Unanticipated CNS Safety Signal in a Placebo-Controlled, Randomized Trial of Co-Administered Atovaquone-Proguanil and Amodiaquine

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Atovaquone-proguanil (ATV-PG) plus amodiaquine (AQ) has been considered as a potential replacement for sulfadoxine-pyrimethamine plus AQ for seasonal malaria chemoprevention in African children. This randomized, double-blind, placebo-controlled, parallel group study assessed the safety, tolerability, and pharmacokinetics (PKs) of ATV-PG plus AQ in healthy adult males and females of Black sub-Saharan African origin. Participants were randomized to four treatment groups: ATV-PG/AQ (*n* = 8), ATV-PG/placebo (*n* = 12), AQ/placebo (*n* = 12), and placebo/placebo (*n* = 12). Treatments were administered orally once daily for 3 days (days 1–3) at daily doses of ATV-PQ 1000/400 mg and AQ 612 mg. Co-administration of ATV-PG/AQ had no clinically relevant effect on PK parameters for ATV, PG, the PG metabolite cycloguanil, AQ, or the AQ metabolite N-desethyl-amodiaquine. Adverse events occurred in 8 of 8 (100%) of participants receiving ATV-PG/AQ, 11 of 12 (91.7%) receiving ATV-PG, 11 of 12 (91.7%) receiving AQ, and 3 of 12 (25%) receiving placebo. The safety and tolerability profiles of ATV-PG and AQ were consistent with previous reports. In the ATV-PG/AQ group, 2 of 8 participants experienced extrapyramidal adverse effects (EPAEs) on day 3, both psychiatric and physical, which appeared unrelated to drug plasma PKs or cytochrome P450 2C8 phenotype. Although rare cases are reported with AQ administration, the high incidence of EPAE was unexpected in this small study. Owing to the unanticipated increased frequency of EPAE observed, the combination of ATV-PQ plus AQ is not recommended for further evaluation in prophylaxis of malaria in African children.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ New antimalarial drug combinations are needed for seasonal malaria chemoprevention (SMC) in African children to overcome and anticipate parasite resistance to existing agents. Atovaquone-proguanil (ATV-PG) is a registered antimalarial with both chemoprotective and therapeutic activity and requires investigation as a potential component of SMC with amodiaquine (AQ).

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This randomized study evaluated the tolerability, safety, and pharmacokinetics (PKs) of ATV-PG and AQ alone and in combination vs. placebo in African adults.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Transient extrapyramidal symptoms were observed in two of the eight participants who received the combination. Similar adverse events have been reported rarely with AQ administration, but not for ATV-PG. However, there were no important differences in the PKs of ATV-PG/AQ when given alone or in combination.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The combination of ATV-PG/AQ is associated with a central nervous system safety profile which is incompatible with its use in SMC in African children, and the regimen should not be investigated further.

Most malaria deaths occur in African children under 5 years of age, and morbidity impedes child development and increases the susceptibility to other illnesses.[1] In particular, of the ~ 24 million African children under 5 years old infected with *P. falciparum* in 2018, 13.8 million were estimated to suffer from severe or moderate anemia.[2]

Seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine (SP) plus amodiaquine (AQ) is recommended for

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children aged 3–59 months during the malaria season in areas of highly seasonal malaria transmission across the Sahel sub-region of Africa.[3] SMC maintains therapeutic antimalarial blood concentrations throughout the period of greatest risk, preventing around three-quarters of clinical malaria episodes.[3,4] Up to four SP + AQ treatment courses are given at monthly intervals. A single SP + AQ dose is given on day 1 followed by daily AQ for 2 subsequent days. The regimen is well-tolerated, inexpensive, and allows artemisinin-containing regimens to be reserved for the treatment of clinical malaria.[3] However, replacements for SMC are needed because of widespread *P. falciparum* resistance to both SP and AQ.[5–7] Currently, SMC is not recommended in eastern and southern Africa because of high-level resistance to SP.[3]

AQ is a 4-aminoquinoline antimalarial with a similar mechanism of action to chloroquine.[8] AQ has a relatively short half-life (~ 5 hours),[9,10] but is rapidly and almost completely metabolized via cytochrome P450 2C8 (CYP2C8) to the active metabolite N-desethyl-amodiaquine (DEAQ),[11,12] which has a terminal half-life of around 4–18 days.[9,10,13] Antimalarial activity against *P. falciparum* after a standard 3-day treatment course derives primarily from DEAQ, with minimal AQ concentrations synergistically potentiating the DEAQ antimalarial effect.[14]

Atovaquone-proguanil hydrochloride (ATV-PG) is a fixed-dose combination registered for malaria prophylaxis in travelers, and for the treatment of acute uncomplicated *P. falciparum* malaria.[15] The drug is approved for pediatric use, but is not currently used for malaria chemoprevention or treatment in African populations. Common adverse reactions for the curative regimen are abdominal pain, nausea, vomiting, headache, diarrhea, asthenia, anorexia, and dizziness in adults, and in children, vomiting, pruritus, and diarrhea.[15]

A combination of ATV-PG plus AQ (ATV-PG/AQ) is a potential replacement for SP + AQ in SMC. The postdose prophylactic duration of the ATV-PG curative dose,[16,17] and the AQ dose used for SMC,[18,19] suggest a similar dosing regimen to SP + AQ. A 3-day ATV-PG/AQ treatment once a month for 3 to 4 consecutive months may achieve the target 75% reduction in malaria incidence rate in the pediatric population.[3] Experiments in human liver microsomes using validated methods,[20] showed minimal inhibition (half-maximal inhibitory concentration (IC_{50}) > 20 μM) of CYP2C8-mediated AQ N-desethylation by ATV, PG, and its active metabolite cycloguanil (CG), suggesting a low potential for CYP2C8-mediated metabolic drug–drug interactions, and a low risk of adverse reactions associated with reduced CYP2C8 metabolism (Medicines for Malaria Venture, data on file, reference CDCO_MMV_PBPK_20_003).

This phase I study assessed the safety, tolerability, and pharmacokinetics (PKs) of the registered ATV-PG curative dose plus the adult equivalent of the AQ dose approved for SMC when administered alone and in combination, in comparison to placebo.

