Combination Drug Therapy for Cryptosporidiosis in AIDS

Nathaniel H. Smith, Stanley Cron, Luis M. Valdez,* Cynthia L. Chappell, and A. Clinton White, Jr.

Aside from effective antiretroviral therapy, there is no consistently effective antiparasitic therapy for cryptosporidiosis in AIDS. The purpose of this study was to assess safety, efficacy, and durability of combination therapy with paromomycin and azithromycin for chronic cryptosporidiosis. Patients with AIDS, chronic cryptosporidiosis, and <100 CD4 cells/μL were treated with open-label paromomycin (1.0 g twice a day) plus azithromycin (600 mg once a day) for 4 weeks, followed by paromomycin alone for 8 weeks. In 11 patients, median stool frequency decreased from 6.5/day (baseline) to 4.9/day (week 4) and 3.0/day (week 12). Median reductions in 24-h oocyst excretion were 84%, 95%, and >99% at 2, 4, and 12 weeks, respectively. None of the responses were attributable to antiretrovirals. Of 5 survivors at 12–30 months of follow-up, 3 remain asymptomatic off medications, and 2 have chronic, mild diarrhea. Treatment of cryptosporidiosis with azithromycin and paromomycin was associated with significant reduction in oocyst excretion and some clinical improvement.

Cryptosporidium parvum, an intracellular protozoan parasite, is a frequent cause of chronic diarrhea in human immunodeficiency virus (HIV)-infected patients, resulting in significant morbidity and mortality. Chronic cryptosporidiosis occurs when CD4 lymphocyte counts are <180/mm³ [1]. In many AIDS patients, cryptosporidiosis resolves as immune status improves with antiretroviral therapy [2]. However, in patients in whom antiretroviral therapy fails and in developing countries where effective antiretroviral therapy is not available, cryptosporidiosis causes prolonged diarrhea and wasting. Treatment for chronic cryptosporidiosis in HIV-infected patients has been problematic. Paromomycin, a poorly absorbed aminoglycoside antibiotic, has demonstrated some efficacy in the treatment of AIDS-related cryptosporidiosis. A review of >300 patients with chronic cryptosporidiosis noted clinical improvement in about two-thirds [3]. Despite treatment, however, relapse and subsequent biliary disease were common. Two small, placebo-controlled trials have been reported with conflicting results [4, 5].

Azithromycin, an azalide antibiotic, has also been used in human cryptosporidiosis [6]. However, in clinical trials for AIDS-related cryptosporidiosis, azithromycin monotherapy has been ineffective [7, 8]. In clinical practice, azithromycin has frequently been used in combination with paromomycin for treatment of cryptosporidiosis [9], but the safety and efficacy of this combination has not been carefully assessed. The purpose of this study was to obtain data on the safety, efficacy, and durability of a combination regimen of paromomycin and azithromycin in the treatment of AIDS-related cryptosporidiosis.

Methods

Between 1 October 1994 and 31 March 1997, Harris County (Texas) Hospital District patients with AIDS, chronic diarrhea (defined as ≥3 loose stools/day on ≥5 days/week for ≥3 weeks), and Cryptosporidium oocysts in their stools were screened for enrollment. All patients with stool studies negative for other parasites (including nonspecific fluorescent stain for microsporidia), bacterial enteric pathogens, and Clostridium difficile toxin and who were not already being treated with aminoglycosides or macrolides were offered enrollment. Enrollees underwent baseline assessment of symptoms, including the number of stools per day and 24-h fecal excretion of Cryptosporidium oocysts. Subjects were treated with paromomycin (1 g by mouth twice a day) plus azithromycin (600 mg by mouth once a day) for 4 weeks, then paromomycin alone for an additional 8 weeks. Symptoms and 24-h stool collection for stool weight and oocyst excretion were reevaluated at 2, 4, and 12 weeks. Subjects also kept a daily record of stool frequency and consistency and recorded the number of doses of antimotility drugs. Potential toxicity was assessed by physical examination and routine laboratory evaluation, including complete blood count and chemistry panel that included liver enzymes.

Stool oocyst excretion was quantified by immunofluorescence as previously described [4, 10, 11]. Using this method, oocyst excretion varies <1 log from day to day in a single patient and correlates with both pathologic changes and intensity of infection seen on endoscopic biopsy specimens [10, 11].

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Study design was approved by the Institutional Review Board of Baylor College of Medicine, and informed consent was obtained from all patients.
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Primary analysis of efficacy was performed at week 4, and durability of response was assessed by a second analysis at week 12. In addition, changes in stool weight, frequency, consistency, and oocyst excretion and in patient Karnofsky score during the first 2 weeks of combination treatment were compared with those from our previous study of patients treated with paromomycin alone [4]. At the end of the study period, therapy was continued at the discretion of the primary care physician. Statistical analyses were performed using the two-tailed Wilcoxon signed rank test.

