc Tukey’s protected $t$ tests. This dosage of lasalocid (120 mg/kg/day) was close to the 50% lethal dose of this drug and could not be used for longer than 3 days without loss of some of the animals. A lower dose of lasalocid has been found to be effective against *Cryptosporidium* infections in immunosuppressed rats when administered for a longer period of time, as has the same dose of sinefungin (4, 9). When these lasalocid and sinefungin regimens were used with the immuno-deficient mouse model, no significant infection effects were observed when assessed by using oocyst excretion scores (Fig. 1c).

The steroid hormone DHEA has been shown to be effective against *Cryptosporidium* infections in immunosuppressed rats (13, 14). When administered subcutaneously twice a day (120 mg/kg/day) for 8 days, this hormone significantly lowered the oocyst excretion score for a group of animals that had been infected 5 weeks earlier, but it failed to significantly reduce the score for animals that had been infected more than 3 months earlier (Fig. 2a). This difference in the effectiveness of DHEA observed between the animals in the 5-week and the >3-month-groups may simply have been due to the fact that the
parasite load was greater in the latter group. From the linear regression equations given above, one would predict that the animals in the >3-month group would have approximately 10% more ileal crypt cells and approximately 6% more villus enterocytes infected than the animals in the 5-week group. Slow release, 21-day DHEA pellets (Innovative Research Inc., Toledo, Ohio) were implanted subcutaneously in a group of animals that had also been infected for more than 3 months. The estimated continuous DHEA release in these animals was 160 mg/kg/day. This dosage and method of DHEA delivery also had no significant effect on oocyst excretion scores (Fig. 2b). The lack of an effect in this case could have been due to a combination of the factors responsible for the lack of an effect of the 120-mg/kg/day dosage in the animals with long-term infections and receptor downregulation resulting from the continuous hormone administration.

The present study indicates that the apparent anticyryptosporidial activity of an agent depends on the model system used to test for such activity. When testing was performed with an immunodeficient mouse model of cryptosporidiosis, agents were less effective than they were when tested with an immunosuppressed rat or mouse model. This is not surprising given that steroid-immunosuppressed rodents retain some residual T-cell responsiveness and require continued immunosuppression to maintain the infection (e.g., see reference 14), while athymic mice have almost a complete absence of CD4+ T cells and have life-long infections (19). In addition, it was more difficult to demonstrate an anticyryptosporidial effect against a well-established, long-term infection than against a recently produced parasite infection, perhaps because of the heavier parasite load with the former.

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REFERENCES