METHODS

Study design and participants

This randomized, double-blind, placebo-controlled, parallel group, phase I study was conducted between April 4 and October 29, 2019 at Richmond Pharmacology Ltd., London, and registered at ClinicalTrials.gov with the identifier NCT04002687. The study protocol is provided in the supplementary materials (**Protocol S1**). Using a computer-generated randomization schedule, 52 participants were to be randomized (4:3:3:3) to 1 of 4 treatment groups: ATV-PG/AQ (n = 16), ATV-PG/placebo (n = 12), AQ/placebo (n = 12), and placebo/placebo (n = 12). The placebo group was sufficiently large to allow corrected QT (QTc) interval modelling with AQ (to be published separately). Following dose completion for the first 20 randomized participants, the ATV-PG/AQ arm was discontinued because of extrapyramidal adverse effects (EPAEs) in 2 of 8 participants. A further 24 volunteers were included in the study in 3 cohorts of 8 participants, re-randomized so that 12 participants were included in the remaining 3 treatment arms. Thus, the safety population included 44 randomized volunteers (20 men and 24 women): ATV-PG/AQ (n = 8), ATV-PG/placebo (n = 12), AQ/placebo (n = 12), and placebo/placebo (n = 12). One participant in the placebo group did not complete the study after withdrawing consent on day 8 for personal reasons.

Eligible participants were healthy men or women aged 18–45 years, weighing ≥ 50 kg, and with a body mass index 18.0–25.0 kg/m^2 (**Table S1**). To ensure a pharmacogenetically representative population, all participants were of Black sub-Saharan African origin (both parents of Black sub-Saharan African origin). Participants were malaria naïve, without visiting a malaria-endemic region for more than 4 weeks within the previous 12 months. Female participants required a negative pregnancy test and could not be lactating. All participants agreed to use effective contraception. Further requirements were no clinically significant abnormalities based on a medical history, physical examination, vital signs, 12-lead electrocardiogram (ECG), and clinical laboratory evaluation, or any known allergy or hypersensitivity to the study drugs. Key exclusion criteria were any current or recurrent disease or condition that might affect the action, absorption, or disposition of ATV-PG or AQ, a history of photosensitivity or retinopathy, a QT interval corrected using Fridericia's formula (QTcF) > 450 ms, or any other significant disease or disorder which in the investigator's opinion might put the participant at risk, influence study results, or impair the volunteer's ability to participate in the study. Participants were ineligible if they had a positive test for alcohol, drugs of abuse, or a history of alcohol abuse, had used any tobacco or nicotine-containing product within the previous 3 months, any prescription medication within 14 days or 10 half-lives (whichever longer), any nonprescription medication within 7 days or 5 half-lives, any moderate/strong CYP inhibitor or inducer within 30 days or 5 half-lives, or any investigational product or device within 90 days or 5 half-lives before the first dose of study drug.

The study complied with current United Kingdom law, the Good Clinical Practice standards of the International Conference on Harmonization (Topic E6), and the Declaration of Helsinki. Ethical approval was granted by South Central–Berkshire B Ethics Committee. Informed written consent was obtained from all participants before any study-related procedure.

Study drugs and procedures

Participants were screened within 20 days prior to admission to the study unit on day −1, with day 0 to normalize to the environment. Study drugs were administered on days 1, 2, and 3, with discharge on day 4, and outpatient follow-up visits on days 8, 15, 22, 29, and 36 (± 1 day). Study drugs were ATV-PG 250/100 mg tablets (GlaxoSmithKline, Uxbridge, UK), AQ hydrochloride 200 mg tablets containing 153 mg AQ base (Guilin Pharmaceuticals, Guangxi, China), and unmatched ATV-PG and AQ placebo tablets. Four tablets of each drug or unmatched placebo (i.e., 8 tablets in total) were administered in the morning orally once daily for 3 days in the fed state (standard breakfast), with 240 mL of water. Total daily doses were ATV-PQ 1000/400 mg and AQ 612 mg. Blindfolded participants were dosed by an unblinded staff member behind a curtain, with assessments conducted by study staff blinded to treatment.

Safety assessments included standard laboratory tests (hematology, coagulation, biochemistry, and urinalysis), vital signs, physical examinations, triplicate 12-lead ECG, continuous telemetry, and adverse event monitoring. Adverse events were categorized using Medical Dictionary for Regulatory Activities (MedDRA; version 21). Participants completed a Profile of Mood Scales (POMS) questionnaire for evaluation of central nervous system (CNS) subjective symptoms on days 1, 4, 8, and 29. A basic neurological exam was conducted to assess tone, power, coordination, and proprioception in all limbs, gait, cranial nerves examination, and mental status assessment on days 1 to 4 and days 8 and 29.

Blood samples were collected from participants for plasma concentration measurement of ATV, PG, CG, AQ, and DEAQ at the following time points: predose and at 1, 2, 3, 4, 5, 6, 8, and 12 hours postdose on day 1, predose on day 2, predose and 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours postdose on day 3, and on day 8 (120 hours postdose day 3), day 15 (288 hours postdose day 3) and day 22 (456 hours postdose day 3). Plasma concentrations were determined using validated liquid chromatography-tandem mass spectrometry methods at Swiss BioQuant AG, Reinach, Switzerland.[21,22] The lower limit of quantification in plasma was 100 ng/mL for ATV, 5.0 ng/mL for PG, 1.5 ng/mL for CG, 0.5 ng/mL for AQ, and 3.0 ng/mL for DEAQ.

CYP2C8 polymorphisms can affect the biotransformation of AQ to DEAQ and hence exposure. Following the observation of EPAE, a protocol amendment was implemented and a supplementary pharmacogenetic analysis conducted to identify CYP2C8 status in the remaining 15 volunteers recruited. CYP2C8 alleles *1, *2, *3, and *4 were investigated using standard methods.[12,23] CYP2C8 metabolizer status was defined as extensive metabolizer (*1/*1), or intermediate metabolizer (*1/*2).

Outcomes

The primary outcome was an assessment of ATV-PG and AQ safety and tolerability when administered alone and in combination, in comparison with placebo. The PK of ATV, PG, CG, AQ, and DEAQ following administration of ATV-PG and AQ alone or in combination was a secondary outcome. Determination of the relationship between AQ and DEAQ and ECG parameters and any impact of the combination with ATV-PG was also a secondary outcome. A post hoc analysis of the effect of CYP2C8 polymorphism on DEAQ exposure was also conducted.

