A DOUBLE-BLIND, RANDOMIZED STUDY OF AZITHROMYCIN COMPARED TO
CHLOROQUINE FOR THE TREATMENT OF PLASMODIUM VIVAX MALARIA
IN INDIA

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Abstract. Azithromycin has demonstrated activity in a prevention of Plasmodium vivax infection, but no controlled treatment studies have been performed. We conducted a double-blinded trial in P. vivax malaria in which patients were randomized to either azithromycin 1,000 mg q.d. × 3 or chloroquine 600 mg q.d. × 2 then 300 mg on Day 3 followed by primaquine on Days 7 through 20. Eighty-five of 97 (88%) of those on azithromycin and 101 of 102 (99%) of those on chloroquine [difference 11%; 95% CI: –18, –4] were clinically cured at Day 7. The Day 28 results were similar [89% versus 99%, azithromycin versus chloroquine, respectively]. Parasitologic success was seen in 81 of 97 (84%) on azithromycin and 100 of 102 (98%) on chloroquine [difference 14%; 95% CI: –22, –6]. The median parasite clearance time was 55 hours on azithromycin and 20 hours on chloroquine (P = 0.001). Drug-related adverse events were seen in 13 of 98 (13%) on azithromycin and 24 of 102 (24%) on chloroquine (P = 0.062). Resolution of parasitemia was significantly faster with chloroquine compared with azithromycin, but azithromycin was better tolerated. These data provide support for further study of azithromycin to better define its role in the treatment of P. vivax malaria, either alone as second-line treatment or in combination with other active therapies.

INTRODUCTION

The standard of care for treatment of patients with malaria due to Plasmodium vivax remains 3 days of chloroquine and 14 days of primaquine. There have been recent reports of chloroquine resistance, and while these reports are uncommon, it would seem prudent to begin to explore treatment options other than chloroquine for treatment of this infection.

Protein synthesis inhibitors such as doxycycline and clindamycin have been used to treat infections due to various plasmodial species. A recent review of chloroquine resistance in P. vivax was recently published, discussing the merits of alternative therapies to chloroquine. Data has accumulated that suggest that azithromycin may also have clinical utility in this setting. In a study of malaria prophylaxis, azithromycin was found to be >98% effective in prevention of malaria due to P. vivax. Additionally, if azithromycin is found to be effective for treatment of P. vivax malaria, it might be considered a partner for other drugs active against P. vivax, given the interest in combination therapies for treatment of malaria. With these data as support, a clinical trial was undertaken to explore the role of a 3-day course of azithromycin for treatment of vivax malaria.

MATERIALS AND METHODS

This trial was a randomized, double-blind, double-dummy study conducted between July 1998 and October 2001 at six sites in India, specifically New Delhi, Baroda, Berhampur, Karamsad, Jabalpur, and Guwahati. Patients were eligible for enrollment into the trial if they were 18 to 65 years of age, presented with a history of fever within the prior 48 hours, had a Giemsa-stained thin smear of peripheral blood with asexual forms consistent with P. vivax, and a quantitative count of <100,000 parasites/µL as well as a rapid test negative for evidence of Plasmodium falciparum (ParaSight F, Becton Dickinson, India, or ICT, ICT Diagnostics, Australia). Patients were excluded from enrollment into the trial if they had evidence of impaired consciousness, were jaundiced, in respiratory distress, or had a self-report of hematuria. Other exclusion criteria included treatment with any antimalarial drug (chloroquine, quinine, mefloquine, sulfadoxine-pyrimethamine) or antibacterial with known antimalarial activity (macrolide, doxycycline, clindamycin) within 15 days prior to enrollment into the study; laboratory evidence or history of significant cardiovascular, liver, or renal functional abnormality that in the opinion of the investigator would place them at increased risk; a serum glucose level less than the lower limit of normal; a history of allergy to or hypersensitivity to chloroquine, azithromycin, or other macrolides; a transfusion of red blood cells within the prior 28 days and any situation that would prevent the patient from returning to follow-up visits. All patients underwent G-6PD testing at baseline.

The institutional review board of each participating center reviewed and approved the protocol. Patients who met the inclusion and exclusion criteria subsequently provided witnessed, written informed consent in the local language. They then underwent a history and physical examination and subsequently were randomized to either azithromycin 1,000 mg q.d. × 3 days or chloroquine 600 mg q.d. each for 2 days and 300 mg on Day 3. In addition to active drug, patients were also provided matching placebos. Primaquine was provided at Days 7 through 20. Study drug was provided to sites in blocks of four. Sealed envelopes were available at each site to be opened only in the case of emergencies. The integrity of these envelopes was monitored periodically at each site. All parties remained blinded to treatment assignment.
Patients were hospitalized from the time of randomization. Peripheral blood smears and oral temperatures were obtained at baseline and every 8 hours. Patients were discharged when two consecutive blood smears were negative for parasites, returning for follow up visits at Days 7, 14, and 28 at which time a clinical examination and a quantitative parasite count was performed. Patients who the investigator felt were not responding to therapy were given alternative therapies at their discretion, including quinine and mefloquine.

