NOTES

In Vitro Susceptibilities of *Entamoeba histolytica* to Azithromycin, CP-63,956, Erythromycin, and Metronidazole

JONATHAN I. RAVDIN* AND JOANNA SKILOGIANNIS

Divisions of Clinical Pharmacology, Geographic Medicine, and Infectious Diseases, Departments of Medicine and Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Received 28 December 1988/Accepted 8 March 1989

Current therapy of *Entamoeba histolytica* infection requires use of multiple agents effective at different body sites, including the intestinal lumen, intestinal tissue, and liver. Azithromycin and CP-63,956, new extended-half-life macrolides which reach high levels in tissue, exhibit in vitro antiamebic activity at 18 or 48 h of incubation at concentrations comparable to that of erythromycin and slightly higher than that of metronidazole. Azithromycin and CP-63,956 have the potential to be useful therapeutic agents for all types of *E. histolytica* infection.

*Entamoeba histolytica* infects approximately 10% of the world population, causing up to 50 million cases of invasive amebiasis and 100,000 deaths per year (18). *E. histolytica* can cause asymptomatic intestinal infection, acute amebic dysentery, chronic localized intestinal amebiasis, chronic noduleseric colitis (mimicking inflammatory bowel disease), and amebic liver abscess (13). Groups at high risk of infection in the United States include travelers to areas of endemic infection, sexually active male homosexuals (9), individuals with acquired immune deficiency syndrome (16), institutionalized mentally retarded patients (17-19), and emigrants from areas of endemic infection such as Mexico (13). Therapy of invasive amebiasis presently requires use of a luminal agent, such as diloxanide furoate, diiodohydroxyquin, or paromomycin, in combination with a tissue-active agent, such as the nitroimidazoles metronidazole and tinidazole or the more toxic emetine derivatives, including dehydroemetine (10). Erythromycin and tetracycline, reported to be effective in treatment of amebic colitis, are not recommended for eradication of parasites in the intestinal lumen or the liver (1). Availability of a single agent efficacious against all forms of amebiasis and with low levels of toxicity would be a major therapeutic advance, especially if the drug was safe to use during pregnancy.

Azithromycin (CP-62,993) and its analog CP-63,956 are new macrolide antibiotics which differ in structure from erythromycin by a methyl-substituted nitrogen in the macrolide ring (14). Compared with erythromycin, azithromycin has greater stability at low pH, a prolonged elimination half-life of up to 21.0 h, and up to 10-fold-enhanced tissue penetration in studies of experimental animals (5, 14). Azithromycin successfully treated experimental anaerobic bacterial liver abscess in mice (5). The long half-life, ease of oral administration, tissue distribution, and excellent therapeutic index with the low levels of toxicity characteristic of macrolide antibiotics suggest that new agents such as azithromycin might be useful for treatment of all forms of *E. histolytica* infection.

Axenic *E. histolytica* trophozoites (strain HM1:IMSS) were maintained in culture with TYI-S-33 (Biosate, iron, and serum) with penicillin (100 U/ml) and streptomycin sulfate (100 μg/ml) (all from GIBCO Laboratories, Grand Island, N.Y.) as described by Diamond et al. (3). At 48 to 72 h following subculture, trophozoites were harvested by chilling the tube and adjusted to a concentration of 5 × 10⁵ amebae per ml in culture medium containing 10% serum.

Amebae were placed in microdilution wells in 200 μl (total volume) of culture medium with or without the desired concentration of drug to be tested. Azithromycin and CP-63,956 were supplied by Pfizer Inc., New York, N.Y.; erythromycin base (activity 94.2%) was also supplied by Pfizer. Metronidazole hydrochloride (Searle Pharmaceuticals Inc., Chicago, Ill.) was reconstituted in culture medium. The microdilution plates were placed in a chamber filled with 3% O₂, 3% CO₂, and 94% nitrogen (2) for 18 or 48 h of incubation at 37°C. The parasite inoculum per microdilution well was 5 × 10⁴ for 18 h of incubation and 6 × 10⁴ for 48 h of incubation. These inocula were selected because they resulted in optimal parasite survival in control wells and adequate numbers of amebae for hemacytometer counts at the end of the incubation. Aqueous trypan blue solution (4.0%; 20 μl) was added to each well; the viable trophozoites which excluded trypan blue were counted by aspiration of cells and use of a hemacytometer chamber. The data are expressed as percentage of viability in experimental wells with drug present versus viability in control wells; viability (percent of control) = (number of viable amebae in experimental well at the end of incubation/number of viable amebae in control well at the end of incubation) × 100. At least six observations in each of two experiments were made for each drug concentration at both 18 and 48 h of incubation. Statistical comparisons of difference for the paired data were performed by using the Student *t* test.