Statistics

In this exploratory phase I study, sample size was based on the number of participants needed to adequately describe the tolerability, safety, and PKs of the study treatments. The parallel group design and limited sample size are such that this study was not powered to investigate a formal PK drug–drug interaction. The statistical analysis plan was prospectively prepared.

Safety outcomes were reported using descriptive statistics. PK parameters were calculated from ATV, PG, CG, AQ, and DEAQ measured plasma concentrations using noncompartmental analysis. For individual plasma concentration data, the actual blood sampling time relative to the time of study drug administration was used to derive PK parameters. Area under the curve (AUC) values were calculated using the linear/log trapezoidal method, applying the linear trapezoidal rule up to maximum plasma concentrations (C_{max}) and the log trapezoidal rule for the remainder of the curve. AUC was calculated from zero to time t of the last measured concentration above the limit of quantification ($AUC_{0\text{-}t}$) and AUC from predose to 24 hours postdose ($AUC_{0\text{-}24h}$).

Samples below the limit of quantification (BLOQ) prior to the first quantifiable concentration were set to zero. Samples with concentrations BLOQ after the first quantifiable concentration were set to "missing" and omitted from the analysis. Other PK parameters were calculated according to standard equations (Phoenix WinNonlin version 6.3, Pharsight Corporation, Mountain View, CA, USA). Geometric mean ratios for C_{max} and AUC values with 90% confidence limits were calculated by comparing the ATV, PG (and CG) PK following placebo or AQ co-administration. The PK of AQ and DEAQ when co-administered with placebo or ATV-PG were compared similarly.

RESULTS

Safety

Adverse events occurred in 8 of 8 (100%) participants receiving ATV-PG/AQ, 11 of 12 (91.7%) receiving ATV-PG, 11 of 12 (91.7%) receiving AQ, and 3 of 12 (25%) receiving placebo. Adverse events were primarily mild (32/44 (72.7%)) or moderate (4/44 (9.1%)) in severity. No adverse event led to study withdrawal and there were no deaths. When given individually, the safety profiles for ATV-PG and AQ were as expected: dizziness, headache, nausea, and abdominal pain were most commonly reported with ATV-PG[24]; dizziness and nausea with AQ[19] (**Figure 1**). There was one serious adverse event in the ATV-PG/AQ group of EPAE (see below). There was one adverse event of special interest, a decrease in plasma hemoglobin levels in a 39-year-old woman participant receiving ATV-PG/AQ (from 122 g/L to 101 g/L), which recovered by study end, which was not considered drug related. Otherwise, there were no clinically relevant findings in any laboratory results or vital signs. No participant had clinically important ECG findings, QTcF interval prolongation > 480 ms, or a change in QTcF from baseline > 60 msec. QTcF interval prolongation > 450 ms on day 3 at 4–5 hours postdose was noted for 3 of 8 participants in the ATV-PG/AQ group, resolving by 12 hours postdose, and 2 of 12 in the AQ group, resolving by 24 hours postdose.

Extrapyramidal adverse effects

All participants had normal baseline POMS and neurological examinations. In the ATV-PG/AQ group, 2 of 8 participants (participant numbers 11010 and 12013) reported EPAE, both occurring on day 3 and associated with anxiety; an additional participant who received ATV-PG/AQ reported a loss of consciousness (participant 11001; (**Commentary S1**).

Participant 11010 was a man, 25 years old, with the lowest bodyweight among men in the ATV-PG/AQ group (58.6 kg, mean 62.1 kg). The day 3 neurological examination at 15:55 was normal. EPAE started 11 hours 20 minutes after the last dose on day 3 (at 22:05 hours on day 3) and the participant was admitted to the hospital for evaluation. Symptoms were both physical (akathisia and dystonia) and psychiatric (anxiety); all were moderate in intensity. Neurological examination on day 4 noted involuntary tongue movements and related difficulty in speech, present for ~ 2 hours, which did not recur, and no treatment was required. EPAE resolved by day 5, and neurological examination on day 8 was normal. However, mild intermittent anxiety persisted until the participants' last follow-up on day 195.

Participant 12013 was 27 years old, with the lowest body weight among women in the ATV-PG/AQ group (52.6 kg; mean 62.7 kg). As for the previous case, EPAE occurred after the final dose on day 3, were more focused on anxiety, starting at 15:25 on day 3, but with some akathisia (shaking and restlessness). One oral diazepam dose (2 mg) was given on day 3 at 21:00. Symptoms continued before fully resolving on day 5. No persistent anxiety was reported after day 5.

