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PHARMACOKINETICS AND DISPOSITION

Comparative oral bioavailability of non-fixed and fixed combinations of artesunate and amodiaquine in healthy Indian male volunteers

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Abstract
Objective The aim of the present study was to compare the pharmacokinetic properties, bioavailability and tolerability of artesunate (AS) and amodiaquine (AQ) administered as a fixed-dose combination (Amonate FDC tablets; Dafra Pharma, Turnhout, Belgium) or as a non-fixed dose combination of separate AS tablets (Arsuamoon; Guilin Pharmaceutical Co, Shanghai, China) and AQ tablets (Flavoquine; Sanofi-Aventis, Paris, France).
Methods This was a randomized, open label, two-period, two-treatment, two-sequence, cross-over study in which 60 healthy male Indian volunteers were given a single total oral dose of 100 mg AS and 400 mg AQ hydrochloride either as two tablets of Amonate FDC (AS 50 mg and AQ hydrochloride 200 mg) or as two AS tablets of the co-blister Arsuamoon (50 mg AS) together with two Flavoquine tablets (200 mg AQ hydrochloride). Plasma AS and blood AQ concentrations, as well as those of their respective active metabolites dihydroartemisinin (DHA) and desethylamodiaquine (DEAQ), were measured by high-performance liquid chromatography–tandem spectrometry. The pharmacokinetic parameters of AS, DHA, AQ and DEAQ were determined by non-compartmental analysis. Bioequivalence assessment was performed by analysis of variance (ANOVA), and calculation of the 90% confidence intervals of the geometric mean ratio test (fixed)/reference (non-fixed) for $AUC_{0\text{-}t}$ and C_{max} for AS, AQ, DHA and DEAQ.
Results Interim analysis showed that both treatments were not bioequivalent; therefore, statistical analysis was carried out on the results of all subjects for whom blood/plasma concentrations were available for all four analytes (n=26). The C_{max} (maximum plasma/blood concentration) of AS was 67.0±37.1 and 154.8±116.2 ng/mL for the fixed-dose and non-fixed dose administration, respectively. The $AUC_{0\text{-}t}$ (area under the plasma concentration–time curve from time zero to the last measurable concentration) of AS was 60.1±27.5 and 81.8±44.3 ng h/mL for the fixed-dose and non-fixed dose administration, respectively. The 90% confidence intervals for C_{max} and $AUC_{0\text{-}t}$ of AS were outside the 80–125% acceptance range: 37.02–61.62% and 70.10–83.47%, respectively. The C_{max} of AQ was 33.8±13.6 and 31.4±14.1 ng/mL for the fixed-dose and non-fixed dose administration, respectively. The $AUC_{0\text{-}t}$ of AQ was 332.3±116.6 and 329.8±99.5 ng h/mL for the fixed and non-fixed dose administration, respectively. For AQ, the 90% CIs for C_{max} and $AUC_{0\text{-}t}$ were within the 80–125% acceptance range: 99.17–121.71 and 89.53–107.35%, respectively. Bioequivalence assessment based on the active metabolite data supported the bioequivalence conclusions based on the parent compound data. Both the fixed-dose and non-fixed dose administration of 100 mg AS and 400 mg AQ were well tolerated.
Conclusion Bioequivalence of the fixed-dose AS/AQ formulation with the non-fixed dose combination of the same drugs was not demonstrated for AS, but it was shown for AQ for both C_{max} and $AUC_{0\text{-}t}$. The results

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obtained on the active metabolites support this conclusion. Overall, the fixed-dose 50 mg AS/200 mg AQ tablets were not technically bioequivalent with 50 mg AS tablets and 200 mg AQ tablets administered separately. The difference cannot be explained by the pharmaceutical properties of the tablets and seems to be biologically related.

Keywords Artesunate · Amodiaquine · Fixed-dose/non-fixed combination · Pharmacokinetics · Bioequivalence · Tolerability · Healthy male volunteers

Introduction

Artesunate (AS), a semi-synthetic derivative of artemisinin, can be considered a prodrug as it rapidly and extensively hydrolyzes in the body to the active metabolite dihydroartemisinin (DHA). Until the 1990s, AS in monotherapy was used for the curative treatment of malaria. Although very active, a minimum of 600 mg of AS has to be given for at least 5 days in order to avoid recrudescence of the disease. In 2002, the World Health Organization (WHO) emphasized the necessity of using AS combination therapies in order to improve case management and prevent the development of artemisinin resistance by the parasites [1]. From 2006 onwards, the use of monotherapies was officially banned [2].

