Pharmacokinetics of artesunate in the domestic pig

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Objectives: The aim was to study the pharmacokinetic profile of artesunate and its metabolite dihydroartemisinin (DHA) in a pig model.

Methods: Thirteen pigs received either intravenous (iv) or intramuscular (im) artesunate (60 mg), with the alternative preparation given 24 h later in an open crossover design. Five of them also received an additional intra-arterial (ia) artesunate dose (60 mg). The plasma concentrations of artesunate and DHA were determined by high-performance liquid chromatography with electrochemical detection. Population modelling was performed with NONMEM, using a two-compartment model.

Results: Plasma concentration–time profiles were comparable to those observed in humans, with a rapid and biphasic decline for both artesunate and DHA. Following an iv bolus, artesunate had a median maximum plasma concentration (Cmax) of 13.8 μM (interquartile range (IQR), 10.4–22.1 μM), elimination half-life (t1/2) = 18 min (IQR, 16–22 min), total plasma clearance (CL) = 5.58 L/h/kg (IQR, 3.31–5.91 L/h/kg) and volume of distribution (Vd) = 1.85 L/kg (IQR, 1.27–3.20 L/kg). The median Cmax value for DHA was 3.30 μM (IQR, 2.08–5.95 μM), t1/2 = 26 min (IQR, 23–31 min), CL/Fm = 4.37 L/h/kg (IQR, 3.29–6.87 L/h/kg) and Vd/Fm = 2.56 L/kg (IQR, 1.93–4.49 L/kg). Artesunate and DHA pharmacokinetic parameters were similar after ia administration. Following im dosing, median artesunate Cmax was 4.81 μM (IQR, 3.74–5.40 μM), t1/2 = 18 min (IQR, 16–28 min), CL = 4.37 L/h/kg (IQR, 4.13–4.68 L/h/kg) and Vd = 2.07 L/kg (IQR, 1.83–2.79 L/kg); the bioavailability was 100%. For DHA, median Cmax was 1.43 μM (IQR, 1.00–1.92 μM), t1/2 = 27 min (IQR, 25–37 min), CL/Fm = 4.68 L/h/kg (IQR, 3.35–6.73 L/h/kg) and Vd/Fm = 3.31 L/kg (IQR, 2.89–4.27 L/kg).

Conclusions: The pharmacokinetic properties of artesunate and DHA in pigs were similar to those reported in humans, suggesting that the swine model is suitable for determining the preclinical pharmacokinetics of artemisinin derivatives.

Keywords: antimalarials, swine, population pharmacokinetics, preclinical

Introduction

Malaria remains an important public health concern both in countries where transmission occurs regularly and in areas where transmission has been largely controlled or eradicated. The global burden of malaria is estimated to be between 400 and 600 million cases per year, resulting in 1.5–2.7 million deaths.1 Antimalarial drug resistance is one of the greatest challenges of malaria control today. It has been identified as a key factor in the advent of malaria in new areas and in its resurgence in areas where the disease had been eradicated. Resistance has arisen to all classes of antimalarial drugs, with the possible exception of artemisinin derivatives. This has led the World Health Organization to recommend the use of artemisinin...
derivatives in combination therapies (artemisinin-based combination therapy; ACT) as a first-line treatment against Plasmodium falciparum malaria. One of the main benefits of ACT is the potential to delay the spread of antimalarial resistance. This is explained by the advantage of using two drugs with different mechanisms of action, thereby preventing further selection of resistance and resulting in higher cure rates, and by the effect of artesinin derivatives in reducing gametocyte carriage. However, due to the urgent need for new drugs to treat patients with multidrug-resistant parasites, treatment regimens involving these compounds have been developed empirically and evaluated in a variety of different clinical settings.

Choice of combination, administration route, number and frequency of drug dosing with regard to patient compliance, incorrect dosing or unusual pharmacokinetic properties of the drugs may contribute to the rise of resistance. Above all, pharmacokinetic characteristics of partner drugs, their interactions and elimination half-lives are crucial for the selection of resistant parasites.

