

Pharmacokinetics of Azithromycin Administered Alone and with Atovaquone in Human Immunodeficiency Virus-Infected Children

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To evaluate if atovaquone (ATQ) interacts pharmacokinetically with azithromycin (AZ) in human immunodeficiency virus-infected children, 10 subjects (ages, 4 to 13 years) were randomized in a crossover study to receive AZ (5 mg/kg/day) alone (ALONE) or AZ (5 mg/kg/day) and ATQ (30 mg/kg/day) simultaneously (SIM) prior to receiving AZ and ATQ staggered by 12 h. Despite a lack of significant difference in the mean AZ pharmacokinetic parameters, the steady-state values of AZ's area under the concentration-time curve from 0 to 24 h and maximum concentration in serum were consistently lower ($n = 7$ of 7) for the SIM regimen than they were for the ALONE regimen. A larger study will be required to determine if ATQ affects AZ pharmacokinetics and efficacy in a clinically significant manner.

Children infected with human immunodeficiency virus (HIV) have an increased risk of serious and recurrent infections (3, 15, 16, 18, 20), among which the most common is *Pneumocystis carinii* pneumonia (PCP). A new, promising combination, azithromycin (AZ) plus atovaquone (ATQ), is currently under investigation in a phase II/III clinical trial (ACTG 254) to compare its efficacy and safety with those of trimethoprim-sulfamethoxazole in the prophylaxis of multiple opportunistic infections in HIV-infected children. In a preliminary study of HIV type 1 (HIV-1)-infected children (ACTG 254; AZ at 5 mg/kg/day and ATQ at 30 mg/kg/day), we found that 11 of 17 subjects had AZ concentrations in serum below 50 ng/ml by 4 h after dosing. Moreover, the predose concentrations in serum (29 ± 49 ng/ml) were considerably lower than those reported for children not infected with HIV (67 ± 31 ng/ml [13]). Therefore, we initiated a drug-drug interaction study to determine if coadministration of AZ and ATQ leads to a reduction in AZ concentrations in serum and to test if the intestine is the site of potential interaction.

The protocol was approved by the Institutional Review Board at each participating site. Prior to enrollment, written informed consent was obtained from each subject's parent or legal guardian. Subjects were excluded from participation if they had suspected or active PCP; were receiving antimicrobial treatment for active infections, including *Mycobacterium avium* complex, toxoplasmosis, tuberculosis, cryptosporidiosis, and microsporidiosis; had a known history of hypersensitivity to microfluidized ATQ and/or AZ; had grade 2 or worse diarrhea for more than 1 week or other causes of malabsorption; had a low hemoglobin level (≤ 7.0 g/dl), absolute neutrophil count (< 750 cells/mm³), or platelet count ($\leq 50,000$ cells/mm³); had

a total concentration of bilirubin that was ≥ 3 times the upper limit of normal values or serum creatinine that was ≥ 1.7 mg/dl; or were pregnant or lactating. To be eligible for enrollment, subjects should not have received AZ and/or ATQ for more than 3 consecutive weeks up to 2 weeks prior to study entry.

A power analysis, based on the data of Nahata et al. (13), indicated that a minimum of 10 subjects was required to determine if the pharmacokinetics of AZ are significantly affected ($>40\%$ decrease in the area under the concentration-time curve [AUC]) by coadministration of ATQ. Five male and five female HIV-1-infected children (4 to 13 years old; body weight, 14 to 34 kg) requiring PCP prophylaxis were recruited. The study was divided into three phases, each to last for at least 10 days. In phase 1, subjects (five per group) were randomized to receive either AZ (suspension, 5 mg/kg once daily) and ATQ (microfluidized suspension, 30 mg/kg once daily) simultaneously (SIM regimen; group A) or AZ alone (5 mg/kg once daily) (ALONE regimen; group B) in the morning. In phase 2, subjects in group A discontinued ATQ, while subjects in group B began taking ATQ (30 mg/kg once daily) simultaneously with AZ (5 mg/kg once daily) in the morning. In phase 3, all 10 subjects took AZ (5 mg/kg) in the morning and ATQ (30 mg/kg) at night (STAG regimen). Subjects were asked to take all medications with meals. On days 10 to 15 after each drug regimen was initiated, blood samples (2 ml each) were collected just prior to dose administration and at 1, 2, 4, 6, 12, and 24 h after administration. Serum samples were obtained by centrifugation and stored at -70°C until analysis. AZ concentrations were measured by a specific high-performance liquid chromatography-mass spectrometry method (4), and ATQ concentrations were measured by high-performance liquid chromatography (19). Calibration curves were linear over ranges of 10 to 250 ng/ml for AZ and 0.25 to 50 $\mu\text{g/ml}$ for ATQ. The intra- and interday coefficients of variation for precision were $<13\%$ for AZ and $<6.8\%$ for ATQ.

