Randomized, Open-Label Trial of Azithromycin Plus Ethambutol vs. Clarithromycin Plus Ethambutol as Therapy for *Mycobacterium avium* Complex Bacteremia in Patients with Human Immunodeficiency Virus Infection

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Disseminated *Mycobacterium avium* complex (MAC) infection continues to be a common opportunistic infection in patients infected with human immunodeficiency virus (HIV). The optimal therapy for disseminated MAC infection is unclear. We compared azithromycin plus ethambutol with clarithromycin plus ethambutol in the treatment of disseminated MAC infection in HIV type 1–infected patients, examining the frequency of bacteremia clearance, time to clearance, and study drug tolerance after 16 weeks of therapy. Fifty-nine patients for whom blood cultures were positive for MAC were enrolled in the study from 10 university-affiliated Veterans Affairs Medical Centers. Thirty-seven patients were evaluable for determination of quantitative bacteremia and clinical outcomes. Clearance of bacteremia was seen at the final visit in 37.5% of azithromycin-treated patients and in 85.7% of clarithromycin-treated patients (P = .007). The estimated median time to clearance of bacteremia was also significantly different between the two treatment arms: 4.38 weeks for clarithromycin recipients vs. >16 weeks for azithromycin recipients (P = .0018). Only one isolate developed macrolide resistance during therapy. Abatement of symptoms, other laboratory-evident abnormalities, and adverse effects were similar in the two groups. At the doses used in this study, clarithromycin/ethambutol produced a more rapid resolution of bacteremia than did azithromycin/ethambutol, and clarithromycin/ethambutol was more effective at sterilization of blood cultures after 16 weeks of therapy.

Disseminated *Mycobacterium avium* complex (MAC) infection remains the most common opportunistic bacterial infection in adults infected with HIV type 1 in the United States, despite recent advances in antiretroviral therapy and chemoprophylaxis for MAC infection [1]. Infection is characterized clinically by sustained bacteremia, with intermittent fever, night sweats, abdominal pain, and weight loss [2]. Disseminated MAC infection has been shown to be an independent predictor of mortality [3]. Treating disseminated infection can lead to both an improvement in the clinical condition and a decrease in the bacterial load and has been shown to prolong survival [4]. Previous studies have demonstrated that quantitative changes in the level of mycobacteremia are a reliable indicator of therapeutic response [4–6].

In vitro studies and recent human trials suggest that macrolide-based combination antimycobacterial therapy should be used to treat disseminated MAC infection [4–6]. Yet, the optimal therapeutic regimen for treatment of disseminated MAC infection is unknown [4]. Since macrolide monotherapy leads to the occurrence of drug resistance and resultant clinical failure [4], recent studies have evaluated several different macrolide-based combination regimens [4–7]. Ethambutol and clofazimine have the best tolerance patterns of nonmacrolide drugs that have predictable in vitro activity against MAC infection [4], although clofazimine when given with clarithromycin neither prevents emergence of resistance to clarithromycin nor prolongs survival [7]. Results from recent trials support the use of ethambutol with a macrolide as a component of combination therapy [6–8].

Both azithromycin and clarithromycin have undergone extensive in vitro and chemoprophylactic testing in recent years [9–11]. Yet, no information is available that directly compares these two macrolide antibiotics as therapy for disseminated
MAC infection. Therefore, we performed a randomized treatment trial comparing the efficacy and tolerability of azithromycin plus ethambutol with those of clarithromycin plus ethambutol as treatment of disseminated MAC infection in HIV-seropositive patients. Our study was limited to 16 weeks and employed a quantitative microbiological primary outcome measure. Signs, symptoms, and laboratory-evident abnormalities of disseminated MAC infection; study drug tolerance; and antimicrobial resistance were also assessed.