Thus, to optimize current therapies and to develop new co-formulations, there is a need to determine the pharmacokinetic properties of the drugs given alone and in combination. Rodent models are the primary species studied during preclinical development phases. These models are not compatible with extensive blood sampling and are not suitable for complete kinetics. Alternatively, larger animals such as dogs and monkeys can be used for bioavailability and pharmacokinetic studies, but their use is often restricted due to the high cost involved and for ethical reasons. These reasons have led to the development of other large-animal models. Swine have notable anatomical and physiological similarities with humans and their use is of great interest. Moreover, this experimental model allows repeated blood sampling and complete kinetics on the same animal.

In view of these considerations, we evaluated the domestic pig as a potential experimental animal model for determining the pharmacokinetics of the artesinin derivative artesunate and its active metabolite, dihydroartemisinin (DHA). The pharmacokinetic parameters of artesunate and DHA were estimated from plasma concentration–time profiles after intravenous (iv) and intramuscular (im) administrations of artesunate to pigs using empirical Bayes methodology (NONMEM). These routes of administration were chosen referring to a study conducted on children with severe malaria who received iv and im artesunate administrations. To complete the knowledge about the artesunate outcome in the organism, an intra-arterial (ia) administration was then performed to show the hypothetical pulmonary first-pass metabolism.

To our knowledge, this is the first time that the pharmacokinetic properties of artesunate in pigs have been described.

Materials and methods

Chemicals and reagents

DHA and artesunate were kindly donated by Sanofi-Aventis (Gentilly, France). Artemisinin (internal standard) was obtained from Sigma (Saint Quentin Fallavier, France). Stock solutions of the three compounds were prepared in methanol/water (50:50, v/v) at a concentration of 150 mg/L and stored at −80 °C until use. Acetonitrile was of high-performance liquid chromatography (HPLC) grade and de-ionized water was used to prepare buffers. Acetic acid, methanol (both from VWR International, Fontenay-sous-Bois, France) and ethyl acetate (Baker, Philipsburg, NJ, USA) were of analytical reagent grade.

Oasis HLB cartridges (30 mg of sorbent, 1 mL capacity, average particle diameter 30 μm) were supplied by Waters (Waters Corp., Milford, MA, USA).

Animals

This research adhered to the ‘Principles of Laboratory Animal Care’ (NIH publication No. 85-23, revised 1985) and the ‘Guide for Care and Use of Laboratory Animals’. The animal study was approved by the local Animal Use Committee (Institut de Médecine Tropicale, Marseille, France).

The experiment was performed in 13 healthy Landrace X Large White male pigs, weighing 38.3 ± 2.8 kg (~12 weeks old) and purchased from EARL TREBOR (Saint Andiol, France). They were housed individually in pens, in the Surgery and Experimental Physiology Unit of the Tropical Medicine Institute (Marseille, France) and were allowed to acclimatize to the laboratory conditions and human contact for a period of ~1 week. They were fed with a standard pig diet and allowed free access to drinking water.

In order to facilitate multiple blood sampling, the jugular vein of each pig was surgically catheterized and exteriorized 1 day before the experiment. Anaesthesia was maintained with Propofol (Diprivan®, AstraZeneca, UK). Pre-medication was performed using a mixture of ketamine (0.25 g/kg body weight; Imalgene®, Merial, France), acepromazine (0.25 mg/kg body weight; Vétranquili®, Ceva Santé Animale, France) and atropine sulphate (0.025 mg/kg body weight; Audentt, France) given intramuscularly. Analgesia was enhanced by buprenorphine hydrochloride (0.0075 mg/kg body weight; Temgesic®, Schering-Plough, France).

At the end of experiment, pigs were sacrificed by iv injection of pentobarbital (91 mg/kg body weight; Dolethal®, Vétosquinol, France).

Drug administration and blood sampling

The artesunate administered dose was 60 mg corresponding to the current therapeutic dosage used in humans. Thirteen pigs received an im administration and an iv administration; five of them received an ia administration in addition. A 24 h period of wash-out was chosen between each administration. The order of administration was assumed not to be significant due to the fast elimination of artesunate and DHA.

Artesunate was dissolved on the day of administration by mixing the artesunate powder with 1 mL of 5% sodium hydrogen carbonate. This solution was diluted with 5 mL (iv and ia) or 2 mL (im) of 0.9% sterile isotonic saline. For iv and ia administrations, the dose was administrated over 2 min into the ear vein and the femoral artery, respectively. For im administration, the drug was injected into the gluteal muscle.

