PHARMACOKINETICS OF AZITHROMYCIN AND THE COMBINATION OF IVERMECTIN AND ALBENDAZOLE WHEN ADMINISTERED ALONE AND CONCURRENTLY IN HEALTHY VOLUNTEERS

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Abstract. Azithromycin is a critical component of an integrated disease elimination program against trachoma. This study was conducted to evaluate whether azithromycin has a pharmacokinetic interaction with the combination of ivermectin and albendazole. Eighteen healthy volunteers were administered single doses of azithromycin, ivermectin/albendazole, and the combination of the three agents in random, crossover fashion. To assess the presence of interactions, test (combination) and reference (single dose) data were compared using an estimation approach. Compared with reference phases, the geometric mean values for the combination arm’s azithromycin AUC0–t and Cmax were increased approximately 13% and 20%, respectively, albendazole AUC0–t decreased by approximately 3% and Cmax increased approximately 3%, and ivermectin AUC0–t and Cmax were increased 31% and 27%, respectively. Albendazole sulfoxide AUC0–t and Cmax were decreased approximately 16% and 14%, respectively. All treatments were well tolerated. The interactions for azithromycin and albendazole were minimal although the increase in ivermectin exposure requires further study.

INTRODUCTION

A number of public-private partnerships have been created in public health in recent years. One of the challenges in global health is the effective delivery of proven interventions to communities in developing countries. Several of the interventions are focused on single disease control programs, with implementation largely based on delivery through the primary health infrastructure. The demands of various programs on this peripheral level of the health system are tremendous and challenging to health workers, as well as to volunteers in the community. Single disease elimination programs bring value to the community; however, they are in turn dependent on a scarce community resource: the community health worker. This raises an important issue of whether integration of disease elimination programs can improve operating efficiencies at the village and district levels.1–3

Trachoma is caused by ocular strains of Chlamydia trachomatis and the disease persists in its severest forms in the poorest parts of Africa and Asia.4 The case for elimination of trachoma was outlined at a World Health Organization (WHO) meeting in 1996. To mobilize resources and advocacy, WHO founded the Alliance for the Global Elimination of Trachoma by 2020 in 1997. In 1998 a World Health Assembly resolution called for eliminating blinding trachoma by member states by 2020 using the WHO recommended SAFE strategy (Surgery for late stage disease, Antibiotics for acute infection, and improved Facial hygiene and Environmental change [i.e., improved access to water and sanitation]). Pfizer Inc. agreed to donate azithromycin in support of national programs implementing the SAFE strategy, and, with the Edna McConnell Clark Foundation, founded the International Trachoma Initiative (ITI) as a charity dedicated to the elimination of blinding trachoma by 2020. By July 2006, ITI operations have supported the administration of more than 41 million doses of azithromycin and conducted at least 200,000 surgeries (www.trachoma.org), and commitments have been made to scale up the donation to reach 135 million people in the next 3 years.5 Success has been documented in countries where national commitment has been strong (i.e., Morocco)6 and recently in a community in Tanzania where antibiotic coverage rates were nearly 100%.7

In many parts of the developing world trachoma is endemic8 and overlaps with a number of other infectious diseases that are also part of disease elimination and control programs. Onchocerciasis, or river blindness, is caused by Onchocerca volvulus and is transmitted by simulium black fly bites.9 Lymphatic filariasis is a mosquito-transmitted disease caused by Wuchereria bancrofti and Brugia malayi. Annual treatment with ivermectin for onchocerciasis and albendazole/ivermectin for lymphatic filariasis reduces filarial counts and interrupts further transmission. Albendazole/ivermectin is also active against soil-transmitted helminth infections and the case has been made that a package of interventions against a basket of neglected tropical diseases would be cost-effective and compare favorably with interventions targeting acquired immunodeficiency syndrome/human immunodeficiency virus (HIV), tuberculosis, and malaria.10

