

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

GUY W. AMSDEN,* THOMAS B. GREGORY, CHERYL A. MICHALAK, PAUL GLUE, AND CHARLES A. KNIRSCH
Clinical Research Division, Bassett Healthcare Research Institute, Bassett Healthcare, Cooperstown, New York; Clinical Research and Development, Pfizer Inc., New York, New York

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 AUC_{0-t} and C_{max} were increased approximately 13% and 20%, respectively, albendazole AUC_{0-t} decreased by approximately 3% and C_{max} increased approximately 3%, and ivermectin AUC_{0-t} and C_{max} were increased 31% and 27%, respectively. Albendazole sulfoxide AUC_{0-t} and C_{max} 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-

* Address correspondence to Guy W. Amsden, Clinical Research Division Bassett Healthcare, 1 Atwell Road, Cooperstown, NY 13326. E-mail: guy.amsden@bassett.org

label, three-way crossover study. The study was performed at the Bassett Healthcare Research Institute in 2004–2005. Eighteen healthy volunteers ≥ 18 years of age and within 30% of their ideal body weight for their sex, height, and frame were recruited into the study. After providing written informed consent, subjects were deemed healthy by medical history, physical examination, vital signs (including heart rate, blood pressure and temperature), 12-lead electrocardiogram, and laboratory screening (complete blood count with differential; tests for alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, and serum creatinine; urinalysis; qualitative urine toxicologic tests; and tests for HIV, hepatitis B virus [HBV], and hepatitis C virus [HCV]; and urine pregnancy tests for women of childbearing potential [the latter was repeated prior to each study arm]). Women of childbearing potential were to be surgically sterile (ovaries intact) or using a non-hormonal barrier method of birth control. All subjects were to be free of any drug exposure for at least 21 days prior to the start of the study. Subjects with clinically significant abnormal physical examination results, medical histories, electrocardiograms (defined as QTc > 450 msec or evidence of significant conduction disturbances), or laboratory results were excluded from study participation. Other exclusion criteria included history of significant adverse effects, sensitivity or allergy to azithromycin, ivermectin, albendazole, or any related compounds; pregnant or lactating women; evidence of gastrointestinal disease(s) or pathology that may interfere with proper study medication absorption; donation of blood within eight weeks of the start of the study or plans to donate blood during the study or within 8 weeks after study completion; use of nicotine delivery devices within the past 12 months; current alcohol and/or illicit drug abuse; those unwilling to abstain from alcohol, grapefruit juice, orange juice, grape juice, apple juice, and caffeine during the study phases and 48 hours prior to each study phase; and, those who in the opinion of the investigator could not follow instructions.

Clinical procedures. A computer-generated randomization scheme was used to assign subjects to the following oral, single-dose regimens in random order: 1) azithromycin, 500 mg (Zithromax lots 4HP031A and 4HP043E, expiration dates 4/1/07 and 6/1/07; Pfizer Inc., New York, NY); 2) ivermectin, 200 $\mu\text{g}/\text{kg}$ of total body weight rounded to the nearest 3 mg (Stromectol lot HU83280, expiration date 10/06; Merck, West Point, PA) with albendazole, 400 mg (Albenza lot X3 4A30, expiration date 2/06; GlaxoSmithKline, Research Triangle Park, NC); and 3) all three drugs administered concurrently. All dosing was administered concurrently with 240 mL of water and a standardized breakfast consisting of 12 ounces of skim milk and 1 cup (1 serving size) of General Mills (Golden Valley, MN) Cheerios® cereal (nutritional make-up = 235.65 Kcal, 2.09 g fat, 3 g fiber, 15.68 g protein, 40.43 g carbohydrate, 551.5 mg calcium, 8.25 mg iron, and 5.22 mg zinc). Prior to dosing and the standardized breakfasts, subjects fasted for at least eight hours and then abstained from any further food for four hours after study drug administration. Moderate intake of water was permitted during the four-hour post-dosing period. Subjects were ambulatory throughout the study. Study arms were separated by washout periods of three weeks. With the exceptions of HIV, HBV, HCV, and urine toxicology screening, laboratory tests were repeated for safety monitoring after the completion of subjects' first study

arms and then before and after both of their subsequent study phases. Electrocardiograms were repeated as a measure of safety immediately prior and two hours after each study drug administration.

A total of 19 blood samples were collected predose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours after drug administration during each of the study phases. Because sample volume differed depending on collection time, between 5 mL and 10 mL of blood was obtained at each draw. A heparinized cannula was used for blood draws over the first 12 hours, and blood was obtained by individual venipunctures at other time points. Samples were collected into heparinized Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ). Blood samples were centrifuged at 3,000 rpm for 15 minutes and the plasma samples were collected in plain plastic tubes without anticoagulant and then stored at -80°C . At study completion, samples were shipped frozen overnight in a sufficient quantity of dry ice to BAS Analytics (West Lafayette, IN) for sample analyses of azithromycin, albendazole, albendazole sulfoxide, ivermectin H_2B_{1a} , and ivermectin H_2B_{1b} .