Blood samples were withdrawn after an additional physical restraint. Patency of the catheter (Hassle cath 6F 60 cm × 0.2 cm, 14G; Plastimed division Prodlim, Saint-Leu-la-foret, France) was maintained by flushing with small volumes of heparinized saline (50 U/mL). Blood samples were drawn before and (i) 1, 3, 5, 10, 15, 30, 45, 60 and 90 min, and 2, 4, 6, and 8 h after the end of iv or ia administration; and (ii) 5, 10, 15, 30, 40, 50, 60, 70, 80 and 90 min, and 2, 4, 6, and 8 h after the im administration. These samples were immediately centrifuged at 2000 g for 10 min, and aliquots of separated plasma were frozen at −80 °C until analysis.
Assay method

The assay of artesunate and DHA in plasma samples (1 mL) was performed using a specific and selective HPLC method with electrochemical detection operating in the reductive mode. The HPLC standard curves (5–400 ng/mL) and quality control samples (125, 190 and 350 ng/mL) were prepared by spiking plasma with methanol/water solutions (50:50, v/v) of artesunate and DHA. The internal standard used was artemisinin at a concentration of 3000 ng/mL.

The precision for HPLC measurement of artesunate and DHA was <7.4% and accuracy was between 85% and 115%. Dilutions were used for samples with concentrations of >400 ng/mL. The ability to dilute samples originally above the upper limit of the standard curve was demonstrated by accuracy and precision parameters in the method validation. The lower limit of quantification was 5 ng/mL for both compounds.

Population pharmacokinetic analysis

Individual pharmacokinetic parameters were estimated using an empirical Bayes methodology. In this analysis, population characteristics of the parameters to be estimated were used as prior information to estimate each individual pharmacokinetic parameter. Plasma concentrations less than the limit of quantification were omitted from the analysis. The maximum plasma drug concentration (Cmax) and time to maximum concentrations were obtained from the plasma drug concentration curve. Bioavailability was estimated from the population analysis. Conversion of artesunate into DHA was assumed to be complete.

Structural model building. Pharmacokinetic analyses were performed using the non-linear mixed-effect modelling approach as implemented in the NONMEM computer program (Version 5.0) through the Visual-NM graphical interface. The Fortran compiler used was Compaq Visual Fortran Standard Edition, Version 6.5. The population characteristics of the pharmacokinetic parameters (fixed and random effects) were estimated using the subroutine ADVAN-2 or ADVAN-4 from the library of programs provided with the NONMEM-PREDPP package. One- and two-compartment disposition models with first-order absorption and elimination rate constants were evaluated. The presence of an absorption delay was also investigated. The structural model was chosen on the basis of changes in –2 log likelihood and qualitative assessment of diagnostic plots. Because –2 log likelihood is approximately χ² distributed and the addition of one compartment increases the degrees of freedom by 2, a change of 5.99 in –2 log likelihood was required at the 5% significance level to select the more complex model. Both first-order (FO) and first-order conditional estimation (FOCE) methods were used to estimate population pharmacokinetic parameters (fixed and random effects).

Model for inter-individual variability. The pharmacokinetic parameter (PK) of the jth individual animal was modelled with the use of an exponential inter-individual variability error model:

\[ PK_j = PK_{\text{mean}} \times \exp(\eta_{PK}) \]  

where PKmean is the population mean and ηPK is the deviation from the log of PKmean and jth pig parameter value. The ηPK was assumed to be a Gaussian random variable with mean zero and variance of σ²PK. Using this approach, the model parameters were partitioned in fixed-effect (PKmean), random effect (σPK) and residual error (σ) parameters. All the parameters (fixed and random) were estimated using all of the collected measurements.