The first attempts to manufacture artemisinin-based combination therapy (ACT) consisted simply in co-packaging separate tablets of two different active substances in one blister (co-blister packaging). Fixed-dose combinations (FDCs) of active pharmaceutical ingredients in a single tablet were subsequently developed to increase treatment compliance and simplify dose administration. Currently, ACT-based fixed drug combinations of AS/amodiaquine (AQ), artesunate/mefloquine, artemether/lumefantrine and of artesunate/sulfamethoxypyrazine/pyrimethamine are available to treat malaria infection.

The formulation of AS/AQ as a fixed-drug combination represents a daunting pharmaceutical challenge at the technical level. In fact, because AS is an alpha ester of DHA and succinic acid, with one carboxylic acid function in its structure, and because AQ is usually present as a dihydrate dihydrochloride salt, unfavorable interactions between these two active molecules are expected to occur. This risk of chemical instability has been previously discussed in several papers [3–6]. In response to this challenge, we have developed a tablet formulation in which AQ hydrochloride particles are physically isolated by coating (and then protected from possible interactions with AS). Our fixed-dose AS/AQ combination tablet (brand name Amonate FDC) contains 50 mg AS and 200 mg of AQ hydrochloride (153.1 mg AQ base).

In the study reported here, we compared the pharmacokinetic properties, bioavailability and tolerability of AS/AQ Amonate FDC to those of the same drugs given concomitantly as separate tablets (non-fixed dose combination) in a randomized open label cross-over bioequivalence (BE) study in healthy Indian male subjects. The results are discussed in the light of previously published comparative studies and with regards to their potential clinical relevance.

Material and methods

Study subjects and study design

This study used a randomized, open label, two-period, two-treatment, two-sequence, cross-over design to compare the pharmacokinetic properties, bioavailability and tolerability of AS and AQ administered as a fixed-dose combination (Amonate FDC tablets; Dafra Pharma, Turnhout, Belgium) or as a non-fixed dose combination of separate AS tablets (Arsuamoon; Guilin Pharmaceutical Co, Shanghai, China) and AQ tablets (Flavoquine; Sanofi-Aventis, Paris, France). The study was conducted by Accutest Research Laboratory, Ahmedabad, India, in accordance with the declaration of Helsinki, WHO, Medicines Control Council (MCC) and relevant National Laws and Regulations, current International Conference on Harmonization (ICH)—Good Clinical Practice (ICH–GCP) guidelines and Indian Council of Medical Research (ICMR) guidelines. The protocol was approved by the Siddhant Independent Ethics Committee (Ahmedabad, India).

A total of 60 normal, healthy, male subjects, aged between 18 and 55 years, were randomized to receive either the fixed-dose or non-fixed dose combination regimen in study period I. The number of study subjects was selected based on an intra-individual variability [CV; analysis of variance (ANOVA)] of 40% for AS. After a wash-period of 30 days, subjects entered study period II and received the alternative treatment.

Drugs were administered under fasting conditions (at least 10-h overnight fast; see details below). All subjects were instructed to abstain from any xanthine-containing beverages (chocolate, tea, coffee or cola drinks) or food, fruit juice/grapefruit juice 48 h prior to drug dosing and until the last blood sample collection of each study period. Up to 240 mL of water was allowed on the morning of the dosing up to 1 h before drug administration. Two hours after drug administration, 240 mL of xanthine-free fluids was allowed. At all other times, water was given ad libitum. Subjects were provided standard meals the night before drug administration (in such a way to maintain the criterion of a 10-h pre-dose fasting period), and at 4, 8, and 12 hours post-dose in each study period. Each subject was given a

single total oral dose of 100 mg AS and 400 mg AQ hydrochloride either as two tablets of Amonate FDC (AS 50 mg and AQ hydrochloride 200 mg) or as two AS tablets of the co-blister Arsuamoon (50 mg AS) together with two Flavoquine tablets (200 mg AQ hydrochloride). The medications were taken under supervision and with 240 mL of water.

Pharmaceutical quality control of Amonate FDC tablets showed that the product met all of the specifications at the time of the study. Specifically, in terms of dissolution, both AS and AQ attained 92% after 45 and 30 min, respectively, which is in compliance with European Pharmacopoeia (AS; at least 75% dissolution within 45 min) and U.S. Pharmacopoeia (the Monograph of AQ tablets; at least 75% dissolution within 30 min) specifications.