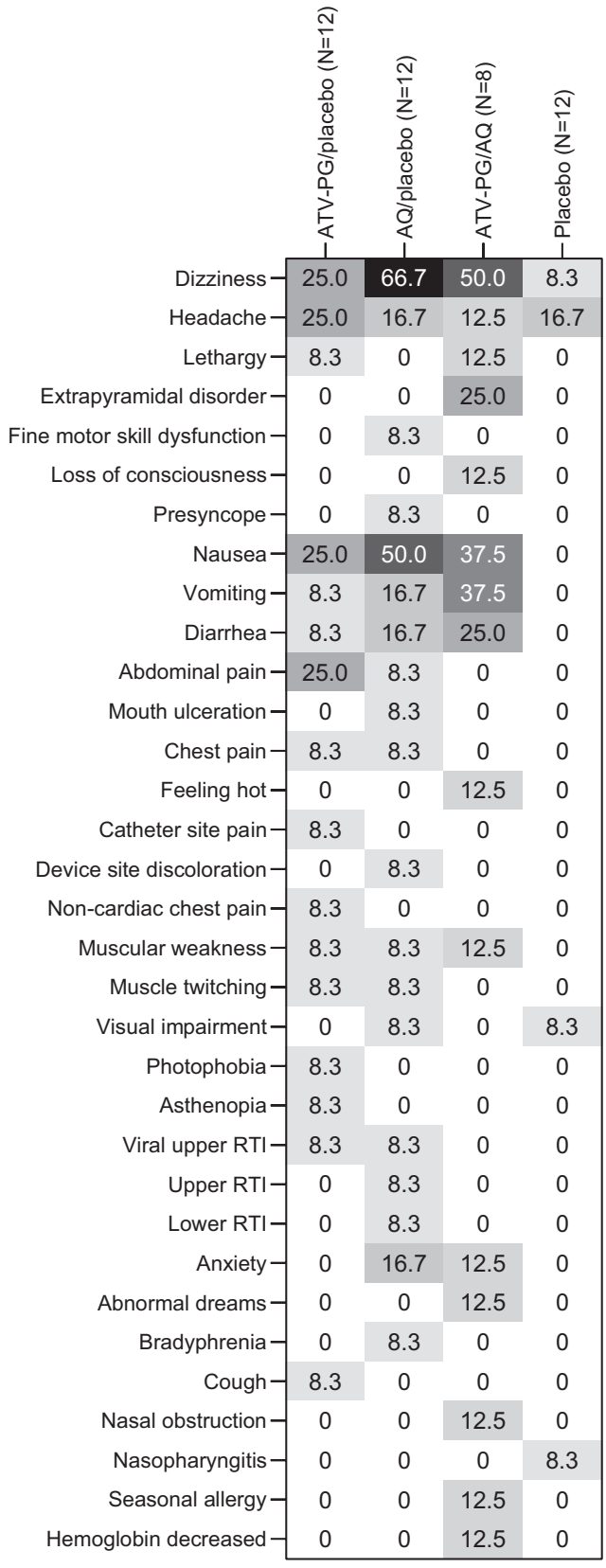

Figure 1 Frequency of adverse events following administration of atovaquone-proguanil (ATV-PG), amodiaquine (AQ) or ATV-PG/AQ. Values are the percentage of participants experiencing the adverse event.

Participant 11001, a 27-year-old woman, developed lightheadedness 3.5 hours after dosing on day 3, and fainted on standing, waking up on the floor seconds later. The episode was unwitnessed and no concurrent vital signs were recorded. After assessment by staff, ~ 10 minutes after the event, the participant walked back to bed unassisted. There was no history of fainting, and she also experienced diarrhea, dizziness, nausea, and vomiting, which were considered related to study drug administration.

Both participants who experienced EPAE had the lowest bodyweight by gender, and some of their PK parameters were outliers in the group receiving ATV-PG/AQ (**Figure 2**). Participant 11010 had the highest ATV C_{max} on day 1 (5,000 ng/mL) and day 3 (7,970 ng/mL), the highest ATV $AUC_{0\text{-}t}$ on day 1 (74902 ng.h/mL) and $AUC_{0\text{-}24h}$ on day 3 (147,889 ng.h/mL), the highest AQ C_{max} on day 3 (30.5 ng/mL), and AQ $AUC_{0\text{-}24h}$ on day 3 (174 ng.h/mL). Participant 12013 had the highest CG C_{max} on day 3 (136 ng/mL), $AUC_{0\text{-}t}$ on day 1 (1,635 ng.h/mL), and $AUC_{0\text{-}24h}$ on day 3 (2,081 ng.h/mL). Both participants had the highest ATV C_{max} and $AUC_{0\text{-}24h}$ and AQ C_{max} and $AUC_{0\text{-}24h}$ on day 3 when adjusted for bodyweight (**Figure S1**).

Pharmacokinetics

Plasma concentration data were available for all 44 participants. On day 3, one participant in the AQ group experienced vomiting on 2 occasions after dosing, and their PK values for day 3 were excluded from the analysis. Geometric mean plasma concentrations–time profiles and PK parameters are shown for ATV, PG, and CG in **Figure 3**, **Figure S2**, and **Table 1** and for AQ and DEAQ in **Figure 4** and **Table 2**. PK values for the placebo group were BLOQ and are not shown. Overall, in this parallel group design, the PK of ATV, PG, CG, AQ, and DEAQ were not affected to a clinically relevant extent following ATV-PG/AQ co-administration versus ATV-PG or AQ administered individually.

Compared with ATV-PG, following ATV-PG/AQ co-administration the C_{max} and $AUC_{0\text{-}t}$ for ATV were 12.0% and 11.0% higher on day 1, but 18.2% and 16.0% lower on day 3, respectively, and time to maximum concentration (T_{max}) was longer on both day 1 and day 3 (**Table 3**). As the ATV T_{max} was 24.0 hours on day 3, the longer T_{max} was probably not related to the initial absorption of ATV. Following ATV-PG/PQ administration, PG C_{max} and $AUC_{0\text{-}t}$ were higher on day 1, whereas both parameters were about 11% lower on day 3 (**Table 3**). For CG, C_{max} and $AUC_{0\text{-}t}$ were higher on both day 1 and day 3 following ATV-PG/PQ administration (**Table 3**).

Compared with AQ alone, following co-administration with ATV-PG, the AQ C_{max} and AUC were 8.0% and 19.0% higher on day 1 and 29.0% and 12.0% higher on day 3, respectively (**Table 3**). For DEAQ, C_{max} was similar on day 1, but increased by 23.0% on day 3, whereas $AUC_{0\text{-}t}$ was higher on day 1 by 14.0% and day 3 by 10.0%, following ATV-PG/PQ administration (**Table 3**).

Pharmacogenetics

CYP2C8 metabolizer status was determined for 15 of 44 study participants. CYP2C8*2 was present at an allelic frequency of 16.7%, noted in 5 of 15 volunteers, who were all heterozygous