Azithromycin, the sole member of the 15-membered macrolide structured sub-class known as the azalides, has currently become the standard for mass treatment of villages in trachoma-endemic areas.11 Because azithromycin and ivermectin are likely to be substrates of the drug transporter P-glycoprotein (PGP) in humans,12,13 studies measuring drug interactions and pharmacokinetic profiles of the drugs involved in combination therapy are required for community disease elimination programs. This exploratory healthy volunteer trial was undertaken to investigate whether the combination of azithromycin, ivermectin, and albendazole could be safely co-administered without significant pharmacokinetic interactions prior to further study in areas endemic for trachoma, onchocerciasis, lymphatic filariasis, and intestinal helminth infections.12,13

METHODS

Study subjects. The Bassett Healthcare Institutional Review Board reviewed and approved this randomized, open-
Drug analysis. Azithromycin plasma samples were assayed for azithromycin concentrations using a validated proprietary liquid chromatography system with electrochemical detection (LCEC). Briefly, azithromycin was extracted from plasma samples by liquid/liquid extraction at alkaline pH with methyl-t-butyl ether. Before the extraction, the N-propargyl derivative of azithromycin was added as an internal standard. After extraction, the organic layer was collected, transferred to a clean tube, and evaporated to dryness. The residue was reconstituted with a buffer/acetonitrile mixture and washed with hexane. The extract was then injected into an LCEC system set up with a hydrocarbon-coated zirconium oxide stationary phase and an alkaline phosphate/buffer/acetonitrile mobile phase. The linear range of the assay was 12.5–1,000 µg/L with an overall accuracy and precision of > 90% and < 10%, respectively.

Albendazole and albendazole sulfoxide samples were assayed for their respective concentrations using a validated high-pressure liquid chromatography assay with mass spectrometry detection (HPLC/MS/MS). Briefly, albendazole and albendazole sulfoxide were extracted using liquid/liquid extraction with ethyl acetate. Before the extraction, oxibendazole was added as an internal standard. The organic layer was collected and evaporated to dryness. The residue was then reconstituted with mobile phase. Analysis was performed using a Synergi Polar-RP column (Phenomenex, Torrance, CA) with an ammonium formate/methanol/acetonitrile/formic acid mobile phase. The linear range of the two assays was 1.00–200 ng/mL and 10.0–2000.0 ng/mL, respectively. Precision and accuracy of both assays was < 10% and > 90%, respectively.

Ivermectin H$_2$B$_{1a}$ and H$_2$B$_{1b}$ were assayed using a validated HPLC system with LC/MS detection. Briefly the two ivermectin components were extracted using liquid/liquid extraction at alkaline pH with methyl-t-butyl ether. Before the extraction, abamectin was added as an internal standard. The organic layer was collected, transferred, and evaporated to dryness. The residue was then reconstituted using an ammonium bicarbonate buffer/methanol mixture. The reconstituted samples were then injected into the assay system that used a Luna C8 (2) column (Phenomenex) with an ammonium hy-
droxide/water/methanol mobile phase. The assays were linear over the ranges of 2.5–1,000.0 ng/mL and 2.5–20.0 ng/mL, respectively. The precision values for both assays were < 10%. In terms of accuracy, although the bias was not exceeded (± 15%) for H₂B₁a, for either the high-quality or low-quality control (QC) samples, they were exceeded for H₂B₁b during long-term stability testing (–21.8% at the low QC and –17.3% for the high QC).

For all assays, the low value in the standard curve was also the lower limit of quantification for the assay. None of the assays were cross-reactive with each other.