Each subject was informed of the objectives, nature and possible risks of the trial. Written informed consent was required before the subjects were allowed to participate in the study. Based on a complete medical history and the results of the physical examination (including electrocardiogram) and laboratory tests (hematology, biochemistry, serology and urinalysis), all subjects who met all the inclusion and none of the exclusion criteria were enrolled in the study. The presence of any significant diseases or clinically significant abnormal findings were ruled out during the screening process

Blood sampling and handling

A total of 25 8-mL blood samples were collected during both study periods at pre-dose and at 0.16, 0.33, 0.5, 0.66, 0.83, 1, 1.25, 1.50, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 10, 12, 24, 48, 72 and 96 h post-dose. After collection, 0.5-mL aliquots of the whole blood samples were kept and treated as analytical samples for measuring the concentrations of AQ and its metabolite desethylamodiaquine (DEAQ), while 6 mL of the samples was centrifuged at 3,500 rpm for 10 min at 5°C±3°C to obtain plasma for the determination of AS and DHA concentrations. Samples (1-mL aliquots) of the plasma were then treated as analytical samples. Both sets of analytical samples and back-up samples (fraction of whole blood/plasma not tested) were stored at −70°C±5°C until analysis.

Drug assay

The concentrations of AS and AQ and their respective pharmacologically active metabolites DHA and DEAQ in the plasma/blood samples were determined using validated high performance liquid chromatography–tandem mass spectrometry (LC-MS/MS) assays at the bioanalytical facility of Accutest Research Laboratories PVT.LTD, Ahmedabad, India.

AS and DHA

Solid-phase extraction of plasma samples was performed using Lichrosep DVD-HL extraction cartridges (Merck, Whitehouse Station, NJ). The cartridges were preconditioned by sequential flushing with 1 mL methanol and 1 mL of 1 M acetic acid prior to loading with plasma samples. After washing twice with 1 mL of 1 M acetic acid followed by 1 mL of water, samples were eluted with 1 mL of acetonitrile. The eluates were subsequently evaporated to dryness under nitrogen gas at 45°C and reconstituted in 200 μL of mobile phase [2 mM ammonium acetate buffer (pH 3.00±0.05):methanol mixture (30:70 v/v)]. Chromatographic separation of AS, DHA and metaxolone (the internal standard) was carried out at ambient temperature on a Hypurity C18 column (50×4.6 mm; particle size 5 μm; Thermo Fisher Scientific, Waltham, MA) using a Shimadzu LC-20AD pump and a Shimadzu SIL-HTc autosampler. The flow rate of mobile phase was 0.6 mL/min. MS/MS detection (API3000; Applied Biosystems, Foster City, CA) was performed in the ion spray-positive mode.

The standard/calibration curve for AS was linear in the range 2–300 ng/mL and for DHA, in the range 5–500 ng/mL. The mean overall extraction recoveries of AS, DHA and metaxolone were all above 66%. The lower limit of quantification of AS and DHA was 2 ng/mL and 5 ng/mL, respectively. The intra- and inter-day precision for both AS and DHA at low, mid and high concentrations was below 15%, and the intra- and inter-day accuracy for both AS and DHA at low, mid and high concentrations was within ±15% of the respective nominal concentration.

AQ and DEAQ

Solid-phase extraction of blood samples was performed using Lichrosep DVD-HL extraction cartridges (Merck). The cartridges were preconditioned by sequential flushing with 1 mL methanol and 1 mL water prior to loading with blood samples. After washing twice with 1 mL of 30% methanol in water (v/v), samples were eluted with 1 mL of mobile phase [2 mM ammonium formate buffer (pH 2.50± 0.05):acetonitrile mixture (40:60 v/v)]. Chromatographic separation of AQ, DEAQ and propranolol (the internal standard) was carried out at 40°C on a Hypersil Gold column (100×4.6 mm; particle size 5 μm; Thermo Fisher Scientific, Waltham, MA) using a Shimadzu LC-20AD pump and Shimadzu SIL-HTc autosampler. The flow rate of mobile phase was 0.6 mL/min. MS/MS detection (API3000, Applied Biosystems) was performed in the ion spray positive mode.

The standard/calibration curve for AQ and DEAQ was linear in the range 0.5–70 and 4–560 ng/mL, respectively.

For AQ, DEAQ and propranolol, the mean overall extraction recovery was above 62%. The lower limit of quantification of AQ and DEAQ was 0.5 and 4 ng/mL, respectively. The intra- and inter-day precisions for both AQ and DEAQ at low, mid and high concentrations were below 15%; the intra- and inter-day accuracies for both AQ and DEAQ at low, mid and high concentrations were within ±15% of the respective nominal concentration.

Pharmacokinetic and statistical analysis

Interim results midway the analytical stage of the study showed that the fixed-dose combination of AS and AQ Amonate FDC was not bioequivalent to the non-fixed dose combination of separate AS tablets (Arsuamoon (Guilin Pharmaceutical Co) and AQ tablets (Flavoquine; Sanofi-Aventis). It was therefore decided not to continue with the analysis of the remaining samples. At that point in the study, from the 54 volunteers who had completed both clinical phases, plasma/blood concentrations were available for 38 subjects for AS, for 37 for DHA and AQ, and for 33 for DEAQ. We therefore included all subjects for whom the complete data set was available (plasma/blood concentrations of the four analytes AS, AQ, DHA and DEAQ) in the BE analysis, without any other type of selection (n=26).