The study was designed as a noninferiority trial, and the level of significance was 0.049, adjusted for an interim analysis. The two regimens would be considered equivalent if the lower limit on the difference in clinical response rates of the 95.1% confidence interval was greater than or equal to −10%. If the true success rates were the same for both treatments and as low as 95%, 200 subjects provides a probability of 0.845 of concluding that azithromycin is noninferior to chloroquine. Missing data were imputed by using the method of the last observation carried forward. Data were analyzed on a modified intent to treat basis, defined as including patients with a positive smear for malaria and a negative rapid test who were randomized into the study. The primary end point was clinical response, defined as resolution of fever, without relapse, at Day 7. Secondary end points included clinical response rate at Days 3, 14, and 28, parasitological response rate at Days 3, 7, 14, and 28, time to resolution of fever, and time to clearance of parasitemia. Parasite clearance times for 50% and 90% of the treated population (PC_{50}, PC_{90}) were also calculated for patients remaining on study therapy. Parasitologic failure was defined as RI (recrudescence after clearance of parasitemia); RII (reduction in parasitemia by > 75% of baseline without clearance); RIII (failure to reduce parasitemia to < 25% of baseline). A regression analysis was performed to assess the correlation between baseline parasitemia and time to parasite clearance.

### RESULTS

The demographic characteristics of each group were evenly matched (Table 1). By the Day 7 assessment, more patients given chloroquine had resolved their fever compared with those who received azithromycin [99% (101 of 102) versus 88% (87 of 97), respectively; 95.1% CI on the difference: −18, −4] (Table 2). An assessment of the resolution of fever by Day 3 revealed that the difference in clinical response was evident at that earlier timepoint (94% versus 74% for chloroquine and azithromycin treated patients, respectively).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Azithromycin (N = 97)</th>
<th>Chloroquine (N = 102)</th>
<th>Difference (95.1% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>72 (74%)</td>
<td>96 (94%)</td>
<td>20 (−30, −10)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Day 7</td>
<td>85 (88%)</td>
<td>101 (99%)</td>
<td>11 (−18, −4)</td>
<td>0.003</td>
</tr>
<tr>
<td>Day 28</td>
<td>86 (89%)</td>
<td>101 (99%)</td>
<td>10 (−17, −3)</td>
<td>0.006</td>
</tr>
<tr>
<td>PC_{50} (h)</td>
<td>55</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC_{90} (h)</td>
<td>96</td>
<td>40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The clinical outcome measures are supported by the assessment of parasitologic response. Patients receiving azithromycin were slower to resolve their parasitemia compared with those getting chloroquine with 44% and 93% having negative peripheral blood smears at Day 3, respectively (Table 3; Figure 1). The median time to clearance of parasitemia was 20 hours for patients receiving chloroquine compared with 55 hours for those getting azithromycin. The parasite clearance time for azithromycin and chloroquine treated subjects was proportional to the initial parasite count [correlation coefficients of 0.31 (P = 0.005) and 0.25 (P = 0.001), respectively].

No patients given chloroquine required another antimarial therapy, though three patients were slow to resolve their fever. One patient given chloroquine had a significant parasitemia (45% of baseline) 48 hours after starting therapy and as low as 95%, 200 subjects provides a probability of 0.845 of concluding that azithromycin is noninferior to chloroquine. Missing data were imputed by using the method of the last observation carried forward. Data were analyzed on a modified intent to treat basis, defined as including patients with a positive smear for malaria and a negative rapid test who were randomized into the study. The primary end point was clinical response, defined as resolution of fever, without relapse, at Day 7. Secondary end points included clinical response rate at Days 3, 14, and 28, parasitological response rate at Days 3, 7, 14, and 28, time to resolution of fever, and time to clearance of parasitemia. Parasite clearance times for 50% and 90% of the treated population (PC_{50}, PC_{90}) were also calculated for patients remaining on study therapy. Parasitologic failure was defined as RI (recrudescence after clearance of parasitemia); RII (reduction in parasitemia by > 75% of baseline without clearance); RIII (failure to reduce parasitemia to < 25% of baseline). A regression analysis was performed to assess the correlation between baseline parasitemia and time to parasite clearance.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Azithromycin (N = 97)</th>
<th>Chloroquine (N = 102)</th>
<th>95.1% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3</td>
<td>43 (44%)</td>
<td>95 (93%)</td>
<td>−60, −38</td>
</tr>
<tr>
<td>Failure (RI; RII)</td>
<td>26; 28</td>
<td>6; 1</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>81 (84%)</td>
<td>100 (98%)</td>
<td>−22, −6</td>
</tr>
<tr>
<td>Failure (early RI; RII; RIII)</td>
<td>1; 7; 8</td>
<td>0; 2; 0</td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td>85 (88%)</td>
<td>101 (99%)</td>
<td>−18, −4</td>
</tr>
<tr>
<td>Failure (RI; RII; RIII)</td>
<td>1; 3; 8</td>
<td>0; 1; 0</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The purpose of this study was to explore potential options for treatment of vivax malaria in anticipation that chloroquine resistance to \textit{P. vivax} could eventually become widespread. This is the first randomized, double-blind, comparative trial to examine the effects of azithromycin for treatment of \textit{P. vivax} and, relative to chloroquine, a number of observations can be made.