Model for intra-individual variability. Various error models were tested (additive, proportional, and combined additive and proportional). Both for artesunate and DHA, the error on the concentration measurements of the individual animal j was best described by a combined additive and exponential model, as shown in Equation (2):

\[ C_{ij} = C_{\text{pred}ij} \times \exp(\varepsilon_{ij}) + \varepsilon_{2ij} \]  

where Cij is the jth observed concentration for the jth individual, Cpredij is the plasma concentration predicted from the pharmacokinetic parameters of individual j, and εij (the difference between Cij and Cpredij) is assumed to be random Gaussian variable with a mean of zero and a variance of σ². Such error arises from factors such as assay variability, model misspecification, inaccurate recording of dosing or sampling times and intra-individual pharmacokinetic variability. The uncertainty (coefficient of variation) in estimating fixed and random parameter values was determined by expressing the standard error of estimation (calculated in NONMEM) as a percentage of the estimated value. The individual predicted plasma concentrations (Cpredij) were computed, for each individual, using the empirical Bayes estimate of the pharmacokinetic parameters using the POSTHOC option in the NONMEM program.

At each step of the model building, diagnostic plots were analysed for closeness to and randomness along the line of identity on the observed (DV) versus predicted (PRED) and individual predicted (IPRED) concentration plot, as well as randomness along the residual (DV–PRED) and weighted residual zero line on the predicted concentrations or time versus residual or weighted residual plot. PRED concentrations were computed based on population parameter estimates; IPRED concentrations were computed based on individual parameter estimates. The model was accepted when (i) plots showed no systematic pattern, and (ii) descriptive statistics did not show any systematic deviation from the initial hypothesis.

Statistical analysis

A two-way analysis of variance was performed to compare pharmacokinetic parameters evaluated after iv administration to the ones evaluated after im administration. A P value <0.05 was taken as the threshold of probability. In a last step, for the five pigs receiving ia, iv and im administrations of artesunate at 24 h intervals, pharmacokinetic parameters were compared between the three routes using a two-way analysis of variance. A simple contrast test was used to compare 2-by-2 each route of administration; in this case, the level of significance was corrected for multiple comparisons and fixed at 0.017.

The statistical analysis was carried out using the computer package Pk-fit.

Results

Semi-logarithmic plots of the artesunate and DHA plasma concentration–time profiles are illustrated in Figure 1(a and b), respectively. Following ia and iv administrations (60 mg), the median artesunate Cmax reached at the first blood sampling (1 min) was 6994 ng/mL [18.2 μM; interquartile range (IQR), 11.2–24.8 μM] and 5308 ng/mL (13.8 μM; IQR, 10.4–22.1 μM), respectively. There was a wide variation between animals in peak artesunate concentrations in plasma (range, 5.3–25.7 μM after ia administration; 7.2–33.4 μM after iv administration). The median
Artesunate pharmacokinetics in a pig model

$C_{\text{max}}$ concentration values for DHA were 809 ng/mL (2.84 μM; IQR, 2.14–3.46 μM) and 937 ng/mL (3.30 μM; IQR, 2.08–5.95 μM) after ia and iv artesunate administration, respectively. The time of $C_{\text{max}}$ ranged from 1 to 10 min (median value, 3 min) after ia administration and from 1 to 5 min after iv administration (median value, 1.5 min). After im administration, artesunate was absorbed very rapidly from the muscle; the median $C_{\text{max}}$ value was 1848 ng/mL (4.81 μM; IQR, 3.74–5.40 μM). This value was reached between 3 and 10 min depending on the pig (median 5 min). For the metabolite, the median $C_{\text{max}}$ of 407 ng/mL (1.43 μM; IQR, 1.00–1.92 μM) was reached between 3 and 15 min (median 10 min) after artesunate injection.

After artesunate administration, a rapid decrease in artesunate and DHA concentrations occurred during the first 10 min followed by a slower decrease. A two-compartment model with first-order absorption and elimination without a lag-time improved the fit of data markedly relative to a one-compartment model, as judged by plots of weighted residuals versus time and a lowering of the objective function by over 450 U. Thus, the six-dimensional vectors, $\theta$, of pharmacokinetic parameters considered in the population analysis consist of clearance ($\theta_1 = CL (L/h)$), initial volume of distribution ($\theta_2 = V_1 (L)$), transfer rate constants ($\theta_3 = k_{12}$ and $\theta_4 = k_{21}$ (h$^{-1}$)), absorption rate constant ($\theta_5 = k_a$ (h$^{-1}$)) and bioavailability ($\theta_6 = F$). From the individual (Bayesian estimates) primary pharmacokinetic parameters, the following secondary pharmacokinetic parameters were calculated: the half-life of the terminal part of the curve ($t_{1/2\alpha}$), the half-life of the distribution phase ($t_{1/2\beta}$), the volume of distribution at steady-state ($V_d = CL/t_{1/2\alpha}$) and the area under plasma concentration versus time curve (AUC = dose/CL).