Pharmacokinetic parameters of AS, DHA, AQ and DEAQ were determined by non-compartmental analysis of individual concentration–time data using WinNonlin Professional ver. 5.2.1 (Pharsight Corp, Mountain View, CA). C_{max} (maximum plasma concentration) and T_{max} (time to reach C*max*) were obtained directly from the observed blood/plasma concentration–time data. $AUC_{0\text{-}t}$ (area under the plasma concentration–time curve from time zero to the last measurable concentration) was calculated by the linear trapezoidal rule from measured data points from the time of administration until the time of the last quantifiable concentration. Total area under the curve ($AUC_{0-\infty}$) was calculated as the sum of $AUC_{0\text{-}t}$, and $AUC_{t-\infty}$. $T_{½}$ (terminal plasma half-life) was estimated from the slope of the terminal phase of the semi-logarithmic plot the plasma/blood concentration versus time curve.

Statistical evaluations of $AUC_{0\text{-}t}$, $AUC_{0-\infty}$ and C_{max} were performed after ln-transformation using ANOVA for a two-way crossover design and the general linear model (GLM) procedure to assess the effect of sequence, subject within sequence, period and formulation on these BE metrics (WinNonlin Professional ver. 5.2.1, Pharsight Corporation). For the assessment of BE, the 90% confidence intervals (CI) of the geometric mean ratio test (fixed)/reference (non-fixed) for $AUC_{0\text{-}t}$, $AUC_{0-\infty}$ and C_{max} were calculated according to the two one-sided tests procedure of Schuirmann [7]. BE was concluded when the 90% confidence interval for $AUC_{0\text{-}t}$ and C_{max} fell within the 80–125% acceptance range.

Safety

Physical, vital (blood pressure, pulse rate, temperature and respiratory rate) and laboratory (hemogram, biochemistry and serology) tests were performed at the time of screening, check-in, check-out and at the last ambulatory visit of each study period or on discontinuation of the subject from the study. Blood pressure and pulse rate were measured before (pre-dose) and at 1, 2, 6, 10, 48 and 72 h following drug administration during each treatment period. Twelve lead electrocardiograms were carried out at the time of screening, at 2 h post-dose (during each treatment period) and at the last blood sample collection during study period II.

Safety was also evaluated by monitoring serious adverse events (SAE), adverse events (AE) and complaints throughout the course of the study. Standard definitions for AE and SAE were used. All adverse events that occurred during the study were properly documented: description of the event, details of timing, frequency, severity of the event, any treatment or diagnostic steps taken in relation to the event, outcome of event, judgment by the medical officer of any relationship of the event to the study medication or procedures.

Results

Subject demographics and flow chart

A total of 60 adult healthy subjects were enrolled in the study, and 54 subjects successfully completed both clinical phases of the study (i.e. they received both the fixed and the non-fixed dose of AS/AQ in two consecutive administration periods). Six subjects did not complete the two study phases: three subjects were withdrawn from the study after re-screening of period II due to safety reasons (out of reference range hematological counts), two subjects dropped-out from the study during the wash-out period between the two study phases, and one subject dropped-out during study period II for personal reason (Fig. 1).

The mean (range) age, body weight and height of the 54 subjects who successfully completed the clinical phase of the study were 28.9 (20–40) years, 57.5 (50–73) kg and 165.7 (154–179) cm, respectively.

Pharmacokinetics and BE assessment

The results of all 26 subjects who completed both study periods, and whose blood/plasma concentration data set was complete for all four analytes were used to calculate the pharmacokinetic parameters of both parent compounds