Azithromycin was slower to resolve parasitemia. In parallel to these parasitologic findings, fever was slower to resolve in azithromycin-treated patients. While, in general, patients showed gradual reduction in their daily maximum temperature elevation, there may have been a tendency for investigators to switch treatment of patients not resolving their fever by Day 3, based on an anticipated rapid response to chloroquine. This slower resolution of fever and parasitemia was not unanticipated as clindamycin, another member of the macrolide/lincosamide family of antibiotics, has also been shown to resolve parasitemia more slowly than chloroquine.\cite{5,7} The reason for this slower resolution is not clear. The effects of interference with protein synthesis, possibly in the apicoplast, should be explored more fully.

As is demonstrated in Figure 1, the rate of resolution of parasitemia, on average, was slower for patients treated with azithromycin. The reason for this delay in action is not clear but may be a result of the time it takes for the parasite to die after inhibition of protein synthesis. Partnering azithromycin for the first day or two with a more rapidly cidal agent may cause a faster parasitologic and symptomatic response and improve the overall treatment effect. These data also point out the difficulties with using the RI/RII/RIII nomenclature of parasitologic failure for slower acting drugs, as patients deemed RIII failures can go on to be cured.

There are two other published reports of an experience with azithromycin for the treatment of \textit{P. vivax} infection. In the first, five patients with vivax malaria were initially cured but three of the five relapsed, suggesting that eradication of hypnozoites may not be possible with azithromycin.\cite{8} In the second experience, 16 patients were randomized to open-label azithromycin or other antibacterial agents, such as tetracycline, clindamycin, or doxycycline. Again, parasite clearance was noted in those treated with azithromycin but most patients relapsed within 28 days.\cite{6} Although similar parasite clearance times are reported, this study was smaller, did not use blinded therapy, did not provide primaquine, and used a 500-mg dose of azithromycin, half of the dose used in the current report. In both of these experiences, the initial cure rates through Day 7 are consistent with the results of this study.

This study has certain limitations. No conclusions can be drawn around the likelihood of late relapse on azithromycin compared with chloroquine as all patients received primaquine on Day 7 and the follow-up ended at Day 28. Studies to explore the activity of azithromycin on gametocytes are warranted. Also, this study was performed in only one geographic area. Conclusions about treatment effects in other regions will require studies from those areas.

In conclusion, azithromycin given as 1 g per day for 3 days resulted in an 88% clinical response rate by Day 7 but was not as active as chloroquine. Although it appeared to be better tolerated overall, the slower onset of action of azithromycin delayed the time to clinical improvement. These data provide support for further study of azithromycin to better define its role in the treatment of \textit{P. vivax} malaria, either alone as second-line treatment or in combination with other active therapies.

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\begin{table}
\centering
\caption{Adverse events}
\begin{tabular}{|l|c|c|c|}
\hline
 & \textbf{Azithromycin} & \textbf{Chloroquine} & \textbf{\textit{P} value} \\
 & \textit{(N = 97)} & \textit{(N = 102)} & \\
\hline
Patients with treatment-related adverse events & 13 & 24 & 0.06 \\
Discontinued for adverse events & 0 & 2* & \\
Number of adverse events & 15 & 35 & 0.002 \\
Headache & 0 & 1 & \\
Nausea & 0 & 5 & \\
Vomiting & 0 & 8 & \\
Musculoskeletal & 2 & 0 & \\
Respiratory & 2 & 0 & \\
Rash & 0 & 3 & \\
Pruritus & 1 & 8 & \\
\hline
\end{tabular}
\footnote{* One patient with a maculopapular rash, the other with severe pruritus.}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Parasitemia (mean) for patients treated with azithromycin and chloroquine.}
\end{figure}
AZITHROMYCIN FOR VIVAX MALARIA

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