The maximum concentration of drug in serum (C_{max}), the concentration just prior to dose administration (C_{predose}), the

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TABLE 1. Summary of AZ pharmacokinetic parameters when AZ (5 mg/kg once daily) was administered alone or in combination with ATQ (30 mg/kg once daily) to HIV-1-infected children for at least 10 days

Subject ^a	Age (yr)	Study phase (n)	Body wt (kg)	C _{max} (ng/ml)	T _{max} (h)	C _{predose} (ng/ml)	C ₂₄ (ng/ml)	C _{SS} (ng/ml)	AUC ₀₋₂₄ (ng · h/ml)	CL/F (liters/h/kg)
1	12	ALONE ^b								
		SIM	30.1	131	1	16	28	38	949	5.25
		STAG	31.1	473	1	17	23	56	1,401	3.44
2	10	ALONE	26.8	126	2	29	46	62	1,543	3.14
		SIM	26.8	111	4	23	25	49	1,153	4.21
		STAG	26.4	196	1	27	47	87	2,054	2.40
3	10	ALONE	28.5	203	1	62	46	75	1,891	2.67
		SIM	27.6	90	2	46	37	44	1,195	4.37
		STAG	28.2	129	2	35	31	43	1,104	4.63
5	10	ALONE	24.5	210	2	63	100	129	3,302	1.48
		SIM	25.1	121	2	72	84	93	2,304	2.08
		STAG	23.7	102	6	60	56	82	1,791	2.83
6	6	ALONE	20.7	48	4	26	30	28	695	6.95
		SIM	20.2	27	4	28	18	21	539	9.19
		STAG	20.8	365	1	21	21	64	1,391	3.46
7	4	ALONE	14.2	324	2	56	46	71	1,966	2.44
		SIM	14.4	339	1	20	29	58	1,588	2.97
		STAG	14.2	62	1	61	36	40	1,120	4.27
8	5	ALONE	17.8	250	1	22	35	46	1,343	3.68
		SIM	17.1	127	2	33	30	41	1,035	4.97
		STAG ^c								
9	9	ALONE	28.7	447	2	103	254	222	5,561	0.93
		SIM	29.0	315	4	68	104	137	3,812	1.34
		STAG	28.1	220	1	62	60	66	1,844	2.86
Mean ± SD		ALONE (7)	23.0 ± 5.6	230 ± 130	2 ± 1	52 ± 29	80 ± 80	91 ± 66	2,329 ± 1,632	3.04 ± 1.96
		SIM (8)	23.8 ± 5.8	158 ± 110	2 ± 1	38 ± 22	44 ± 32	60 ± 37	1,572 ± 1,043	4.30 ± 2.41
		STAG (7)	24.6 ± 5.7	221 ± 149	2 ± 2	40 ± 20	39 ± 16	62 ± 18	1,529 ± 371	3.41 ± 0.81

^a Subjects 10 and 4 were excluded from pharmacokinetic analyses due to either noncompliance with the dosing schedule (subject 10) or inexplicably low (below the quantification limit of 10 ng/ml) serum AZ concentrations throughout the sampling period (subject 4).

^b Study not conducted.