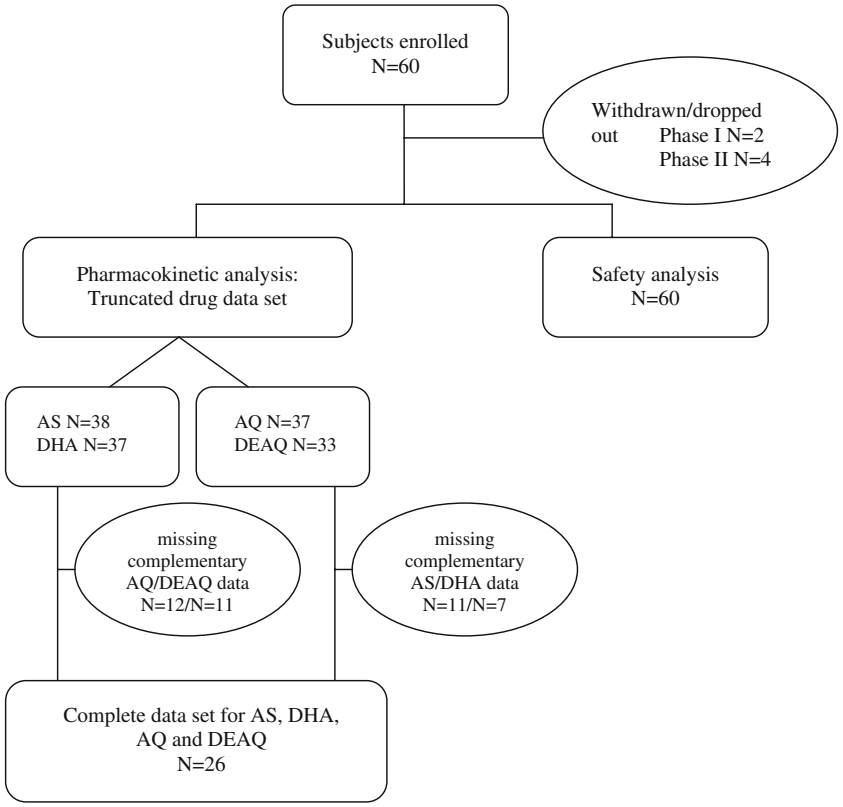

Fig. 1 Study flow chart. *DHA* Dihydroartemisinin, *DEAQ* desethylamodiaquine, *AS* artesunate, *AQ* amodiaquine

and their respective active metabolites, DHA and DEAQ (see Material and Methods). The mean concentrations of AS and DHA (in plasma) and of AQ and DEAQ (in whole blood) as a function of time following a single administration of a fixed and non-fixed dose of AS/AQ are shown in Figs. 2 and 3, respectively. Pharmacokinetic parameter estimates [mean ± standard deviation (SD) value of C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0-\infty}$, median and range of T_{max} and $T_{½}$ (half-life)] of AS, DHA, AQ and DEAQ following administration of the fixed-dose and non-fixed dose AS/AQ combination are summarized in Table 1.

After oral administration, AS and AQ were rapidly absorbed and underwent fast metabolism to their respective metabolites (DHA and DEAQ), irrespective of the type of AS/AQ combination product administered. The plasma/blood concentrations (AUC) of DHA and DEAQ were roughly six- and 30-fold higher, respectively, and persisted longer than their respective parent compounds, regardless of the AS/AQ combination product administered. For AS, DHA and AQ, the $AUC_{0\text{-}t}$ covered, on average, at least 89% of the $AUC_{0-\infty}$. However, for DEAQ, on average only 55.4% (fixed-dose administration) and 56.2% (non-fixed dose administration) of the $AUC_{0-\infty}$ was located in the experimentally determined $AUC_{0\text{-}t}$.

The analysis of comparative bioavailability of the fixed-dose versus the non-fixed dose AS/AQ combination is

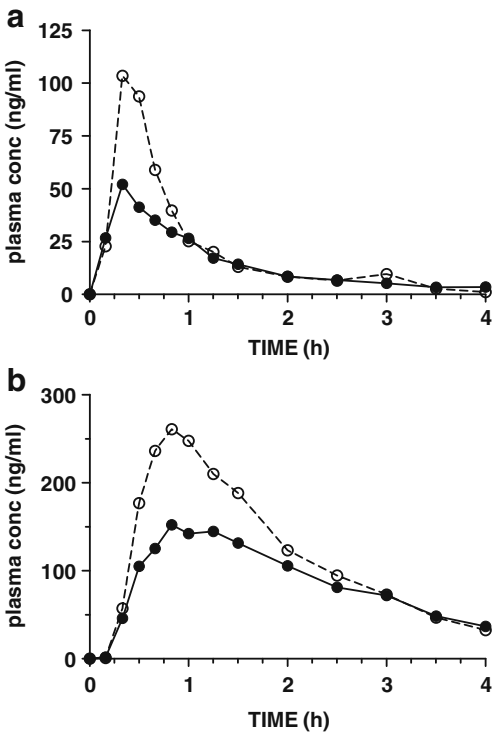

Fig. 2 Profiles of mean plasma concentrations versus time of AS (**a**) and DHA (**b**) following a single oral administration of a fixed-dose (*filled circles*) or a non-fixed (*open circles*) dose AS/AQ combination