Results

During the study period, 13 patients met inclusion criteria and all consented for enrollment. One withdrew immediately after enrollment, and 1 was excluded due to no detectable oocysts in stool at baseline. An additional patient did not provide a baseline stool specimen and was excluded from analyses of stool weight and oocyst quantitation. Baseline characteristics are shown in Table 1. All had advanced immunosuppression and chronic diarrhea. All but 1 had previously been treated with zidovudine. At baseline, only 4 of 11 were receiving antiretroviral therapy: 2 on stavudine monotherapy, 1 on stavudine/lamivudine combination therapy, and 1 on stavudine/indinavir (20 CD4 cells/mm² and HIV-1 plasma viremia of >100,000 copies/mL). Four of 11 patients remained off antiretroviral therapy, and 3 remained on stable antiretroviral regimens throughout the study period. In 3 patients, stavudine monotherapy was initiated during weeks 2, 5, and 11. One patient, who was on stavudine/lamivudine at baseline, began indinavir at week 3; however, diarrhea and oocyst excretion had already resolved by week 2.

The major clinical and laboratory outcomes are summarized in Table 2. The 24-h oocyst excretion decreased significantly by week 4 of paromomycin/azithromycin combination therapy, with further decreases between weeks 4 and 12 (both \( P < .05 \) vs. baseline). The median decrease in oocyst excretion was 84% at week 2, 95% at week 4, and >99% at week 12. By week 12, 24-h oocyst excretion was undetectable in 3 patients, decreased >10-fold in 3 others, decreased 51%–85% in 3, and increased in 1. One patient did not provide a baseline stool specimen and was evaluated for symptoms only. In our previous study of patients treated with paromomycin alone [4], median decrease in 24-h oocyst excretion at 2 weeks was only 50%, and oocysts were undetectable in only 1 of 9.

There were also significant reductions in stool frequency by weeks 4 and 12 (both \( P < .05 \) vs. baseline). Of interest, the median 24-h stool weight actually increased during the first 4 weeks but decreased between weeks 4 and 12 (after azithromycin was stopped). Daily loperamide use was reported by 5 patients and remained low (median, 0; range, 0–7) throughout the study period.

The paromomycin/azithromycin combination was well tolerated, with only 2 of 11 (18%) discontinuing azithromycin early due to abdominal cramping. Two patients with persistent diarrhea discontinued paromomycin between weeks 4 and 12 with immediate resolution of the diarrhea, and a third experienced resolution of mild residual diarrhea with cessation of paromomycin after completion of the study. No drug interactions, otoxicity, or nephrotoxicity were noted. Also, no significant changes in serum albumin, alkaline phosphatase, body weight, or Karnofsky scores were seen.

After study completion, subjects were followed for up to 30 months. Five have survived, of whom 2 have chronic, mild diarrhea and continue to take paromomycin and/or azithromycin, and 3 have experienced complete resolution of diarrhea off medications. Four of the 11 developed biliary disease. Two of these 4 died; the other 2 experienced marked relief of abdominal pain and, in 1, complete resolution of diarrhea after cholecystectomy for acalculous cholecystitis. *Cryptosporidium* was not found in either gallbladder. In 1, cytomegalovirus (CMV) inclusions were found; the other showed only nonspecific acalculous cholelithiasis on histopathology.

Six patients have died, 3 from progressive diarrhea. In 1 of these, colonoscopy with biopsy revealed gastrointestinal CMV inclusions, and biliary disease was also present. In another, CMV retinitis was documented and gastrointestinal CMV disease suspected (no *Cryptosporidium* oocysts were detectable in the stool). The third refused endoscopy, and coinfection could not be excluded. Of the remaining 3 patients, 1 died of pancreatobiliary disease from suspected cryptosporidiosis, and 2 died from unrelated causes (body cavity lymphoma and suspected primary central nervous system lymphoma) >1 year after resolution of their diarrhea.

Discussion

In this small open-label study, treatment of cryptosporidiosis in AIDS with the combination of azithromycin and paromomycin was associated with a dramatic reduction in oocyst excretion. The median decrease of 24-h oocyst excretion was 95% by 4 weeks and >99% at 12 weeks. Five of 9 and 6 of 10 patients had a >10-fold reduction in oocyst excretion by weeks 4 and 12, respectively. This contrasts with our previous study.
of paromomycin, in which there was a median reduction in oocyst excretion of only 50% and only 3 of 9 had a reduction of \( >1 \) log \([4]\). Previous studies of untreated patients have shown some variability in oocyst excretion. When 24-h collections have been obtained and quantitated, the day-to-day variability in oocyst excretion is generally \(<1 \) log \([4, 11]\). For example, 40 patients in the placebo arm of a trial of bovine anti-\( \text{Cryptosporidium} \) immunoglobulin, only 1 had \( >1 \) log decrease in oocyst excretion \([12]\) (Crabb J, ImmuCell, personal communication). Thus, the changes noted here are well outside of the range expected from random variation in excretion.