Methods

Study Patients

Patients were eligible for enrollment in the study if they were HIV-1-seropositive, had a blood culture positive for MAC within 2 weeks, and were at least 18 years old. Patients were excluded from the study if they had received azithromycin, clarithromycin, or ethambutol within 4 weeks before enrollment, had hypersensitivity to any of the study agents, had other concurrent mycobacterial disease such as tuberculosis, had a life expectancy estimated to be <16 weeks, or were unable to take or comply with the oral study regimen. Patients were enrolled at 10 university-affiliated Veterans Affairs Medical Centers and at affiliated institutions. The study was approved by the ethics committees at each center, and each participant gave informed consent.

Study Drug Therapy

Patients were randomly assigned to one of two treatment arms by using an automated phone-in registration process: 600 mg of azithromycin once daily plus ethambutol or 500 mg of clarithromycin twice daily plus ethambutol. The dose of ethambutol was either 800 mg daily if the patient’s weight was <60 kg or 1,200 mg daily if the patient’s weight was ≥60 kg.

Clinical and Microbiological Evaluation

Clinical symptoms, Karnofsky scores, adherence to the study drug regimen, and adverse events were recorded at 2 weeks, 4 weeks, and then every 4 weeks thereafter for the 16-week study duration. Clinical symptoms that were evaluated included fever, night sweats, and abdominal pain; laboratory parameters included levels of hemoglobin, alkaline phosphatase, and alanine or aspartate aminotransferase. Quantitative blood cultures for determination of mycobacteremia were performed in duplicate at baseline before study therapy was initiated and then every 4 weeks for the 16-week study period. Specimens were sent to the Mycobacteriology Clinical Reference Laboratory at the National Jewish Medical and Research Center for Immunology and Respiratory Medicine in Denver and were assessed by previously reported methods [12]. Specimens were shipped at ambient temperature by overnight mail to Denver; shipping of mycobacterial cultures in this manner does not affect viability or quantitative results [12]. Susceptibility testing of isolates to clarithromycin and azithromycin was performed by methods previously described by Heifets [13].

Assessment of Efficacy

Investigators were blinded to results of quantitative blood cultures and susceptibility testing but not to treatment group. The primary study outcome was the proportion of patients with clearance of bacteremia at the final 16-week study visit. A subgroup analysis by level of baseline bacteremia (<10³ and ≥10³ cfu/mL) of this primary end point was a planned part of the study design. Secondary outcomes included the rate of decrease in bacterial load in quantitative blood cultures, the duration in weeks to the first negative blood culture, clinical response, and laboratory findings. Patients were considered evaluable with regard to bacteriologic and clinical responses to therapy if they had a positive quantitative culture at baseline, had at least one subsequent quantitative culture performed while receiving therapy, and received study drugs for a minimum of 4 weeks.

Statistical Analyses

Analyses were done to compare baseline characteristics between groups and to compare treatment outcomes. Differences in categorical variables were assessed with the continuity-corrected χ² test. Normally distributed continuous variables within groups were compared with the paired t test, and skewed variables were compared with the Wilcoxon rank-sum test. The median time to negative blood cultures was estimated by using the product limit method, with differences assessed by the logrank test. An interim analysis of the primary outcome was conducted after approximately one-half the projected sample size of 108 patients had been randomized; the committee performing the interim analysis was blinded to specific study regimen assignments. All adverse reactions were reviewed by a data management and safety board whose members were blinded to the study arm assignment.

Results

Study Patients

Between November 1991 and October 1994, 59 patients were enrolled in the study: 35 in the clarithromycin arm and 24 in the azithromycin arm. The study was stopped after an interim analysis demonstrated a significant difference (P = .028) between study arms in the proportion of patients for whom cultures were negative at 16 weeks. A skew in the randomization between study arms resulted from the termination of the study earlier than originally projected. Of 59 patients enrolled in the study, 37 (21 in the clarithromycin arm and 16...
in the azithromycin arm) were evaluable for microbiological and clinical outcomes. The remaining 22 patients either had inadequate documentation of microbiological response or did not receive adequate therapy to evaluate efficacy. Twelve patients received drug therapy for <4 weeks, and eight patients had negative repeated blood cultures at study enrollment; for an additional two patients, study drug therapy was not initiated.