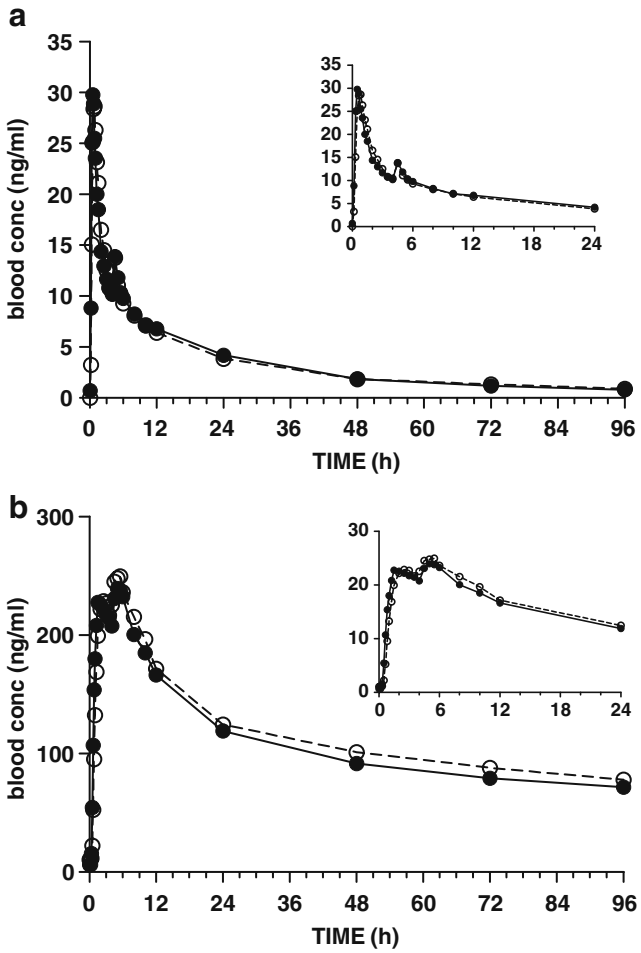

Fig. 3 Profiles of mean blood concentrations versus time of AQ (**a**; *insert* 0–24 h time frame) and DEAQ (**b**; *insert* 0–24 h time frame) following a single oral administration of a fixed-dose (*filled circles*) or a non-fixed (*open circles*) dose AS/AQ combination

shown in Table 2. For AS, the geometric mean ratios obtained for C_{max} and $AUC_{0\text{-}t}$ were 47.8 and 76.5%, respectively. The 90% CI for C_{max}, i.e., 37.0–61.6%, fell clearly outside the acceptance interval of 80–125%. The 90% CI for $AUC_{0\text{-}t}$ was 70.1–83.5%, and exceeded the lower limit (80%) of the acceptance range. Plasma C_{max} and $AUC_{0\text{-}t}$ were lower following administration of the fixed-dose combination by 57 and 27%, respectively. The within-subject variability (CV%) of C_{max} was very high, i.e. 56.0%. The T_{max} of AS was slightly shorter in subjects given the fixed-dose combination (median 0.33 h vs. 0.50 h for non-fixed dose combination). Similar results were obtained for DHA, the active metabolite of AS. The 90% CI of the geometric mean ratio for C_{max}, i.e. 45.5–67.9%, fell outside the acceptance interval of 80–125%. The 90% CI for $AUC_{0\text{-}t}$ was 69.0–86.0% and exceeded the lower limit (80%) of the acceptance range. Plasma C_{max} and $AUC_{0\text{-}t}$ were 46 and 24% lower, respectively, following administration of the fixed-dose combination compared to the non-fixed dose combination. The within-subject variability (CV%) of C_{max} was high, i.e. 43.9%. The T_{max} of DHA was similar following administration of the fixed-dose compared to the non-fixed dose combination.

Individual AS and DHA plasma concentration–time profiles occasionally showed an irregular time course during the so-called elimination phase. Consequently, it was not always possible to obtain a reliable estimate of plasma half-life and elimination rate constant in each subject. Therefore, the median $T_{1/2}$ of AS and DHA following the administration of both combination products are shown for informative purposes only, and the BE assessment was restricted to C_{max} and $AUC_{0\text{-}t}$.

For AQ, the geometric mean ratios obtained for C_{max} and $AUC_{0\text{-}t}$ were 110.0% and 98.0%, respectively. The 90% CIs for C_{max} and $AUC_{0\text{-}t}$ were 99.2–121.7% and 89.5–107.4%, respectively, both within the acceptance interval of 80–125%. The within-subject variabilities of C_{max} and $AUC_{0\text{-}t}$ were between 18–22%. The T_{max} of AQ was similar following the administration of the fixed-dose and the non-fixed dose combination, with a median time of 0.50 vs. 0.83 h, respectively. Similar results were obtained for DEAQ, the active metabolite of AQ. The 90% CI of the geometric mean ratios for C_{max} (92.7–108.1%) and $AUC_{0\text{-}t}$ (91.0–99.0%) were both within the 80–125% acceptance interval. The within-subject variabilities of C_{max} and $AUC_{0\text{-}t}$ were low (8–16%). The T_{max} of DEAQ was similar following the administration of the fixed-dose and the non-fixed dose combination, with a median time of 4.75 vs. 4.50 h, respectively.