^c Data were excluded because the patient missed the AZ dose 1 day prior to the pharmacokinetic study.

concentration at 24 h after oral dosing (C₂₄), and the time to achieve C_{max} (T_{max}) were obtained from serum-time profiles. The area under the serum concentration-time curve for a 0- to 24-h dosing interval (AUC₀₋₂₄) and oral clearance (CL/F,

where F is bioavailability) were computed by noncompartmental analysis (LAGRAN program). A pairwise comparison was performed by using the paired Wilcoxon signed-rank test with Bonferroni's correction at a level of significance of 0.05.

TABLE 2. Comparison of AZ pharmacokinetic parameter estimates in HIV-1-infected and non-HIV-1-infected children

Group and infection (regimen)	No. of subjects	Mean ± SD (range)				
		C _{max} (ng/ml)	C _{SS} (ng/ml)	T _{max} (h)	AUC ₀₋₂₄ (ng · h/ml)	CL/F (liters/h/kg)
HIV infected (5 mg/kg q.d.)	7	230 ± 130 (48-447)	91 ± 66 (28-222)	2.0 ± 1.0	2,329 ± 1,632 (695-5,561)	3.04 ± 1.96 (0.93-6.95)
Non-HIV infected						
Acute otitis media ^a (day 1, 10 mg/kg; days 2-5, 5 mg/kg q.d. ^b)	13	224 ± 120 (65-496)	77 ± 27 ^d (40-110)	1.8 ± 0.4	1,841 ± 651 (951-2,646)	3.03 ± 1.28 ^d (1.82-6.10)
Streptococcal pharyngitis ^c (day 1, 10 mg/kg; days 2-5, 5 mg/kg q.d.)	14	383 ± 142 (165-623)	130 ± 43 ^d (44-214)	2.4 ± 1.1	3,109 ± 1,033 (1,062-5,134)	1.89 ± 1.01 ^d (0.84-4.90)

^a Data are from reference 14.

^b q.d., once a day.

^c Data are from reference 13.

^d Values are estimated based on dose and/or AUC₀₋₂₄ data provided in the reference.

TABLE 3. Summary of ATQ pharmacokinetic parameters when ATQ (29.9 ± 0.9 mg/kg once daily) was simultaneously administered with AZ (4.9 ± 0.2 mg/kg once daily) to HIV-1-infected children for at least 10 days

Subject ^a	Age (yr)	Body wt (kg)	C_{\max} ($\mu\text{g/ml}$)	T_{\max} (h)	C_{predose} ($\mu\text{g/ml}$)	C_{24} ($\mu\text{g/ml}$)	C_{SS} (ng/ml)	AUC_{0-24} ($\mu\text{g} \cdot \text{h/ml}$)	CL/F (ml/min/kg)
2	10	26.8	36	2	31	37	35	822	0.59
3	10	27.6	29	2	26	21	19	529	0.99
4	8	23.1	15	24	9	15	14	327	1.59
5	10	25.1	40	4	32	20	17	413	1.20
6	6	20.2	30	2	27	22	23	583	0.85
7	4	14.4	18	2	8	10	10	266	1.82
8	5	17.1	16	2	11	7	10	239	2.14
9	9	29.0	35	4	31	31	26	724	0.69
Mean \pm SD ($n = 8$)		24.8 ± 6.1	27 ± 10	5 ± 8	22 ± 11	20 ± 10	19 ± 9	448 ± 214	1.24 ± 0.56

^a Data from subject 10 were excluded from analysis due to noncompliance with the dosing schedule. Data from subject 1 were excluded from analysis because the serum ATQ concentrations achieved throughout the 24-h sampling period were inexplicably below the quantification limit ($0.25 \mu\text{g/ml}$) of the assay.