Baseline characteristics of nonevaluable patients were balanced between treatment arms, except for initial level of bacteremia. Most nonevaluable patients for whom blood cultures were negative at baseline were in the clarithromycin arm, with the resultant baseline level of bacteremia trending toward a lower value in the nonevaluable patients in the clarithromycin arm than in those in azithromycin arm (median level, 0 vs. 480 cfu/mL, respectively; \( P = .07 \)). Study drug tolerance and adverse reactions were evaluated in all 59 patients.

### Baseline Characteristics

Baseline characteristics of the 37 evaluable patients are shown in table 1. The two treatment arms were balanced with respect to the CD4 lymphocyte count at study entry, the quantitative level of mycobacteremia at baseline, and Karnofsky scores. Eight of 16 and 10 of 21 evaluable patients in the azithromycin and clarithromycin arms, respectively, had levels of bacteremia of \( \geq 10^4 \) cfu/mL at baseline; baseline characteristics were balanced between the treatment arms for low and high level bacteremia strata. Baseline symptoms and laboratory evident abnormalities were also similar between study drug arm.

### Response to Therapy

The study time for evaluable patients was similar in the two study treatment arms (median study time, 12 weeks for the azithromycin arm and 16 weeks for the clarithromycin arm) (table 2). Treatment was discontinued before 16 weeks for 19% of azithromycin recipients and 33% of clarithromycin recipients. Reasons for discontinuation of therapy before 16 weeks included the following: investigator’s concern with possible clinical failure (occurring for two azithromycin recipients and one clarithromycin recipient); patient request to withdraw from the study on three occasions; two episodes of increasing...
disability; and death at 8 weeks of therapy in two patients. Adherence to the study drug regimen, as assessed by pill counts, was similar for the two arms and improved after the 2-week study visit.

Microbiological Response

MAC bacteremia was cleared in 65% of the 37 evaluable patients at the final study visit. The proportion of patients with clearance of bacteremia at the final study visit was 37.5% in the azithromycin arm and 85.7% in the clarithromycin arm ($P = .007$). The proportion of patients with negative blood cultures was significantly different at weeks 8 ($P = .006$) and 16 ($P = .028$) but not at week 12 ($P = .29$; figure 1). The estimated median time to clearance of bacteremia (figure 2) was also significantly different ($P = .0018$, by the logrank test) for the two treatment groups: 4.38 weeks for the clarithromycin arm vs. >16 weeks for the azithromycin arm. The median level of bacteremia for the two treatment groups did not differ significantly at any study visit (table 2). At 4 weeks of therapy, the geometric mean reduction in colony counts from baseline was 1.2 and 1.4 logs for the azithromycin and clarithromycin arms, respectively. At 12 weeks, the geometric mean reduction was 1.2 and 1.6 logs, respectively.

An intent-to-treat analysis of the 59 enrolled patients demonstrated a significant difference ($P = .011$) in the proportion of patients with clearance of bacteremia at 16 weeks: 45.5% for the azithromycin arm vs. 94.4% for the clarithromycin arm. A subgroup analysis by level of baseline bacteremia demonstrated a significant difference between treatment arms in the rapidity of blood sterilization for those patients with a level of bacteremia of $\geq 10^1$ cfu/mL at baseline that favored the clarithromycin regimen ($P = .0132$, by the logrank test). This difference between treatment arms remained but did not reach statistical significance for those patients with entry levels of bacteremia of $<10^1$ cfu/mL ($P = .0782$).

