Intermittent Azithromycin for Treatment of *Mycobacterium avium* Infection in Beige Mice

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The activity of azithromycin (AZI) was evaluated in the beige mouse model of disseminated *Mycobacterium avium* infection. Mice were infected intravenously with approximately 10⁷ viable *M. avium* ATCC 49601. AZI at 50, 100, or 200 mg/kg of body weight or clarithromycin (CLA) at 200 mg/kg was given by gavage 5 days per week for 4 weeks. Groups of treated mice were compared with untreated control animals. A dose-related reduction in cell counts in organs was observed with AZI treatment. AZI at 200 mg/kg was more active than CLA at 200 mg/kg against organisms in spleens. The activities of these two agents at 200 mg/kg were comparable against organisms in lungs. In a second study, AZI at 200 mg/kg was given daily for 5 days; this was followed by intermittent AZI treatment for the next 3 weeks. The activities of AZI given on a three-times- and five-times-per-week basis in the continuation phase were comparable. AZI given on a once-weekly basis was less active. The regimen of AZI given in combination with rifapentine on a one-weekly basis for 8 weeks showed promising activity. Clinical evaluation of AZI and rifapentine will help to define the roles of these agents in the treatment of disseminated *M. avium* complex infection.

Azithromycin (AZI) and clarithromycin (CLA) are promising new agents for the treatment of disseminated *Mycobacterium avium* complex (MAC) infection in individuals with AIDS (7, 19, 23). We have previously reported on the activities of AZI and CLA in the beige mouse test system (5, 17) and have compared the in vivo activities of these two agents against eight MAC isolates (6). Comparative trials of AZI and CLA in combination with other agents in patients with disseminated MAC infections will be necessary to determine any clinically significant differences in their efficacies and tolerances in this patient population.

One pharmacokinetic advantage of AZI and CLA in the treatment of disseminated MAC infection is the high concentration of drug achieved in tissues (2, 14, 21). AZI also has a long half-life in serum and tissues, making it potentially useful for intermittent administration (9, 10).

The purpose of the present study was to compare the activities of AZI and CLA over a longer treatment period than we used in our previous studies and to explore the activity of AZI administered on an intermittent basis against MAC in the beige mouse test system. The activity of AZI alone and in combination with rifapentine (RPT) against MAC was assessed.

**MATERIALS AND METHODS**

**Drugs.** AZI was provided by Central Research Division, Pfizer, Inc., Groton, Conn. CLA was provided by Abbott Laboratories, Abbott Park, Ill. RPT was provided by Merrell Dow Research Institute-Lepetit Research Center, Geranzano, Italy. AZI and CLA were dissolved in absolute ethanol, with subsequent dilution in distilled water. RPT was dissolved in dimethyl sulfoxide (DMSO), with subsequent dilution in distilled water. The final concentration of ethanol or DMSO in the drug preparations was 0.5%. Drugs were freshly prepared each morning prior to administration.

**Isolate.** *M. avium* ATCC 49601 (serotype 1) was obtained as a clinical isolate from a patient with AIDS at the State University of New York Health Science Center in Syracuse. This isolate has been used previously in beige mouse studies in our laboratory (5, 6, 17). The MICs of AZI and CLA were determined in modified Mueller-Hinton broth (pH 7.4) supplemented with 5% Middlebrook oleic acid-albumin-dextrose-catalase (OADC) enrichment (Difco Laboratories, Detroit, Mich.) (11, 20). The MIC of RPT was determined in modified 7H10 broth (7H10 agar formulation with agar and malachite green omitted; pH 6.6) supplemented with 5% OADC enrichment (4). The MICs of AZI, CLA, and RPT (in micrograms per milliliter) for *M. avium* ATCC 49601 are 8, 4, and 0.125, respectively. A cell suspension with a predominantly (>95%) transparent colonial morphology was used for MIC determinations and infection. The organisms were passaged through beige mice every 3 months to maintain the virulence of the organisms.

**Media.** The organisms were grown in modified 7H10 broth with 10% OADC enrichment-0.05% Tween 80 on a rotary shaker at 37°C for 3 days. The culture suspension was diluted in modified 7H10 broth to yield 10 Klett units per ml (Klett-Summerson colorimeter; Klett Manufacturing, Brooklyn, N.Y.), or approximately 5 × 10⁷ CFU/ml. The size of the inoculum was determined by titration and counting from triplicate 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% Middlebrook OADC enrichment. The plates were incubated at 37°C for 3 weeks prior to counting.

**Infection studies.** Four- to 6-week-old beige (C57BL/6J bg/bg) mice, bred at our facility, were infected intravenously through a caudal vein. Mice of the same sex were used in separate experiments. Each mouse received approximately 10⁷ viable organisms suspended in 0.2 ml of modified 7H10 broth. There were six mice per group.