Safety evaluation

All 60 subjects were included in the safety evaluation. Both combinations were well tolerated. Four hematological counts out of the reference range were reported over the course of the study in four of the 60 subjects. Two of these events were observed during study period I: one in a subject treated with the fixed-dose combination (white blood cell: $14.8\times10^3/mm^3$), and one in a non-fixed dose combination-treated subject (eosinophils: $1.30\times10^3/mm^3$). The other two AEs were observed during study period II in two subjects treated with the non-fixed dose combination (platelets: $74\times10^3/mm^3$ and eosinophils: $1.17\times10^3/mm^3$). All events were mild in severity and resolved spontaneously. They were classified as "possible or not" related to the study medication. No serious AEs occurred during the study.

Discussion

The results of our study show that both AS and AQ when co-administered either as loose products (non-fixed

Table 1 Main pharmacokinetic parameters for AS, AQ and their respective metabolites following co-administration of AS and AQ as a fixed-dose or a non-fixed dose combination

Compound	Combination type	T_{max}[a](h)	$T_{1/2}$[a] (h)	C_{max}[b] (ng/mL)	$AUC_{0\text{-}t}$[b] (ng h/mL)	$AUC_{0-\infty}$ (ng h/mL)[c]
Artesunate (AS)	Fixed	0.33 (0.16–3.00)	0.59 (0.23–2.86)	67.0±37.1	60.1±27.5	66.6±27.7
	Non-fixed	0.50 (0.33–3.00)	0.42 (0.08–3.00)	154.8±116.2	81.8±44.3	89.0±47.4
Dihydroartemisinin (DHA)	Fixed	1.00 (0.66–4.00)	0.82 (0.47–11.53)	180.4±90.3	390.2±158.5	433.2±173.1
	Non-fixed	1.00 (0.50–4.50)	0.75 (0.38–1.27)	337.1±188.2	511.6±242.5	521.5±243.1
Amodiaquine (AQ)	Fixed	0.50 (0.33–4.50)	31.9 (17.8–48.1)	33.8±13.6	332.3±116.6	373.7±134.7
	Non-fixed	0.83 (0.33–4.50)	29.4 (16.2–61.8)	31.4±14.1	329.8±99.5	371.5±124.5
Desethylamodiaquine (DEAQ)	Fixed	4.75 (1.00–8.00)	76.9 (39.1–528.1)	277.9±109.2	10.5±3.4	21.4±14.5
	Non-fixed	4.50 (1.50–6.00)	72.1 (39.5–193.3)	271.6±82.6	11.2±3.7	20.9±9.3

T_{max}, Time to reach maximum plasma concentration; C_{max}, maximum plasma concentration; $AUC_{0\text{-}t}$, area under the plasma concentration–time curve from time zero to the last measurable concentration; SD, standard deviation; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time zero to infinity

[a] Data are presented as the median with the range in parenthesis

[b] Data are presented as the mean ± standard deviation (SD)

[c] For desethylamodiaquine, AUC is given as μg h/mL

combination) or as a fixed-dose combination were readily absorbed. The large inter-individual variation observed in the pharmacokinetic profiles of AS and DHA is consistent with the results of previous studies [8–11]. In terms of safety, both AS/AQ combinations were well tolerated, and no serious adverse events were reported.

According to the European drug-registration guidelines, BE should be established based on the blood/plasma concentrations of the parent compounds because it is believed that they better reflect the in vivo performance of medicinal products [12]. However the blood/plasma concentrations of the active metabolites may be used as supportive evidence. Since it is well known that both AS and AQ are rapidly converted to DHA and DEAQ, respectively, and that those metabolites significantly contribute to the antimalarial activity of the parent compounds [13, 14], both parent compounds and metabolites were evaluated separately for BE in our study.

Our calculations showed that both AS/AQ combinations were bioequivalent for AQ but not for AS. Indeed, the plasma concentrations of AS and DHA were substantially lower (47–56% for C_{max} and 24–27% for AUC) following the administration of the fixed drug combination compared to the non-fixed dose combination. As a result, the BE of both regimens (fixed-dose combination vs. non-fixed) could not be established.