The patient with the highest level of bacteremia at baseline (mean level revealed by duplicate blood cultures, 2,884 cfu/mL) was in the azithromycin arm. This patient’s culture remained positive at the 16-week study visit, and a level of bacteremia of $>10^3$ cfu/mL was demonstrated at each study visit. Intercurrent cytomegalovirus retinitis was recognized at week 12. Susceptibility testing of this patient’s isolates from baseline and week 16 demonstrated susceptibility to azithromycin, and adherence to study drug therapy was reported to be 100% except at the 2-week study visit. Excluding this patient from the analysis did not change the significant difference observed between treatment arms at 16 weeks in either the frequency or rate of bacteremia clearance that favored the clarithromycin arm ($P = .032$ and $P = .0056$, respectively).

Azithromycin and clarithromycin susceptibility testing was performed on all baseline and final study visit isolates from those patients whose cultures remained positive at the time of study termination. Only one 16-week isolate from a patient in the clarithromycin arm was definitely resistant to the study macrolides (MIC of clarithromycin, $>32$ mg/mL; MIC of azithromycin, $>256$ mg/mL). The baseline isolate from this patient was susceptible to both macrolides (MIC of clarithromycin, $\leq 2.0$ mg/mL; MIC of azithromycin, $\leq 16$ mg/mL). Although a progressive decline in the quantitative level of bacteremia was demonstrated at each study visit for this patient, he was the only patient in the clarithromycin arm whose culture...
remained positive at the 16-week study visit (baseline, 554 cfu/mL; week 16, 7 cfu/mL).

Three patients in the azithromycin arm had isolates with an MIC equal to 64 μg/mL and were considered susceptible by the central reference laboratory [13]. Two of these three isolates were recovered from baseline cultures for patients whose final study visit isolates were definitely susceptible to azithromycin (MIC, <16 μg/mL); a progressive decline in the quantitative level of bacteremia during therapy was demonstrated for both patients. The third isolate for which the MIC of azithromycin was 64 μg/mL was from a patient at the final study visit who had a baseline isolate for which the MIC was <16 μg/mL; a progressive decline in the quantitative level of mycobacteremia during therapy also was demonstrated for this patient. All three isolates for which MICs of azithromycin were 64 μg/mL were susceptible to clarithromycin (MIC, ≤2 μg/mL).

Clinical Response

At the final study visit, the frequency of patients with symptoms of fever and night sweats decreased from that at baseline in both arms (table 3). The effect on abdominal pain was less marked. Little change in laboratory-evident abnormalities, weight, or Karnofsky score was observed over the 16-week study duration in either arm. At study termination, the frequency with which the hemoglobin level was <8 g/dL had increased over that at baseline in both arms. No patient in either arm received transfusions during the study. At the final study visit, there was no significant difference between treatment arms in the frequency of patients with symptoms of MAC disease or laboratory-evident abnormalities. There was no significant difference between treatment arms in the frequency with which other intercurrent illnesses occurred during the 16-week study duration.

Adverse Events

Neither study drug therapy was withheld frequently, occurring only for 18 of 1,432 aggregate study days in the azithromycin arm and 22 of 2,048 study days in the clarithromycin arm. Adverse reactions were recorded for seven (29%) of 24 azithromycin-treated patients and 10 (29%) of 35 clarithromycin-treated patients. The most common adverse events reported were nausea and gastrointestinal intolerance, responses that occurred with similar frequency in the two treatment arms. Of the adverse events designated possibly or probably related to study drugs, grade 3 or 4 reactions were reported for two and five events in the azithromycin and clarithromycin arms, respectively. Discontinuation of therapy because of suspected drug toxicity occurred in two (8%) of 24 azithromycin recipients and three (9%) of 35 clarithromycin recipients.