A total of 226 and 385 concentrations were used to compute population parameters of artesunate and DHA, respectively. Results are presented in Table 1. After im administration, the median absolute bioavailability of artesunate was 1.13 (range, 0.67–1.90; IQR, 1.05–1.32).

Weighted residual plots did not show any pattern and plots representing population or individual predictions versus observed data did not show either any substantial or systematic deviations from the line of identity (Figure 2). Overall, the proposed model correctly described the data. The mean weighted residual values of 0.034 ng/mL (artesunate) and 0.078 ng/mL (DHA) were not significantly different from zero (Student’s $t$-test). In a last step, individual predicted concentrations were

![Figure 1. (a) Mean plasma artesunate versus time profiles after iv (filled circles), ia (filled triangles) and im (filled squares) administrations of 60 mg artesunate. (b) Mean plasma DHA versus time profiles after iv (open circles), ia (open triangles) and im (open squares) administrations of 60 mg artesunate.](https://academic.oup.com/jac/article-abstract/62/3/566/733813)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Artesunate</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean value</td>
<td>CV (%)</td>
<td>mean value</td>
</tr>
<tr>
<td>CL (L/h)</td>
<td>172 (11%)</td>
<td>27.3</td>
</tr>
<tr>
<td>$V_1$ (L)</td>
<td>9.49 (20.6%)</td>
<td>68.6</td>
</tr>
<tr>
<td>$k_{12}$ (h$^{-1}$)</td>
<td>9.9 (28%)</td>
<td>51.5</td>
</tr>
<tr>
<td>$k_{21}$ (h$^{-1}$)</td>
<td>2.79 (17%)</td>
<td>46.8</td>
</tr>
<tr>
<td>$k_a$ (h$^{-1}$)</td>
<td>8.37 (19%)</td>
<td>80.2</td>
</tr>
<tr>
<td>$F$</td>
<td>1.1 (21%)</td>
<td>74.2</td>
</tr>
<tr>
<td>Intra-individual variability</td>
<td>28.9%; 14.9</td>
<td>22.5%; 317</td>
</tr>
<tr>
<td>Objective function</td>
<td>2337.4</td>
<td>3466.9</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; CL, total plasma clearance; $V_1$, initial volume of distribution; $k_{12}$ and $k_{21}$, two transfer rate constants; $k_a$, absorption rate; $F$, bioavailability.

Standard errors of estimates [expressed as CV (%)] are given in parentheses.

*Not corrected to the amount of artesunate metabolized to DHA (Fm).
compared with observed concentrations by computing the bias. Results are as follows: artesunate bias –5.36 ng/mL, 95% CI 31.2–20.5; DHA bias –4.45 ng/mL, 95% CI 9.7–0.80.

Typical posterior individual fittings are displayed in Figure 3(a) for artesunate and in Figure 3(b) for the metabolite.

Mean pharmacokinetic parameters calculated by Bayesian estimation according to the route of administration are given in Table 2 for artesunate and in Table 3 for DHA.

For the parent drug and its metabolite, the two-way analysis of variance performed on $V_1$, $V_d$, $CL$, $AUC$, $t_{1/2L1}$ and $t_{1/2L2}$ showed no statistically significant difference between iv and im administrations ($P > 0.05$). For the five pigs receiving ia, iv and im administrations of artesunate, the pharmacokinetic parameters were not statistically different between the three routes of administration (data not shown).

The molar AUC values for DHA were 1.30- to 1.73-fold those for artesunate.