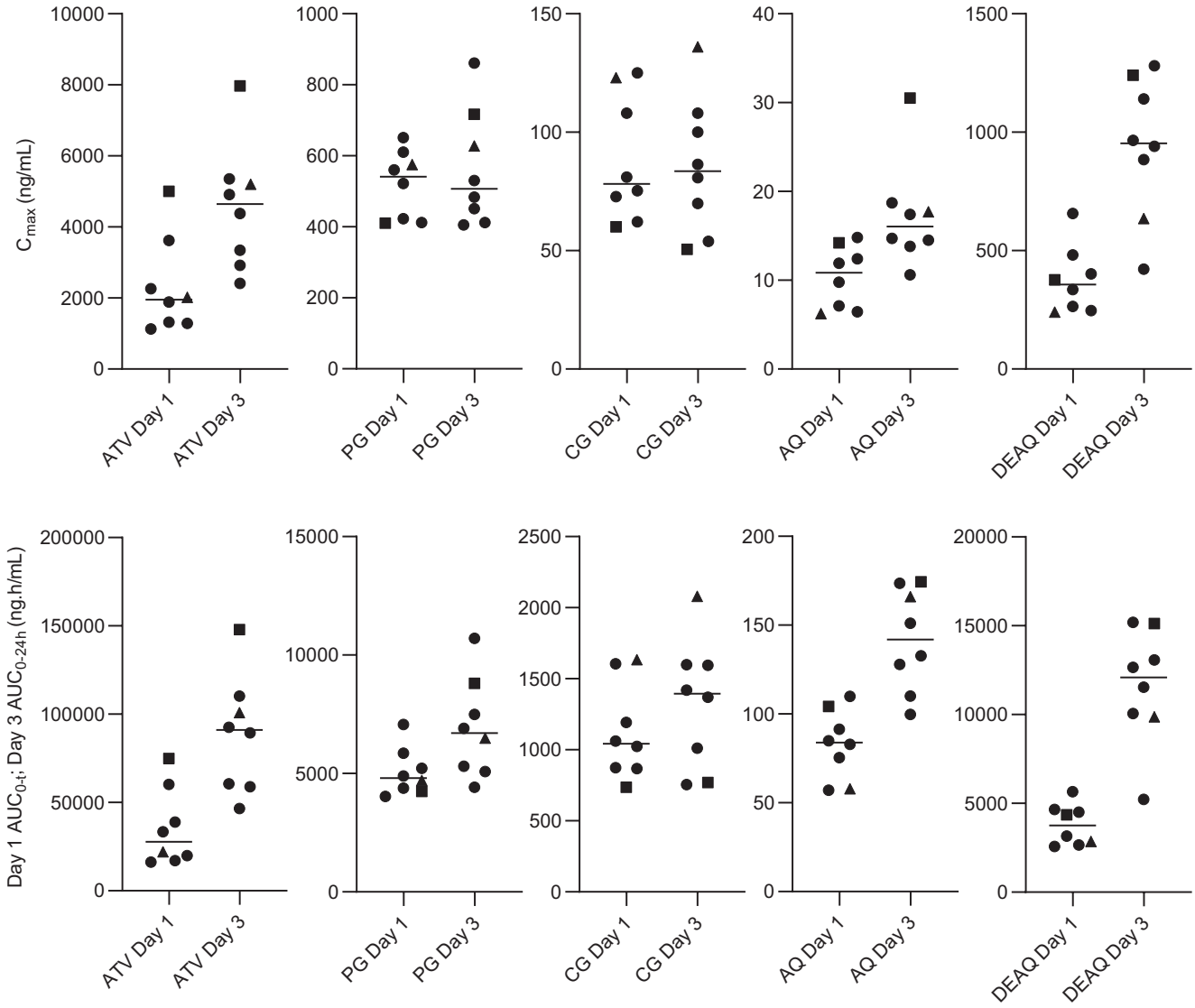

Figure 2 Individual participant values for maximum plasma concentration (C_{max}) and area under the concentration–time curve (AUC) for atovaquone (ATV), proguanil (PG), cycloguanil (CG), amodiaquine (AQ), and N-desethyl-amodiaquine (DEAQ) following administration of ATV-PG/AQ. Participants that experienced extrapyramidal adverse effects (EPAEs) are shown with a black square (participant 11010) and a black triangle (participant 12013). Participants without EPAE are shown with black circles.

(*1/*2) intermediate metabolizers (**Table S2**). None were CYP2C8 poor metabolizer homozygous (*2/*2). For the 2 participants with EPAE, CYP2C8 metabolizer status could not be analyzed for participant 11010 owing to the timing of the protocol amendment, but participant 12013 was an extensive metabolizer (**Table S2**). Of the five intermediate metabolizers, four received AQ and one received ATV-PG/AQ. Of the 10 extensive metabolizers, 5 received AQ and 5 ATV-PG/AQ. There was no relationship between CYP2C8 metabolizer status and AQ or DEAQ exposure (**Table S2**, **Figure S3**).

DISCUSSION

This investigation examined the safety, tolerability, and PK of ATV-PG and AQ when administered individually and in combination to healthy adult volunteers as a preliminary assessment of the combination for SMC in African children. Adverse events for ATV-PG and AQ when administered individually were consistent with their known safety profiles.[15,19] However, the occurrence of EPAE at a high incidence (2/8 participants) when the drugs were co-administered was not anticipated. A few antimalarial agents, including chloroquine and AQ, are associated with EPAE.[25–27] To our knowledge, this adverse event has not been reported for ATV-PG, and it is not noted on the summary of product characteristics.[24]

In 2015–2016, 37.6 million SMC SP + AQ doses were administered to children in sub-Saharan Africa, with one EPAE case recorded.[28] EPAEs are also a rare adverse event (< 0.1%) with AQ in combination with artesunate (AS + AQ) for the treatment of uncomplicated malaria.[26–30] However, pharmacovigilance in malaria endemic regions is not well-developed, and EPAE in patients with