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 $\mu\text{g}/\text{L}$ 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_2B_{1a} and H_2B_{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-

dioxide/ 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 ($\pm 15\%$) for H₂B_{1b} for either the high-quality or low-quality control (QC) samples, they were exceeded for H₂B_{1a} 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 H₂B_{1a} and H₂B_{1b}) were analyzed using the Win-Nonlin 4.1 software program (Pharsight Corporation, Mountain View, CA) and non-compartmental methods with a uniform weighting scheme. Maximum observed concentrations (C_{max}) and time of C_{max} (T_{max}) 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 (AUC_{0-t}), and extrapolated to infinity using the elimination rate constant (λ_z) extrapolated from the last measurable timepoint (AUC_{0-∞}); half life (T_{1/2}), calculated as the reciprocal of the terminal phase rate constant λ_z ; total oral clearance (CL_t/F, with F denoting bioavailability, calculated as dose/AUC_{0-∞}); and apparent volume of distribution during the terminal phase (V_z/F, with F denoting bioavailability, calculated as CL_t/λ_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, subject during

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 C_{max}, AUC_{0-t}, and AUC_∞. Lack of interaction would be concluded if the 90% CI were contained within the equivalence limits of 80% and 125%.¹⁴ Power calculations indicated that on the basis of variability of ~50% in AUC or C_{max}, 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.¹²

RESULTS

Twenty healthy Caucasian volunteers were screened for this study with 18 enrolled and completing the study (9 men and 9 women, mean \pm SD age = 39.4 \pm 10.5 years, mean \pm SD weight = 78.2 \pm 12.4 kg, mean \pm SD ivermectin dose = 15.5 \pm 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 AUC_{0-t} and C_{max} were increased approximately 13% and 20%, respectively, albendazole AUC_{0-t} was decreased approximately 3% and C_{max} was increased approximately 3%, and ivermectin AUC_{0-t} and C_{max} were increased approximately 31% and 27%, respectively. Albendazole sulfoxide AUC_{0-t} and C_{max} 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 \pm SD pharmacokinetic parameter values and geometric mean ratios (test/reference) and 90% confidence intervals for azithromycin, albendazole, albendazole sulfoxide, and ivermectin*

Parameter	Phase	Azithromycin	Albendazole	Albendazole sulfoxide	Ivermectin
C _{max} (μg/L)	Test	403 \pm 165	37.4 \pm 26.7	426.9 \pm 182.1	60.3 \pm 18.8†
	Reference	335 \pm 148	38.5 \pm 33.9	495.6 \pm 218.2	48.8 \pm 17.1
	GM ratio (90% CI)	119.8% (97.4, 147.3)	103.1% (77.5, 137.0)	86.1% (71.9, 103.1)	126.9% (111.9, 144.0)
T _{max} (hr)	Test	2.62 \pm 1.57	1.72 \pm 0.67‡	2.56 \pm 0.82§	3.79 \pm 1.69§
	Reference	2.78 \pm 1.21	2.20 \pm 1.03	3.54 \pm 1.20	5.06 \pm 1.98
T _{1/2} (hr)	Test	57.7 \pm 37.9	12.2 \pm 12.4	20.3 \pm 16.6	66.2 \pm 41.3
	Reference	44.1 \pm 13.2	9.4 \pm 9.8	19.4 \pm 13.5	58.1 \pm 47.0
AUC _{0-t} (μg.hr/L)	Test	4,122 \pm 1,550	139.9 \pm 117.2	6,070 \pm 3,385‡	1,132 \pm 550‡
	Reference	3,724 \pm 1,564	164.8 \pm 160.9	7,547 \pm 4,737	923.8 \pm 522
	GM ratio (90% CI)	113.3% (98.6, 130.2)	96.8% (73.4, 127.8)	83.5% (73.5, 94.8)	130.7% (107.5, 158.8)
AUC _∞ (μg.hr/L)	Test	5,300 \pm 2,131	163.1 \pm 128.3	6,683 \pm 3,829‡	1,432 \pm 715‡
	Reference	4,634 \pm 1,712	190.1 \pm 174.1	8,122 \pm 4,876	1,194 \pm 738
	GM ratio (90% CI)	113.0% (99.3, 128.6)	99.7% (76.2, 130.4)	84.3% (74.9, 95.0)	129.1% (107.4, 155.1)
CL _t /F (L/hr)	Test	111.5 \pm 50.0	4,055 \pm 2,829	75.5 \pm 34.2‡	13.5 \pm 6.5‡
	Reference	124.4 \pm 51.8	5,288 \pm 6,356	66.4 \pm 38.0	19.2 \pm 14.8
V _z /F (L)	Test	7,470 \pm 2,090	48,646 \pm 42,742	1,863 \pm 952‡	985 \pm 355
	Reference	7,396 \pm 2,757	33,975 \pm 26,759	1,579 \pm 1,030	991 \pm 383

* GM = geometric mean; CI = confidence interval.

† $P < 0.01$.

‡ $P < 0.05$.

§ $P < 0.001$.

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 structured 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.²⁰ 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–t}, 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 blood-brain barrier that prevents the drug from entering the central nervous system (CNS) and its limited affinity for CNS receptors.^{13,20} Theoretically, competition for the PGP efflux pump from a molecule such as azithromycin¹² 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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Authors' addresses: Guy W. Amsden, Thomas B. Gregory, and Cheryl A. Michalak, Clinical Research Division, Bassett Healthcare, 1Atwell Road, Cooperstown, NY 13326, Telephone: 607-547-3399, Fax: 607-547-6914, E-mail: guy.amsden@bassett.org. Paul Glue and Charles A. Knirsch, Clinical Research and Development, Pfizer Inc. New York, NY 10017.

Reprint requests: Guy W. Amsden, Clinical Research Division, Bassett Healthcare, 1Atwell Road, Cooperstown, NY 13326.

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