The pharmacokinetics of AZ were quite variable. However, the mean (\pm standard deviation [SD]) values were comparable to those reported previously (Tables 1 and 2). In the present study, steady state was attained in all dose regimens as indicated by a lack of difference between C_{predose} and C_{24} of AZ ($P > 0.05$) (Table 1). We found that the mean C_{\max} , AUC_{0-24} , and CL/F values for AZ were not significantly different between the ALONE and SIM regimens (mean \pm SD, 230 ± 130 versus 162 ± 118 ng/ml, $2,329 \pm 1,632$ versus $1,661 \pm 1,093$ ng \cdot h/ml, and 3.04 ± 1.96 versus 4.16 ± 2.57 liters/h/kg, respectively; $n = 7$; $P > 0.05$). These values are only for the matched pairs and therefore differ from the group means (\pm SD) provided in Table 1. However, when the SIM regimen was compared with the ALONE regimen, there was a reduction in AZ AUC_{0-24} values (by 19 to 37%) and an increase in AZ CL/F values (by 1.2- to 1.6-fold) in seven of seven evaluable subjects (Table 1). The mechanistic basis for this consistent change is not clear. Post hoc analysis of our data indicated that, due to the larger-than-expected variability of AZ pharmacokinetics, about 14 subjects would be needed to definitively ascertain (with 80% power) if there is indeed an interaction between AZ and ATQ ($>40\%$ decrease in AUC). In humans, metabolic and renal clearances do not seem to play a significant role in the elimination of AZ (6, 8) or ATQ (1). AZ is cleared primarily by active biliary secretion and transintestinal secretion (6, 11), whereas ATQ is extensively excreted in bile, followed by enterohepatic recycling (1). Therefore, we had hypothesized that if these two drugs do interact, the site of interaction is most likely to be the intestine. However, this hypothesis was not substantiated by our results, which showed no difference in AZ kinetics whether AZ was given simultaneously or in a staggered manner with ATQ (paired analysis [mean \pm SD]: C_{\max} , 162 ± 118 versus 221 ± 149 ng/ml; AUC_{0-24} , $1,648 \pm 1,102$ versus $1,529 \pm 371$ ng \cdot h/ml; and CL/F , 4.20 ± 2.59 versus 3.41 ± 0.81 liters/h/kg, respectively [$n = 7$; $P > 0.05$]).

At present, the concentrations of AZ in serum required to achieve prophylaxis of multiple opportunistic infections in HIV-1-positive children are not yet defined. Also, the efficacy of AZ is highly correlated with its tissue concentrations rather than its concentrations in serum (2, 17), and AZ concentrations in these two compartments do not appear to be directly correlated (2, 5, 7, 8, 12). Collectively, these observations make it difficult to draw definitive conclusions about the clinical significance of any ATQ-induced reduction in AZ kinetics. However, in the absence of tissue concentration measurements and assuming that at least a 40% change in the AUC of serum AZ concentration is clinically important, our data suggest that

ATQ does not have a significant clinical effect on the pharmacokinetics of AZ.

Concentration profiles of ATQ in serum were highly variable (Table 3). The mean steady-state concentrations (C_{SS}) and AUC_{0-24} for ATQ given simultaneously with AZ were 49 and 45% lower, respectively, than those obtained in a cohort of three HIV-1-infected children (2 to 12 years old; ACTG 227) who also received multiple doses of microfluidized ATQ (30 mg/kg once daily [9]). It is possible that concurrent intake of AZ with ATQ might influence the disposition of ATQ. The clinical significance of such an observation is not clear because the serum ATQ concentrations required for effective prophylaxis of PCP are not known; however, they are expected to be lower than those required for successful treatment ($\geq 15 \mu\text{g/ml}$ [10]). Such ATQ concentrations were maintained in five of eight evaluable subjects in our study (Table 3).

We report here the first pharmacokinetic study in HIV-1-infected children receiving AZ alone or in combination with ATQ. It provides preliminary evidence that concentrations of AZ and ATQ in serum may be reduced when these drugs are coadministered to HIV-infected children. However, a larger number of subjects will need to be studied to definitively determine if these changes are clinically and statistically significant. If the magnitude of change in the AUC of AZ in serum observed here is replicated in a larger study, such a change is not likely to be clinically significant.

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APPENDIX

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