**Pharmacokinetic and statistical analysis.** Plasma concentration versus time data for azithromycin, albendazole, albendazole sulfoxide, and ivermectin (concentration = concentration total of H₂B₁a and H₂B₁b) were analyzed using the WinNonlin 4.1 software program (Pharsight Corporation, Mountain View, CA) and non-compartmental methods with a uniform weighting scheme. Maximum observed concentrations (Cmax) and time of Cmax (Tmax) were obtained by direct observation of the plasma data. Areas under the concentration-time curve (AUC) were calculated using the linear-log trapezoidal rule, from time zero to the time of last measurable concentration (AUC₀₋ₙ₀), and extrapolated to infinity using the elimination rate constant (lambda z) extrapolated from the last measurable timepoint (AUC₀₋ₙ₀); half-life (T₁/₂), calculated as the reciprocal of the terminal phase rate constant lambda z; total oral clearance (CL/F, with F denoting bioavailability, calculated as dose/AUC₀₋ₙ₀); and apparent volume of distribution during the terminal phase (V₁/F, with F denoting bioavailability, calculated as CL/F/lambda z). Statistical analysis of the data was performed using SigmaStat version 3.1 (SPSS Inc., Chicago, IL). Descriptive statistics were completed for each pharmacokinetic parameter as well as for subject demographics and ivermectin dosage. Log-transformed pharmacokinetic parameters were analyzed by an analysis of variance incorporating sequence, period, and treatment effects. The magnitude of interaction was estimated using ratios of the population geometric means and associated 90% confidence intervals (CIs) for the test and reference phases for Cmax, AUC₀₋ₙ₀, and AUC₀₋ₙ₀. Lack of interaction would be concluded if the 90% CI were contained within the equivalence limits of 80% and 125%.

Power calculations indicated that on the basis of variability of ~50% in AUC or Cmax, to detect a 25% change in either parameter of azithromycin at an α of 0.05, a sample size of 18 subjects would have > 90% power to detect it.

**RESULTS**

Twenty healthy Caucasian volunteers were screened for this study with 18 enrolled and completing the study (9 men and 9 women, mean ± SD age = 39.4 ± 10.5 years, mean ± SD weight = 78.2 ± 12.4 kg, mean ± SD ivermectin dose = 15.5 ± 2.6 mg). One subject diagnosed with influenza 24 hours after the start of her third study phase did not complete blood sampling for that arm; however, the data were still included in the analyses. Summary pharmacokinetic parameters for the test and reference phases for each drug, as well as the geometric mean ratios and 90% CIs, are shown in Table 1. Compared with reference phases, during the combination arm, azithromycin AUC₀₋ₙ₀ and Cmax were increased approximately 13% and 20%, respectively, albendazole AUC₀₋ₙ₀ was decreased approximately 3% and Cmax was increased approximately 3%, and ivermectin AUC₀₋ₙ₀ and Cmax were increased approximately 31% and 27%, respectively. Albendazole sulfoxide AUC₀₋ₙ₀ and Cmax were decreased 16% and 14%, respectively.

In general, all treatments were safe and well tolerated. Only 2 of 18 subjects reported mild adverse effects that were possibly related to study medications. One patient had mild indigestion after taking the ivermectin/albendazole combina-

### Table 1

Mean ± SD pharmacokinetic parameter values and geometric mean ratios (test/reference) and 90% confidence intervals for azithromycin, albendazole, albendazole sulfoxide, and ivermectin*  