There were eight deaths during study drug therapy, four in each of the treatment arms. Six deaths occurred within 3 weeks of study enrollment. MAC infection was not listed as the primary cause of death for any patient. Six deaths were classified as not related to study therapy. Two deaths in the azithromycin arm were due to sudden cardiac arrest without a certain etiology, and it was not possible to determine a relationship to the study drug. Both patients had other ongoing diseases that may have contributed to sudden death: cytomegalovirus retinitis in one case and recently diagnosed Pneumocystis carinii pneumonia in one case. Alanine or aspartate aminotransferase levels were measured in all patients at each study visit to assess possible study drug hepatotoxicity. Only two patients in each treatment arm had alanine or aspartate aminotransferase levels that increased ≥20% between the baseline and the final study visit; no value was greater than three times the upper limit of normal at any study visit.

Discussion

Our study confirmed that macrolide-based two-drug therapy results in significant reduction in the level of mycobacteremia in HIV type 1–seropositive patients with disseminated MAC infection. As in prior studies, the microbiological response was accompanied by a decrease in constitutional symptoms of MAC disease [4]. Although the primary end point of our study was clearance of mycobacteria at 16 weeks and thus our study did not extend to a longer follow-up, previous trials have correlated short-term improvement as evidenced by microbiological and clinical responses with increased survival [4–7, 14–18].
To our knowledge, this is the first randomized trial to directly compare azithromycin with clarithromycin as therapy for disseminated MAC disease.

At the macrolide doses employed in this study, the bacteriologic response in the azithromycin/ethambutol arm was delayed in comparison with that in the clarithromycin/ethambutol arm, and the clarithromycin/ethambutol arm was more effective at sterilization of blood cultures after 16 weeks of therapy. The two treatment arms were comparable in terms of clinical and other laboratory findings over the 16-week treatment duration. Drug tolerance also appeared equivalent in the two study drug arms. Acquisition of macrolide resistance in vitro after 16 weeks of combined treatment with a macrolide and ethambutol was infrequent, thus confirming the finding of a protective effect of adding ethambutol that was recently reported in a California Collaborative Treatment Group trial [6]. Our observed time to clearance of bacteremia in the clarithromycin/ethambutol arm was shorter than that reported in prior trials that evaluated three- and four-drug treatment regimens lacking a macrolide component [4]. Our observations are consistent with other recently reported studies employing macrolide-based therapies [5–8].

The optimal dose of either clarithromycin or azithromycin for treating MAC infection is unknown. A dose-ranging monotherapy trial with clarithromycin demonstrated more rapid clearance of bacteremia with higher doses [14]. We evaluated a dosage of clarithromycin of 500 mg twice daily on the basis of studies reported by Chaisson and colleagues [14] who compared three dosages of clarithromycin monotherapy (500 mg, 1,000 mg, and 2,000 mg twice daily) and found that the lowest mortality rate during the first 12 weeks was associated with the dosage of 500 mg twice daily. A second randomized trial, conducted by the Community Program for Clinical Research in AIDS, was modified after an interval analysis showed that survival was better with 500 mg of clarithromycin twice daily than with 1,000 mg twice daily [19]. In a recent study where patients were randomized to a dosage of clarithromycin of 1,000 mg twice daily, 54% of patients required dosage reduction because of gastrointestinal side effects [6]. The dose of azithromycin chosen for this trial was based on more limited data [20], but it was our opinion that the daily dose of 600 mg was comparable with the twice daily dose of 500 mg employed in the clarithromycin arm.

The microbiological response that we observed in both treatment groups was comparable with that reported previously for clarithromycin and azithromycin monotherapies [14, 20]. At the 8-week study visit, we noted clearance of bacteremia in 21% and 75% of patients randomized to the azithromycin and clarithromycin arms, respectively. Chaisson and colleagues [14] reported that 41% of patients had negative blood cultures after 8 weeks of clarithromycin monotherapy, and Berry et al. [20] reported that 54% of patients had negative blood cultures after 6 weeks of treatment with 600 mg of azithromycin once daily. In the recent California Collaborative Treatment Group trial [6], 23 (82%) of 28 patients randomized to clarithromycin, clofazimine, and ethambutol had sterile blood cultures after 16 weeks of therapy. At 12 weeks, we noted that the geometric mean reduction in colony counts was 1.2 and 1.6 logs for the azithromycin and clarithromycin arms, respectively. These results are similar to those reported for 500 mg of clarithromycin twice daily [14] and 600 mg of azithromycin once daily [20]. Differences between studies in the level of baseline bacteremia influence the size of the treatment effect seen and preclude any direct comparison of our findings with historical controls.