In each experiment, a group of infected but untreated mice (designated early controls) was sacrificed at the initiation of therapy. A group of infected but untreated mice (designated late controls) was sacrificed at the conclusion of therapy and was compared with the treated groups of mice. All agents were
were sacrificed by cervical dislocation 3 to 5 days after administration of the last dose of drug. Spleens and lungs were aseptically removed and were ground in a tissue homogenizer. The number of viable organisms was determined by titration on 7H10 agar plates.

Statistical evaluation. The viable cell counts were converted to logarithms, which were then evaluated by one- or two-variable analyses of variance. Statistically significant effects from the analyses of variance were further evaluated by the Tukey honestly significant difference test (13) to make pairwise comparisons among means. The results of the statistical evaluations are summarized in the following section.

RESULTS

Dose-response study of AZI and comparison with CLA. AZI (50, 100, or 200 mg/kg of body weight) or CLA (200 mg/kg) was given 5 days per week for 4 weeks to female mice which had been infected with $3.2 \times 10^7$ viable M. avium (Fig. 1).

The increase in cell counts between the early control group and the late control group was significant for organisms in spleens ($P < 0.01$). Treatment with AZI (at all doses) and CLA reduced organism cell counts in spleens in comparison with the counts in the spleens of mice in both the early and the late control groups ($P < 0.01$ for all comparisons). The decrease in splenic cell counts between groups receiving AZI at 50 mg/kg and AZI at 100 mg/kg was not significant ($P > 0.05$); however, the decrease in cell counts in spleens between groups receiving AZI at 50 mg/kg and AZI at 200 mg/kg was significant ($P < 0.01$). The decrease in cell counts in spleens between groups receiving AZI at 100 mg/kg and AZI at 200 mg/kg was also significant ($P < 0.01$). There was no significant difference in activity between groups receiving either AZI at 50 mg/kg or AZI at 100 mg/kg and the group receiving CLA at 200 mg/kg ($P > 0.05$). AZI at 200 mg/kg was more active than CLA at 200 mg/kg against organisms in spleens ($P < 0.01$).

The increase in cell counts between the early control group and the late control group was significant for organisms in lungs ($P < 0.01$). Only treatment with AZI at 200 mg/kg and CLA at 200 mg/kg reduced organism cell counts in lungs in comparison with the counts in the lungs of mice in the early control group ($P < 0.05$ for AZI; $P < 0.01$ for CLA). Treatment with AZI (at all doses) and CLA reduced cell counts in lungs in comparison with the counts in the lungs of mice in the late control group ($P < 0.01$ for all comparisons).

A dose-related reduction in organism cell counts in lungs was noted with AZI at 50, 100, and 200 mg/kg ($P < 0.05$ for reductions in the number of organisms between the groups receiving AZI at 50 and 100 mg/kg; $P < 0.01$ for reductions in the number of organisms between the groups receiving AZI at organisms 100 and 200 mg/kg). There was no significant difference in activity against organisms in lungs between groups receiving AZI at 200 mg/kg and CLA at 200 mg/kg ($P > 0.05$).

Activity of AZI given on an intermittent basis. AZI at 200 mg/kg was given to male mice which had been infected with $1.3 \times 10^7$ viable M. avium. AZI was given 5 days per week for the first week and then 1, 3, or 5 days per week for the remaining 3 weeks. A group of AZI-treated mice was sacrificed at the end of the first week of therapy. Mice in control groups were sacrificed at the start of treatment, at the end of the first week of treatment, and at the completion of treatment (Fig. 2A and B).

The increase in cell counts between the early control group, the control group sacrificed after 1 week, and the late control group was significant for organisms in spleens ($P < 0.05$ for the
FIG. 2. Activity of AZI given on an intermittent basis. Treatment groups received AZI at 200 mg/kg 5 days per week for the first week and then AZI at 200 mg/kg 1, 3, or 5 days per week for the remaining three weeks. (A) Results for spleens; (B) results for lungs.

increase in the number of organisms between the early control group and the control group sacrificed after 1 week; \( P < 0.01 \) for the increase in the number of organisms between the control group sacrificed at 1 week and the late control group). One week of AZI therapy reduced organism cell counts in spleens in comparison with the counts in the spleens of mice in each control group (\( P < 0.01 \)). Treatment with AZI for the remaining 3 weeks at each dosing schedule further reduced organism cell counts in spleens in comparison with the counts in the spleens of the group receiving 1 week of treatment (\( P < 0.01 \) for each). There was no significant difference in organism cell counts in spleens between mice given AZI 3 days per week and AZI 5 days per week (\( P > 0.05 \)). AZI given 1 day per week was less active than AZI given 5 days per week (\( P < 0.01 \)), but there was no significant difference between AZI given 1 day per week and AZI given 3 days per week (\( P > 0.05 \)).