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase</th>
<th>Azithromycin</th>
<th>Albendazole</th>
<th>Albendazole sulfoxide</th>
<th>Ivermectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/L)</td>
<td>Test</td>
<td>403 ± 165</td>
<td>37.4 ± 26.7</td>
<td>426.9 ± 182.1</td>
<td>60.3 ± 18.8‡</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>335 ± 148</td>
<td>38.5 ± 33.9</td>
<td>495.6 ± 218.2</td>
<td>48.8 ± 17.1</td>
</tr>
<tr>
<td>GM ratio (90% CI)</td>
<td>Test</td>
<td>119.8% (97.4, 147.3)</td>
<td>103.1% (77.5, 137.0)</td>
<td>86.1% (71.9, 103.1)</td>
<td>126.9% (111.9, 144.0)</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>115.9% (97.1, 146.5)</td>
<td>98.7% (77.8, 129.2)</td>
<td>83.9% (71.0, 100.5)</td>
<td>126.7% (111.8, 144.1)</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>Test</td>
<td>2.62 ± 1.57</td>
<td>1.72 ± 0.67‡</td>
<td>2.56 ± 0.82§</td>
<td>3.79 ± 1.69§</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>2.78 ± 1.21</td>
<td>2.20 ± 1.03</td>
<td>3.54 ± 1.20</td>
<td>5.06 ± 1.98</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>Test</td>
<td>57.7 ± 37.9</td>
<td>12.2 ± 1.2</td>
<td>20.3 ± 1.66</td>
<td>66.2 ± 4.13</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>44.1 ± 13.2</td>
<td>9.4 ± 1.9</td>
<td>19.4 ± 1.35</td>
<td>58.1 ± 4.70</td>
</tr>
<tr>
<td>AUC₀₋ₙ₀ (µg.hr/L)</td>
<td>Test</td>
<td>4,122 ± 1,550</td>
<td>139.9 ± 117.2</td>
<td>6,070 ± 3,385§</td>
<td>1,332 ± 530§</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>3,724 ± 1,564</td>
<td>164.8 ± 160.9</td>
<td>7,547 ± 4,737</td>
<td>923.8 ± 522</td>
</tr>
<tr>
<td>GM ratio (90% CI)</td>
<td>Test</td>
<td>113.3% (98.6, 130.2)</td>
<td>96.8% (73.4, 127.8)</td>
<td>83.5% (73.5, 94.8)</td>
<td>130.7% (107.5, 158.8)</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>110.0% (99.5, 120.0)</td>
<td>96.3% (73.2, 119.2)</td>
<td>83.5% (73.4, 94.8)</td>
<td>129.7% (107.4, 155.1)</td>
</tr>
<tr>
<td>AUC₀₋ₙ₀ (µg.hr/L)</td>
<td>Test</td>
<td>5,300 ± 2,131</td>
<td>163.1 ± 128.3</td>
<td>6,683 ± 3,829§</td>
<td>1,432 ± 715§</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>4,634 ± 1,712</td>
<td>190.1 ± 174.1</td>
<td>8,122 ± 4,876</td>
<td>1,194 ± 738</td>
</tr>
<tr>
<td>GM ratio (90% CI)</td>
<td>Test</td>
<td>113.0% (99.3, 128.6)</td>
<td>99.7% (76.2, 130.4)</td>
<td>84.3% (74.9, 95.0)</td>
<td>129.1% (107.4, 155.1)</td>
</tr>
<tr>
<td>CL/F (L/hr)</td>
<td>Test</td>
<td>111.5 ± 50.0</td>
<td>4,055 ± 2,829</td>
<td>75.5 ± 34.2‡</td>
<td>13.5 ± 6.5‡</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>124.4 ± 51.8</td>
<td>5,288 ± 6,356</td>
<td>66.4 ± 38.0</td>
<td>19.2 ± 14.8</td>
</tr>
<tr>
<td>V₁/F (L)</td>
<td>Test</td>
<td>7,470 ± 2,090</td>
<td>48,646 ± 42,742</td>
<td>1,863 ± 952‡</td>
<td>985 ± 355</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>7,396 ± 2,757</td>
<td>33,975 ± 26,759</td>
<td>1,579 ± 1,030</td>
<td>991 ± 383</td>
</tr>
</tbody>
</table>

* GM = geometric mean; CI = confidence interval.
‡ P < 0.01.
§ P < 0.001.
tion, whereas the other patient noted mild dysequilibrium eight days after the administration of the three-drug combination. Neither event required treatment. There were no clinically significant changes in laboratory test values, vital signs, or electrocardiograms during the study.

**DISCUSSION**

This study was conducted to evaluate pharmacokinetic interactions in healthy volunteers between drugs that are under consideration for combination therapy of diseases in community disease elimination programs. Exposures of all three drugs were increased to varying degrees.