**Discussion**

For the last decade, malaria chemotherapy has relied on a limited number and type of drugs due to the development and spread of parasite resistance to nearly all antimalarials in current use and also to the cross-resistance between closely related drugs. However, most of these drugs still have a place, and their lifespan could be prolonged if they are better deployed and used, and also by rationally combining them based on pharmacodynamic and pharmacokinetic properties. Swine are increasingly used as a model in a wide array of biomedical research, including regulatory pharmacokinetics in the pharmaceutical industry. The pressure from society to reduce the number of non-human primates and dogs in biomedical research has undoubtedly led to this increase. Beyond this argument, the anatomy and physiology of swine have notable similarities to those of man.11 – 14 The use of pigs can be more relevant than other models in some phases of the evaluation of new drugs15 or in toxicological studies.10 The size of the animal enables repeated blood sampling without significant change in the haematocrit and haemodynamics; this allows estimation of intra- and inter-individual variability. Furthermore, during surgical preparation, most human anaesthetic products and anaesthesia materials can be used without technical adaptation. Finally, the metabolism of xenobiotics and the total cytochrome P450 enzymatic system are very similar between pig and human.24,25 For all these reasons, the swine model is relevant for studying drug pharmacokinetics as has been demonstrated in previous studies.26 – 28 The present study was designed to develop the pig as a suitable model for studying the pharmacokinetic properties of antimalarial drugs. Nevertheless, the major limitation of this experimental model is that it cannot be infected by *Plasmodium*. Fluctuation of
pharmacokinetic parameters of antimalarial drugs between healthy and infected people is a known fact. It is suggested that the clearance of artesunate is reduced during infection and that the severity of infection has no influence on artesunate pharmacokinetics. In spite of this, the swine model is still relevant during non-clinical drug evaluation.

To evaluate the relevance of the pig model, we chose to determine the pharmacokinetic profiles of the artemisinin derivative artesunate and of its bio-active metabolite DHA after iv, ia and im administrations of artesunate. The population approach is increasingly used in the early phase of the toxicokinetic programme owing to the many advantages it offers in relation to conventional analysis approaches. In the present study, an empirical Bayes methodology was used to estimate individual pharmacokinetic parameters. Since the data were not sparse, a population approach might not be required. However, such an analysis avoided a possible bias (i.e. underestimation) in the estimation of the elimination half-life of artesunate. Indeed, in some animals, 0.2 or 0.8 h after drug administration, artesunate concentrations were below the lower limit of quantification of the analytical method (Figure 1a); so, using a conventional non-compartmental or compartmental approach, the half-life of the terminal part of the curves cannot be suitably estimated in all animals. Moreover, this approach provides a better estimation of inter-individual variability in pharmacokinetic parameters and allowed us to confirm that the selected model in pigs properly fits the entire set of the available data.

Plasma concentration–time profiles following artesunate administrations were comparable in pigs and in humans with a rapid and biphasic decline for both artesunate and its metabolite DHA. Across the three routes of administration, ia, iv and im, the pharmacokinetic parameters of artesunate were comparable. The artesunate AUC values were similar following iv and im administrations (Table 2), indicating that artesunate is well absorbed from the im site and subsequently converted into DHA. Compared with iv administration, the $C_{max}$ for artesunate was 5.5-fold lower following im administration, suggesting that the elimination of artesunate is absorption-rate-dependent. The elimination half-life ($t_{1/2}$) estimates for artesunate are similar for the three routes of administration tested (median, 18 min). This result is consistent with values previously reported after iv administration in other animal models and in humans. However, other studies from different populations of patients reported an elimination half-life of 2–5 min following this route of administration. A reason for this

![Figure 3](https://academic.oup.com/jac/article-abstract/62/3/566/733813)

**Figure 3.** Typical posterior individual fittings after iv administration of artesunate. Artesunate (a) and DHA (b). Filled symbols, individual predicted concentration (IPRED); open symbols, observed concentrations (DVs).