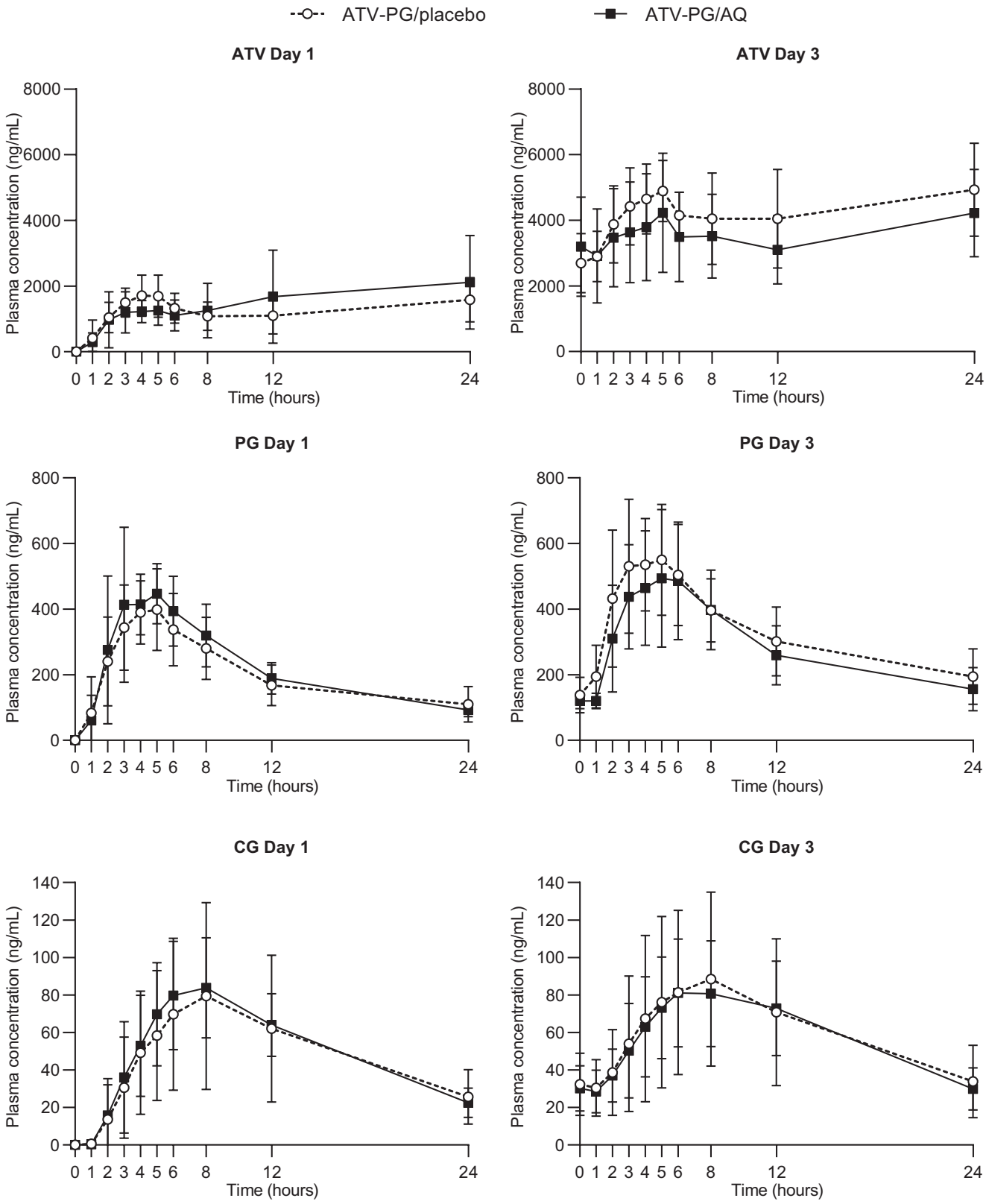

Figure 3 Plasma concentration–time profiles for atovaquone (ATV), proguanil (PG), and cycloguanil (CG) following administration of ATV-PG or ATV-PG/amodiaquine (AQ). See also Figure S2 for the extended plasma concentration–time profile for ATV up to day 22.

malaria could be masked by the symptoms of acute infection. Thus, AQ-induced EPAE in African pediatric populations are likely under-reported.[31] For AQ and other drugs associated with EPAE, cases tend to be in children and young adults, more commonly male patients, with symptoms starting on the third or fourth day after AQ commencement, including transient involuntary contractions

Table 1 PK parameters of ATV, PG, and CG in plasma following administration of ATV-PG or ATV-PG/AQ

		ATV-PG (*N* = 12)			ATV-PG/AQ (*N* = 8)		
Parameter	Time	ATV	PG	CG	ATV	PG	CG
$AUC_{0\text{-}t}$ (ng.h/mL)	Day 1	28,956 (34.4)	4,475 (32.2)	836 (117)	35,355 (63.3)	4,972 (18.7)	1,083 (29.4)
	Day 3	662,532 (40.8)	–	–	556,234 (36.8)	–	–
$AUC_{0\text{-}24h}$ (ng.h/mL)	Day 3	99,060 (26.3)	7,483 (28.4)	1,122 (113)	83,048 (39.7)	6,639 (30.2)	1,253 (37.9)
C_{max} (ng/mL)	Day 1	1,820 (32.5)	421 (28.3)	60.8 (123)	2,040 (55.8)	512 (18.8)	85.1 (30.0)
	Day 3	5,229 (26.4)	614 (21.0)	72.1 (123)	4,279 (39.8)	542 (27.8)	81.5 (35.0)
λz (/h)	Day 3	0.008 (31.3)[a]	0.032 (18.6)[b]	0.031 (5.03)[c]	0.008 (31.3)[d]	0.029 (12.1)[e]	0.032[f]
$t_{1/2}$ (h)	Day 3	90.4 (30.5)[a]	21.8 (18.8)[b]	22.7 (4.5)[c]	88.9 (39.0)[d]	23.7 (13.9)[e]	21.4[f]
T_{max} (h)	Day 1	4.00 (1.00–23.6)	4.50 (2.00–6.00)	8.00 (6.00–11.3)	8.51 (2.00–23.5)	3.00 (3.00–8.00)	6.03 (4.00–8.00)
	Day 3	5.03 (3.00–24.2)	3.50 (2.00–8.00)	8.00 (4.00–11.9)	24.0 (5.00–24.1)	4.50 (2.00–6.00)	6.00 (4.00–12.0)

Values are geometric mean (geometric mean coefficient of variation [CV%]) except for T_{max} which is median (range).
AQ, amodiaquine; ATV, atovaquone; AUC, area under the concentration–time curve; CG, cycloguanil; C_{max}, maximum plasma concentration; PG, proguanil; PK, pharmacokinetic; $t_{1/2}$, terminal elimination half-life; T_{max}, time to reach maximum plasma concentration; λz, terminal elimination rate constant.
[a]*N* = 11; [b]*N* = 5; [c]*N* = 3; [d]*N* = 7; [e]*N* = 2; [f]*N* = 1.

in the muscles of the neck, jaws, tongue, lips, and extremities.[30] These occur in patients with normal DEAQ plasma levels following a standard AQ dose.[32] The AQ dose used in artemisinin combination therapy (AS + AQ 200/540 mg once daily for 3 days) is lower than the AQ chemoprotective dose of 612 mg once daily for 3 days administered in our study.