Reduced oocyst excretion was associated with a decrease in stool frequency. By 12 weeks, 3 patients were asymptomatic off medications, and 2 others had only mild diarrhea, easily controlled with antimotility agents along with azithromycin and/or paromomycin. In 2 others, with acalculous cholecystitis (1 of whom was found to be coinfected with CMV), symptoms resolved promptly after cholecystectomy. Three patients had progressive diarrhea. However, at least 2 of the 3 had active CMV disease. One patient had resolution of diarrhea but died of progressive pancreatobiliary disease.

Improvements in oocyst excretion and stool frequency were not accompanied by consistent reductions in stool volume. Diarrhea is a recognized complication of therapy with azithromycin, even at doses significantly smaller than those used in this study. Stool volume decreased after discontinuation of that drug. Three patients noted clinical improvement when paromomycin was discontinued. Diarrhea has not been previously reported with paromomycin therapy in cryptosporidiosis, but worsening of gastrointestinal function with paromomycin has been recognized \([13]\). If careful parasitologic measures had not been included in our study, drug side effects and coinfections might have been mistaken for treatment failure.

While our results are encouraging, enthusiasm must be tempered by the lack of a placebo control group. Others have noted dramatic responses of cryptosporidiosis to antiretroviral therapy. During our study, most patients were on no antiretroviral therapy or nucleoside monotherapy, and there was no clear relationship between antiretroviral drugs and the responses noted. Only 2 of our patients were treated with protease inhibitors, 1 in whom therapy failed and the other only after resolution of symptoms and oocyst excretion.

Previous studies with paromomycin alone have shown variable responses. Open-label and retrospective studies have shown partial and complete responses in about two-thirds of patients \([3]\). Only two small placebo-controlled trials have been reported. In one, paromomycin was associated with a significant reduction in oocyst excretion and stool frequency compared with placebo \([4]\). The median decrease in oocyst excretion was only 50%. In the second study, responses were noted in 8 of 17 patients treated with paromomycin, a proportion that was not significantly different in the placebo group \([5]\). Dropouts, which only occurred from the placebo arm, were excluded from analysis, and only patients with a reduction in stool frequency of \( \geq 50\% \) were considered to have responded. Furthermore, oocyst excretion was only assessed in a semiquantitative method. Thus, the design made it unlikely that the trial could have detected the modest efficacy noted in the first study.

Azithromycin has also been studied in AIDS patients with cryptosporidiosis. An open-label, expanded-access program noted clinical improvement in most patients but minimal changes in oocyst excretion \([14]\). In a placebo-controlled trial, 85 patients with AIDS and cryptosporidiosis were randomized to 900 mg of either azithromycin or placebo per day \([7]\). There was no significant difference in stool frequency, oocyst shedding, or weight change. Post hoc analysis, however, suggested a decreased oocyst excretion in the subjects with highest azithromycin levels. This association was not confirmed in studies with intravenous azithromycin, though there was a trend toward lower levels of alkaline phosphatase \([15]\).

Combination therapy with paromomycin/azithromycin has not been previously studied.

In summary, we noted a significant reduction in oocyst excretion and stool frequency in \( \text{Cryptosporidium} \)-infected AIDS patients receiving dual therapy with paromomycin and azithro-

### Table 2. Major clinical and laboratory outcomes in 11 patients with cryptosporidiosis and AIDS.

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h oocyst, median*</td>
<td>( 4.28 \times 10^7 )</td>
<td>( 5.85 \times 10^6 )</td>
<td>( 7.30 \times 10^5 )</td>
<td>( 4.00 \times 10^6 )</td>
</tr>
<tr>
<td>No. with ( &gt;1 ) log reduction in 24-h oocyst/no. who collected 24-h stool</td>
<td>NA</td>
<td>4/9</td>
<td>5/9</td>
<td>5/9 (6/10*)</td>
</tr>
<tr>
<td>24-h stool weight, g</td>
<td>469</td>
<td>600</td>
<td>773</td>
<td>350</td>
</tr>
<tr>
<td>No. of stools/day, median(^\dagger)</td>
<td>6.5</td>
<td>5.0</td>
<td>4.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Body weight, kg, mean</td>
<td>68.9</td>
<td>69.8</td>
<td>70.3</td>
<td>69.8</td>
</tr>
<tr>
<td>Karnofsky score, median</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
</tbody>
</table>

**NOTE:** 1 subject did not provide stool and was evaluated for symptoms only.

* Week 2 vs. week 0, \( P = .02 \)

\(^\dagger\) 1 subject with undetectable oocysts at week 4 did not provide stool specimen at week 12.

\(^\dagger\) Week 4 vs. week 0, \( P = .02 \)
mycin. Thus, this study suggests that the combination of paromomycin and azithromycin has significant antiparasitic activity in cryptosporidiosis. After treatment, 8 of 11 patients had resolution or improvement in their diarrhea, but biliary disease remained a problem. Continued diarrhea, when present, was usually due to CMV coinfection or adverse drug effects. Side effects were few but, when noted, were often similar to symptoms of the underlying disease. Further studies with these compounds are warranted, perhaps in combination with other antiparasitic or immunomodulatory agents, in AIDS patients who do not respond to highly active antiretroviral therapy.

References