Results of in vitro susceptibility testing in our study demonstrated minimal macrolide resistance in either treatment arm after 16 weeks of therapy. Isolates from only one of the 37 evaluable patients showed in vitro macrolide resistance. This observation supports the recently reported findings of Dubé et al. [6] who noted that the addition of ethambutol to a macrolide reduced the frequency of acquisition of drug resistance; in that trial, relapse occurred only in patients whose isolates developed clarithromycin resistance, which was seen in <10% of isolates from 25 patients after 16 weeks of therapy. Dubé et al. reported that the time to development of resistance that was associated with the addition of ethambutol to clarithromycin plus clofazimine was significantly longer than the time to development of resistance that was associated with two-drug therapy without ethambutol. These results are consistent with in vitro, ex vivo, and animal studies that demonstrated augmentation of clarithromycin’s antimycobacterial activity by the addition of ethambutol [6, 21–24].

In our trial, the addition of ethambutol did not appear to add to the toxicity that has been previously reported for macrolide therapy. The 8.5% (five of 59) frequency of treatment-terminating toxicities that we observed was consistent with the frequency of 7%–10% that was reported by Dubé et al. [6] for clarithromycin/ethambutol/clofazimine; the duration of their study was approximately twice that of our trial. Therapy in our study was withheld because of possible drug-related adverse effects on only 1.1% of 3,480 aggregate study days, and the overall rate of adherence as assessed by pill counts was >80% for both macrolides.

The major limitation of our trial was the relatively small sample size. We observed a progressive decline in the rate of enrollment as the use of chemoprophylaxis for MAC infection became widespread. A decision to stop our study after 59 patients were randomized rather than proceed to the projected sample size of 108 patients occurred only after an analysis of the range in frequencies of bacteremia clearance that could possibly be observed if the trial was continued. The risk ratio for a reduction in the frequency of clearance of bacteremia at 16 weeks for those patients in the clarithromycin arm, as compared with those in the azithromycin arm, was 2.04 (95% CI, 1.1 to 4.0). Although there is imprecision regarding the possibility of a difference between treatment arms, the likelihood of a reversal in the trend that favored clarithromycin was
thought to be extremely unlikely by the external review committee, and the study was stopped.

Our study was not blinded to study drug assignment. Although this fact should not be expected to influence the primary outcome measure of the quantitative level of mycobacteremia, it could have biased investigators into prematurely discontinuing a study regimen or in classifying possible study drug-related adverse events. However, we observed no differences between treatment arms either in reporting of adverse events or in early study termination. In addition, reporting of adverse events was independently reviewed for consistency of classification by a data management and safety board whose members were blinded to the study drug assignment.

The U.S. Public Health Service Task Force on therapy for MAC infection has recommended that patients treated with a macrolide receive at least one other drug likely to have microbiological activity, such as ethambutol, clofazimine, rifabutin, ciprofloxacin, or amikacin [25]. Yet, few randomized, comparative trials of multidrug treatment for disseminated MAC infection have been reported [4–7, 15–18, 26–28], and several of the prior trials have either not been conducted prospectively or randomized [15–18, 26–28]. Our prospective, randomized study validates the microbiological efficacy and safety of macrolide-based combination therapy for disseminated MAC infection and supports the observation that ethambutol augments the activity of clarithromycin. At the doses we compared in our study, clarithromycin/ethambutol resulted in a more rapid bacteriologic response than was seen with azithromycin/ethambutol, and clarithromycin/ethambutol was more effective at sterilization of blood cultures after 16 weeks of therapy.

Veterans Affairs HIV Consortium Members

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