The EPAE observed in the current study are consistent with previous reports following AQ administration, with symptoms manifest in both cases on day 3 and co-incident with maximal AQ/DEAQ plasma concentrations. However, why this adverse event should appear at high frequency in such a small cohort at normal AQ/DEAQ exposures and only following ATV-PG/AQ co-administration is intriguing.

Both participants with EPAE had low bodyweight, but their AQ and DEAQ exposures were not markedly different from those of heavier participants who received AQ, either alone or in combination. In addition, exposures were similar to those observed for adults following therapeutic AS + AQ doses,[19,33,34] although, in this study, total rather than free AQ/DEAQ plasma concentrations were measured. Thus, the findings cannot be explained by a PK interaction between ATV-PG and AQ/DEAQ, or by inter-individual variability in PK parameters. Neither is a pharmacogenetic explanation probable. In Africans, *CYP2C8*2* is the most common variant related to a poor metabolizer phenotype.[35,36] Individuals homozygous for *CYP2C8*2* show 6-fold lower intrinsic clearance of AQ, a longer AQ half-life, and an increased incidence of drug-induced adverse events vs. wild-type individuals.[12,23,37] CYP2C8 metabolizer status was investigated after the first EPAE case; the second EPAE case had an extensive metabolizer CYP2C8 phenotype. DEAQ exposure showed no consistent relationship to CYP2C8 metabolizer status and was similar across all participants. We did not analyze other enzymes that could impact further DEAQ metabolization.[38] Although AQ is predominantly metabolized by CYP2C8 to DEAQ, there is some evidence of metabolism to an aldehyde formation via CYP1A1 and CYP1B1.[39] Some aldehydes are toxic, and this is another possibility for consideration.

ATV, PG, CG, and AQ are known to cross the blood–brain barrier.[40–42] Studies with agents known to cause EPAE suggest involvement of antagonistic binding of dopaminergic D_2 receptors within the mesolimbic and mesocortical pathways.[43] More specifically, transient aminoquinoline-induced EPAE is associated with a relative excess of dopamine in the basal ganglia, and is spontaneously reversible following drug elimination.[44] Both AQ and chloroquine enhance the functions of the orphan nuclear receptor (Nurr1) in the CNS, leading to activation of midbrain dopaminergic neuron-specific function and beneficial effects in rodent models of Parkinson's disease.[45] Nurr1 is also implicated in levodopa-induced dyskinesia, an adverse effect of dopamine replacement strategies in Parkinson's disease.[45]

Two mechanisms for a potential interaction between AQ/DEAQ and ATV/PG/CG can be hypothesized. A pharmacodynamic interaction could occur at the level of a brain target, such as the dopaminergic DA_2 receptors. For example, if AQ and/or DEAQ and one of the co-administered drugs bind to the same target, or if one drug alters the receptor binding affinity of the

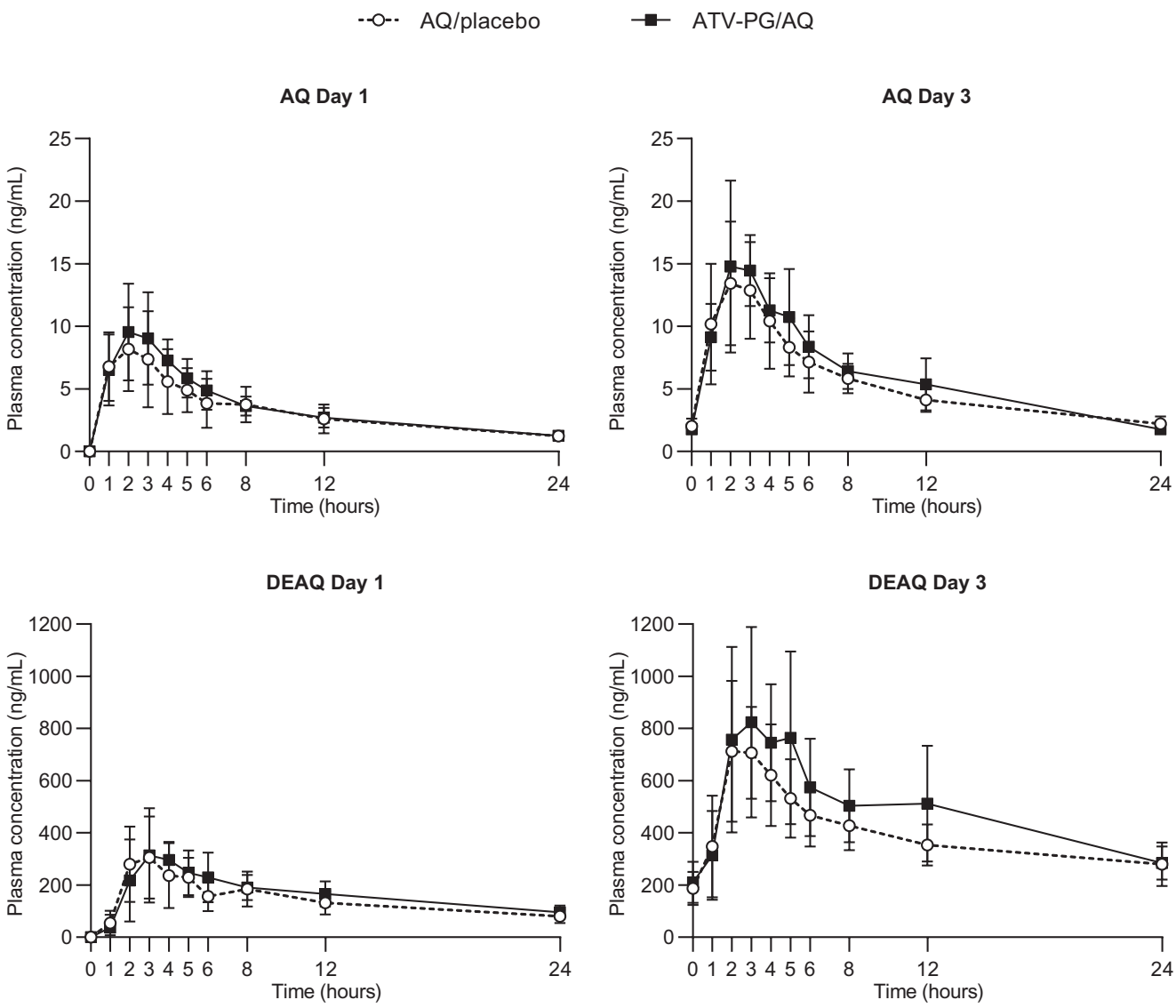

Figure 4 Plasma concentration–time profiles for amodiaquine (AQ) and N-desethyl-amodiaquine (DEAQ) following administration of AQ or atovaquone-proguanil (ATV-PG)/AQ.