Azithromycin is the sole member of the 15-membered macrolide sub-class, the azalides. Its absorption is thought to occur passively and/or actively across the small intestinal lumen via transport channels such as PGP and organic ion transporting protein.\(^{15,16}\) Once absorbed, it becomes a weak substrate for phase I metabolism by the cytochrome p450 3A (CYP3A) system where it undergoes minimal metabolism and does not produce either induction or inhibition of the system.\(^{17}\) The drug then proceeds to the systemic circulation where it is taken up by a variety of cell types and either stored or transported to the site of infection. Within the circulation, azithromycin is bound in a reverse concentration-dependent manner to alpha(1)-acid glycoprotein that is usually a minor contributor to overall total plasma proteins except in times of physiologic stress. Azithromycin is eliminated in the urine to a minor extent and through the intestinal lumen and biliary tree to a major extent. Although a PGP inhibitor would potentially cause a decreased elimination of azithromycin and increased exposure to it, competition between azithromycin and another PGP substrate may lead to accumulation of either compound.\(^{12}\) Mean reference arm results in the current study, including their degree of variability, were comparable to other published healthy volunteer studies with oral azithromycin and as such represent a good basis for test comparison.\(^{12,18,19}\)

Albendazole is a poorly absorbed benzimidazole that has its absorption enhanced 4–5-fold when it is taken with a fatty meal.\(^{20}\) The drug is extensively and rapidly metabolized in the liver to sulfoxide and sulfone metabolites. Large inter-individual variations in plasma concentrations of the sulfoxide metabolite are due either to variation in first-pass metabolism or in the rate of absorption of albendazole. Albendazole sulfoxide is approximately 70% bound to plasma proteins and has a highly variable half-life. Formation of albendazole sulfoxide is catalyzed by both microsomal flavin monooxygenase and isoforms of CYP in the liver and possibly also in the intestine; there is also evidence that benzimidazoles can actually induce their own metabolism. Although the peak concentrations and exposures to albendazole sulfoxide during the reference arm were higher than literature would suggest, this is most likely due to the well-described food effect that the standardized breakfast had on the absorption of the parent compound.\(^{21}\)

In humans, peak ivermectin plasma concentrations are reached approximately 4–5 hours after administration.\(^{22}\) The half-life of ivermectin is reported to range from 12 to 56 hours depending on the population studied.\(^{22,24}\) Ivermectin is highly protein bound (93%) to plasma proteins and is converted to 10 metabolites by CYP3A4, with nearly no unchanged or conjugated ivermectin being found in the patient’s urine. Animal studies have demonstrated that most of the cleared drug is found unchanged in the feces and this is likely to be true in humans. There is no evidence of a drug:drug interaction between albendazole and ivermectin.\(^{25}\) The mean ivermectin reference arm parameter results in the current study and variability are comparable to those in the literature with the half-life at the upper limit of literature reports.

This study showed evidence of mutual interactions, with increased exposures to all three drugs, and a reduction in albendazole sulfoxide. In addition, the variability of the pharmacokinetic data was high, and even if the ratios had been close to 100, it is likely that CIs would have exceeded the 80–125% no-interaction boundaries. The magnitude of interactions for azithromycin and albendazole were minimal and not likely to be clinically relevant. For azithromycin, the slight increase in exposure should mean there is no diminution of its anti-trachoma effects and no safety consequences. The minor decrease in albendazole exposure and decrease in its active metabolite also do not appear to have implications for its safety or tolerability profiles and may be a function of the variability demonstrated in its pharmacokinetics. This study demonstrated a 31% increase in ivermectin AUC\(_{0-4}\) and this may be an underestimate due to possible stability issues with the assay. Although one would expect high concentrations of ivermectin in the brain, extremely low concentrations are actually demonstrated. This finding may be caused by a PGP efflux pump in the brain-blood barrier that prevents the drug from entering the central nervous system (CNS) and its limited affinity for CNS receptors.\(^{13,26}\) Theoretically, competition for the PGP efflux pump from a molecule such as azithromycin\(^{27}\) could increase peak ivermectin CNS concentrations and increase the chances of CNS side effects. It is reassuring that recent data showed no significant CNS toxicity with doses up to 10 times the current standard dose of ivermectin.\(^{26}\)

Further study is needed to evaluate co-administration of albendazole, ivermectin, and azithromycin. Modeling and simulations of dose regimens is underway to evaluate alternative dosing regimens. This will include simultaneous administration and regimens separating azithromycin and ivermectin to predict optimal dose regimens to minimize toxicity.

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