The increase in cell counts between the early control group and the control group sacrificed after 1 week of treatment was not significant for organisms in lungs (\( P > 0.05 \)). The increase in cell counts between the early control group and the late control group was significant (\( P < 0.01 \)). One week of AZI therapy reduced organism cell counts in lungs in comparison
with the counts in the lungs of mice in each control group ($P < 0.01$). AZI given for the remaining 3 weeks at each dosing schedule did not reduce organism cell counts in lungs in comparison with the counts in the lungs of mice in the group receiving 1 week of treatment ($P > 0.05$).

**Intermittent AZI given in combination with RPT.** AZI at 200 mg/kg alone and in combination with RPT at 20 mg/kg was given once a week for 8 weeks to female mice which had been infected with $1.1 \times 10^7$ viable *M. avium*. Another group received RPT at 20 mg/kg once weekly (Fig. 3).

The increase in cell counts between the early control group and the late control group was not significant for organisms in spleens ($P > 0.05$). Treatment with AZI or RPT as single agents reduced organism cell counts in spleens in comparison with the counts in the spleens of mice in both the early and the late control groups ($P < 0.01$). AZI plus RPT was more active than either single agent against organisms in spleens ($P < 0.01$).

The increase in cell counts between the early control group and the late control group was significant for organisms in lungs ($P < 0.01$). Neither AZI nor RPT as single agents reduced organism cell counts in lungs in comparison with the counts in the lungs of mice in the early control group ($P > 0.05$); however, AZI plus RPT reduced organism cell counts in lungs in comparison with the counts in the lungs of mice in the early control group ($P < 0.01$). Treatment with AZI or RPT reduced organism cell counts in lungs in comparison with the counts in the lungs of mice in the late control group ($P < 0.01$), and the combination was more active than either agent used alone ($P < 0.01$).

**DISCUSSION**

In the dose-response experiment, a dose-related reduction in organism cell counts in organs was noted with AZI treatment. AZI and CLA were both more active against organisms in spleens than against those in lungs, perhaps because of the presence of a larger population of extracellular rather than intracellular bacilli in the lungs. AZI at 200 mg/kg was more active than CLA at 200 mg/kg against organisms in spleens, a pattern that we have noted previously in comparative studies in the beige mouse test system (6). The activities of AZI at 200 mg/kg and CLA at 200 mg/kg were comparable against organisms in lungs.

The second study was designed so that AZI treatment was given on a daily basis for the first 5 days and then on an intermittent basis for the next 3 weeks. The activities of AZI on a three-times- and a five-times-per-week basis were comparable. Efficacy decreased when AZI was administered on a once-per-week basis, particularly against organisms in lungs. It would have been useful to design the present study with an additional group that received no continuation therapy after the initial treatment period. This would further clarify the efficacy of the once-weekly AZI treatment in the continuation phase. The plasma elimination half-life of AZI in CD1 mice given a single oral dose of 50 mg/kg is 6.4 h (10), whereas the plasma half-life in humans after administration of a single oral dose of 500 mg is 11 to 14 h (2). Therefore, AZI administered on a once-weekly basis may yet be a tenable approach for the treatment and/or prevention of disseminated MAC infection in patients with AIDS (3). Intermittent therapy may prove to be appropriate for continuation or maintenance therapy only after an initial daily intensive phase of treatment.

Treatment of disseminated MAC infection in individuals with AIDS will involve therapy with drugs in combination in order to increase treatment efficacy and to prevent the development of resistance. RPT is a newer rifamycin derivative with pharmacokinetic advantages, in terms of the concentration...
achievable within tissues and a prolonged half-life, that make it suitable for intermittent administration (1, 8, 12, 22). We have previously reported on the activity of RPT against MAC in the beige mouse test system (15, 16, 18). In our current study, AZI and RPT were administered on a once-weekly basis for 8 weeks. AZI plus RPT was more active than either agent used alone. The combination resulted in mean reductions in spleens and lungs of 3.25 and 3.50 log CFU, respectively. It would be of interest to evaluate the efficacy of rifabutin in a similarly designed study, because rifabutin is currently available for clinical use.

Clinical evaluation of AZI in combination with other long-acting agents will help to define whether intermittent therapy (once or twice weekly) provides effective treatment for disseminated MAC infection. The potential benefits of intermittent therapy may be decreased adverse drug reactions, decreased drug interactions, and reduced treatment costs.

REFERENCES