other.[46] Similarly, there could be a synergistic pharmacological effect on the target. Alternatively, a PK drug–drug interaction at the blood–brain barrier, causing increased exposure to AQ or DEAQ in the CNS could potentiate AQ/DEAQ-associated neurological symptoms. Drug–drug interactions at this interface may be caused by altered activity of influx and efflux transporters, although few cases have been reported. The major efflux transporters described at the blood–brain barrier are the P-glycoprotein (P-gp), multidrug resistance proteins (MRPs), and the breast cancer resistant protein (BCRP).[46,47] *In vitro* studies suggest that AQ is not a substrate of P-gp or BCRP,[48,49] although no information is available for DEAQ. ATV was shown *in vitro* to be a potent BCRP inhibitor, with an IC_{50} of 0.23 μM,[50] significantly lower than total C_{max}, suggesting that it could act as a BCRP inhibitor *in vivo*. However, further *in vitro* work is needed to determine whether DEAQ is a BCRP substrate, and to assess the potential for an interaction between DEAQ and ATV at the blood–brain barrier. Besides, incomplete information is available regarding the clearance pathways for ATV, and hence the risk of this drug to be a victim of co-administered drugs is not fully understood. In addition to *in vitro* transporter studies, physiological-based PK modeling could be used to estimate brain concentrations of the different drugs administered in this study and their major metabolites and to simulate potential drug–drug interactions at the blood–brain barrier, in case *in vitro* data suggest that such an interaction is possible.

The EPAE observed in this study most likely represent an unanticipated CNS pharmacological drug–drug interaction between ATV-PG and AQ, or their metabolites, independent of total plasma drug exposure and CYP2C8 metabolizer status. Despite a favorable PK profile of ATV-PQ and AQ in combination in African adults, the high incidence of transient EPAE in this phase I study is inconsistent with its use for SMC in children in Africa. This study reinforces the need to conduct comprehensive safety assessments for combinations of registered pharmaceuticals, even

Table 2 PK parameters of AQ and DEAQ in plasma following administration of AQ or ATV-PG/AQ

		AQ (*N* = 12)		ATV-PG/AQ (*N* = 8)	
Parameter	Time	AQ	DEAQ	AQ	DEAQ
$AUC_{0\text{-}24h}$ (ng.h/mL)	Day 3	126 (25.3)[a]	9,273 (23.7)[a]	139 (21.4)	11,080 (35.5)
$AUC_{0\text{-}t}$ (ng.h/mL)	Day 1	72.2 (32.3)	3,202 (29.2)	80.9 (24.6)	3,655 (30.8)
	Day 3	–	45,130 (21.6)[a]	–	49,341 (27.3)
C_{max} (ng/mL)	Day 1	9.14 (33.4)	355 (30.2)	9.83 (36.6)	355 (36.2)
	Day 3	14.0 (36.4)[a]	784 (31.4)[a]	16.5 (31.4)	889 (38.8)
λz (/h)	Day 3	–	0.004 (14.9)[b]	–	0.006[c]
$t_{1/2}$ (h)	Day 3	–	196 (13.5)[b]	–	108[c]
T_{max} (h)	Day 1	2.03 (1.00–6.02)	3.00 (2.00–6.02)	3.00 (2.00–5.00)	3.00 (2.00–6.03)
	Day 3	2.00 (1.00–3.00)[a]	3.00 (2.00–5.00)[a]	3.00 (2.00–5.00)	3.52 (2.00–12.0)

Values are geometric mean (geometric mean coefficient of variation [CV%]) except for T_{max} which is median (range).
AQ, amodiaquine; ATV, atovaquone; AUC, area under the concentration–time curve; C_{max}, maximum plasma concentration; DEAQ, N-desethyl-amodiaquine; PG, proguanil; PK, pharmacokinetic; T_{max}, time to reach maximum plasma concentration, $t_{1/2}$, terminal elimination half-life; λz, terminal elimination rate constant.
[a]*N* = 11; [b]*N* = 6; [c]*N* = 1.

Table 3 Comparison of plasma PK parameters following administration of ATV-PG/AQ (test) vs. reference (ATV-PG/placebo or AQ/placebo)

Analyte	Time	C_{max} ratio, % (90% CI)	$AUC_{0\text{-}t}$ ratio, % (90% CI)	$AUC_{0\text{-}24h}$ ratio, % (90% CI)
ATV	Day 1	112 (81.1, 155)	111 (77.7, 158)	83.8 (65.5, 107)
	Day 3	81.8 (63.8, 105)	84.0 (62.2, 113)	–
PG	Day 1	122 (100, 148)	111 (89.6, 138)	–
	Day 3	88.2 (73.2, 106)	–	88.7 (70.8, 111)
CG	Day 1	140 (75.8, 259)	130 (71.5, 235)	–
	Day 3	113 (61.0, 210)	–	112 (61.8, 202)
AQ	Day 1	108 (82.4, 140)	119 (93.4, 151)	–
	Day 3	129 (93.8, 176)	–	112 (89.2, 141)
DEAQ	Day 1	100 (77.8, 129)	114 (90.6, 144)	–
	Day 3	123 (90.0, 168)	110 (91.4, 132)	125 (98.3, 158)

Values are percent point estimates (ratio of test:reference) and 90% confidence limits.
AQ, amodiaquine; ATV, atovaquone; AUC, area under the concentration–time curve; CG, cycloguanil; CI, confidence interval; C_{max}, maximum plasma concentration; DEAQ, N-desethyl-amodiaquine; PG, proguanil; PK, pharmacokinetic.

when the safety liabilities of the individual components are well understood. Further clinical investigation of ATV-PG/AQ for SMC in African children is not recommended.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. S.C., F.M., N.A., and A.M.T. designed the research. S.C., F.M.I., A.H., B.H., U.L., and M.F.C. performed the research. S.C., F.M., N.A., A.H., B.H., U.L., and M.F.C. analyzed the data.

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