After 18 h of incubation, the mean absolute number of amebae in control wells increased from 5 × 10⁴ to 2.7 × 10⁶. Therefore, a reduction in viability in the presence of drug to 19% of control value equalled a complete inhibitory drug effect. Azithromycin or CP-63,956 at ≥20 μg/ml resulted in inhibition of *E. histolytica* growth after 18 h (*P < 0.01; Table 1); at ≥40 μg/ml there was complete inhibition of growth.

* Corresponding author.
After 18 h of incubation, no amebae were detectable (<10³) at ≥100 μg of azithromycin or CP-63,956 per ml (Table 1). In vitro activity of erythromycin was approximately equal to that of azithromycin, with complete inhibition of growth observed at ≥50 μg/ml. Metronidazole, in comparison, demonstrated an inhibitory effect at ≥10 μg/ml, with complete inhibition of growth observed at ≥20 μg/ml (Table 1).

When a smaller parasite inoculum (6 × 10⁴) and longer incubation period (48 h) were used, growth to a mean of 6.7 × 10⁴ amebae was observed; therefore, complete inhibition of growth occurred at 9% of the control value. Azithromycin and CP-63,956 demonstrated significant inhibitory effects over 48 h at ≥20 μg/ml, with complete inhibition of growth at ≥40 μg/ml (Table 1). The inhibitory activity of erythromycin was observed at concentrations identical to that of azithromycin (Table 1). Metronidazole was completely inhibitory at ≥10 μg/ml, demonstrating antiamebic activity at lower concentrations compared with those used in the first set of experiments, which used a larger parasite inoculum and shorter (18-h) incubation period (Table 1).

These studies demonstrate that azithromycin and CP-63,956 have an in vitro activity against *E. histolytica* trophozoites equal to those of erythromycin and, at 18 h, metronidazole. Complete inhibition of parasite growth was observed at ≥40 μg of azithromycin per ml, and at least a 90% amebicidal effect was present at 100 μg/ml. After a 50-ng/kg (body weight) oral dose, azithromycin reached sustained peak concentrations of >70 μg/ml in rat liver compared with <10 μg/ml for erythromycin (5), indicating a potential clinical relevance for the static and amebicidal activities of azithromycin.

In comparison to our findings, Cedeno and Krogstad (2) reported that the 50% growth inhibitory concentration of erythromycin was 24 μg/ml for *E. histolytica* HK9. They found that metronidazole at 1.0 μg/ml provided complete inhibition of amebic growth at 48 h of incubation (2). Eubank and Reeves (4) observed with strain H200:NIH a static effect for metronidazole at 1.7 μg/ml. We utilized strain HM1:1IMSS, which is among the most virulent available in axenic culture. Instead of the radiometric method of Cedeno and Krogstad (2), we preferred to use direct quantitative observations with trypsin blue exclusion criteria to determine parasite viability.

Therapy of amebiasis remains problematic; metronidazole is effective in all tissues but requires at least 10 days at high dosage to eradicate luminal amebae (6, 12, 18). Metronidazole also has substantial gastrointestinal side effects and raises concerns about carcinogenesis and teratogenesis (reviewed in references 7 and 11). Single-dose regimens for treatment of amebiasis with metronidazole or tinidazole have been reported (8, 15), but these regimens are not without drawbacks or toxicity. The extended half-life of azithromycin, the high concentrations which the drug reaches in tissues such as liver, its stability in stomach acid, its high fecal concentrations, and the low level of clinical toxicity observed with other macrolides such as erythromycin suggest that azithromycin may be useful as a single agent for therapy of amebiasis. The in vitro results reported here indicate a need for the study of azithromycin with animal models of invasive amebiasis and luminal intestinal infection, which would further evaluate the potential of azithromycin treatment of human amebiasis.

This work was supported by a grant from Pfizer Inc.

### LITERATURE CITED