To date published data on the pharmacokinetics and safety of AS/AQ combinations either administered as a fixed-drug combination or co-administered as loose products are limited. In a study by Navaratnam et al. [8], the relative bioavailability of a fixed (200 mg AS + 540 mg AQ) versus a loose (200 mg AS + 612 mg AQ) combination of AS and AQ were determined in 24 healthy Malaysian volunteers. In this study, C_{max} and $AUC_{0\text{-}t}$ of AS and DHA were substantially lower when AS and AQ were administered as the fixed-drug combination compared to the non-fixed dose combination. Plasma concentrations of

Table 2 Comparative analysis of bioavailabilities of fixed and non-fixed AS/AQ combinations

Compound	PK parameter	GMR (%)	90% CI	ANOVA CV (%)
Artesunate	C_{max}	47.8	37.0–61.6	56.0
	$AUC_{0\text{-}t}$	76.5	70.1–83.5	18.0
Dihydroartemisinin	C_{max}	55.6	45.5–67.9	43.9
	$AUC_{0\text{-}t}$	77.0	69.0–86.0	22.8
Amodiaquine	C_{max}	110.0	99.2–121.7	21.2
	$AUC_{0\text{-}t}$	98.0	89.5–107.4	18.8
Desethylamodiaquine	C_{max}	100.1	92.7–108.1	15.8
	$AUC_{0\text{-}t}$	94.9	91.0–99.0	8.6

GMR, Ratio of geometric mean fixed/non-fixed drug combination; CI, confidence interval; ANOVA CV, analysis of variance coefficient of variance

AQ and DEAQ were also found to be lower following the administration of the fixed drug combination; however, the authors did not adjust the $AUC_{0\text{-}t}$ of AQ and DEAQ according to the administered dose (which were different). It was concluded that, despite the fact that these two different combination treatments were not bioequivalent, "these differences were not expected to alter clinical responses in patients" [8]. In another study by Orrell et al. [15], the pharmacokinetic interaction and relative bioavailability of AS and AQ administered as a non-fixed dose combination or administered alone were investigated. The results show that the AUC and C_{max} of AS and DHA were decreased when AQ was co-administered compared to when AS was administered alone [15]. A similar result was obtained for AQ and DEAQ when co-administered or not with AS. Taken together, the results of these two studies suggest that a pharmacokinetic interaction takes place between AS and AQ when these drugs are administered at the same time (as loose or as a fixed-dose combination) and that this interaction leads to a decreased exposure to both AS and AQ (and active metabolites) compared to when they are administered alone. Our results are consistent with those published by Navaratnam et al. [8] and Orrell et al. [15] concerning AS and DHA and support pharmacokinetic interaction between AS and AQ. The observed non-bioequivalence for AS cannot be explained by a decreased dissolution of AS when co-formulated with AQ as in the in vitro dissolution test of Amonate FDC 50/200 mg AS/AQ and the Arsuamoon 50 mg AS tablets showed that AS dissolution was nearly complete within 45 min for both products (92 and 94.3%, respectively). As for AQ, the observed BE could possibly be the result of our procedure to manufacture the FDC.

The clinical significance and relevance of the non-bioequivalence between AS/AQ loose products (non-fixed combination) and the fixed-dose combination in healthy volunteers warrant further considerations. In a recent prospective population pharmacokinetic study in pediatric malaria patients, AS and AQ either co-administered as loose tablets or as a fixed-drug combination demonstrated similar bioavailability for DEAQ and DHA and showed equivalent total anti-malarial activity. Although the sum of the molar equivalent plasma concentrations of DHA and AS was used to assess BE in that study (which does not comply with strict BE guidelines), those results are nevertheless very intriguing [16]. In view of clinical relevance, one of the main questions to be addressed is what should primarily drive the final dosing (regimen) of these drugs in a clinical setting: the drug's pharmacokinetics, the drug's therapeutic efficacy or both? Traditionally used anti-malarials must be present at levels greater than the minimum inhibitory concentration (MIC) until eradication of the infection in non-immune patients to ensure cure of the infection [17]. In the case of artemisinin derivatives, available studies suggest that their biological effects extend beyond their presence at therapeutic concentrations in plasma and that two exposures of a few hours in each asexual life cycle are sufficient for a maximal anti-malarial effect [18]. In our study, assuming that most of AS anti-malarial activity derives from its metabolite and taking into account that the mean C_{max} of DHA obtained with a single 100 mg dose was 180 (range 65–362) ng/mL and 337 (range 61–806) ng/mL with the fixed-dose and non-fixed AS/AQ combination, respectively, even the lowest measured values were at least 20-fold higher than the in vitro median DHA IC_{50} of *P. falciparum* parasites (<2.8 ng/mL or <10 nmol/L) [19]. Considering that malaria-infected adults treated with Amonate FDC would receive 200 mg AS three times (as opposed to 100 mg in this study) over a 48-h period and following the reasoning of Navaratnam et al. [8], the maximal anti-malarial effect (and therefore clinical relevance) is predicted.

In conclusion, the results of this study do not support the bioequivalence of the AS/AQ Amonate FDC with the loose dose combination of the same drugs because of the non-bioequivalence of AS. This difference cannot be explained by pharmaceutical properties of the tablets and seems to be biologically related.

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R.K. Verbeeck declares that he has no conflict of interest.

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