**Table 2.** Mean pharmacokinetic parameters of artesunate according to the route of administration

<table>
<thead>
<tr>
<th>Route</th>
<th>$V_1$ (L/kg)</th>
<th>$V_d$ (L/kg)</th>
<th>CL (L/h/kg)</th>
<th>AUC (µmol/L)</th>
<th>$t_{1/2}λ_1$ (h)</th>
<th>$t_{1/2}λ_2$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ia ($n = 5$)</td>
<td>median 0.38</td>
<td>1.79</td>
<td>3.88</td>
<td>1.05</td>
<td>0.032</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>IQR 0.15–0.65</td>
<td>1.46–3.53</td>
<td>3.66–5.56</td>
<td>0.75–1.11</td>
<td>0.022–0.056</td>
<td>0.20–0.50</td>
</tr>
<tr>
<td>iv ($n = 13$)</td>
<td>median 0.15</td>
<td>1.85</td>
<td>5.58</td>
<td>0.73</td>
<td>0.016</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>IQR 0.12–0.17</td>
<td>1.27–3.20</td>
<td>3.31–5.91</td>
<td>0.69–1.23</td>
<td>0.014–0.022</td>
<td>0.26–0.37</td>
</tr>
<tr>
<td>im ($n = 13$)</td>
<td>median 0.17</td>
<td>2.07</td>
<td>4.37</td>
<td>0.93</td>
<td>0.020</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>IQR 0.14–0.27</td>
<td>1.83–2.79</td>
<td>4.13–4.68</td>
<td>0.87–0.99</td>
<td>0.016–0.026</td>
<td>0.27–0.47</td>
</tr>
<tr>
<td>$P$ values</td>
<td>ia/iv/im</td>
<td>0.07</td>
<td>0.90</td>
<td>0.73</td>
<td>0.73</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>iv/im</td>
<td>0.14</td>
<td>0.95</td>
<td>0.26</td>
<td>0.52</td>
<td>0.042</td>
</tr>
</tbody>
</table>

ia, intra-arterial administration; iv, intravenous administration; im, intramuscular administration; $P$ values, statistical comparison between routes; IQR, interquartile range; CL, total plasma clearance; $V_1$, initial volume of distribution; $V_d = CL/λ_2$, steady-state volume of distribution; AUC = dose/CL, area under curve; $t_{1/2}λ_1$, distribution half-life; $t_{1/2}λ_2$, elimination half-life.
discrepancy might be that, in the present study, the limits of quantification were lower than those reported in other published studies, allowing measurement of artesunate and DHA concentrations in plasma for a longer period of time. This explains the use of a two-compartmental pharmacokinetic model and the longer elimination half-life found in this study.

Table 3. Mean pharmacokinetic parameters of DHA according to the route of administration

<table>
<thead>
<tr>
<th>Route</th>
<th>$V_i$/Fm (L/kg)</th>
<th>$V_d$/Fm (L/kg)</th>
<th>CL/Fm (L/h/kg)</th>
<th>AUC ($\mu$mol·h/L)</th>
<th>$t_{1/2}\lambda_1$ (h)</th>
<th>$t_{1/2}\lambda_2$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ia (n = 5)</td>
<td>0.86</td>
<td>3.93</td>
<td>5.27</td>
<td>1.37</td>
<td>0.016</td>
<td>0.58</td>
</tr>
<tr>
<td>IQR</td>
<td>0.64–1.28</td>
<td>3.45–5.68</td>
<td>4.28–6.53</td>
<td>1.17–1.48</td>
<td>0.015–0.017</td>
<td>0.56–0.66</td>
</tr>
<tr>
<td>iv (n = 13)</td>
<td>0.77</td>
<td>2.56</td>
<td>4.37</td>
<td>1.26</td>
<td>0.020</td>
<td>0.44</td>
</tr>
<tr>
<td>IQR</td>
<td>0.51–1.06</td>
<td>1.93–4.49</td>
<td>3.29–6.87</td>
<td>0.81–1.67</td>
<td>0.017–0.021</td>
<td>0.39–0.51</td>
</tr>
<tr>
<td>im (n = 13)</td>
<td>0.87</td>
<td>3.31</td>
<td>4.68</td>
<td>1.18</td>
<td>0.024</td>
<td>0.45</td>
</tr>
<tr>
<td>IQR</td>
<td>0.71–0.88</td>
<td>2.89–4.27</td>
<td>3.35–6.73</td>
<td>0.82–1.80</td>
<td>0.021–0.033</td>
<td>0.42–0.62</td>
</tr>
</tbody>
</table>

$P$ values

| ia/iv/im | 0.79 | 0.37 | 0.99 | 0.58 | 0.36 | 0.38 |
| if/im | 0.69 | 0.73 | 0.17 | 0.13 | 0.37 | 0.77 |

ia, intra-arterial administration; iv, intravenous administration; im, intramuscular administration; $P$ values, statistical comparison between routes; IQR, interquartile range; CL, total plasma clearance; $V_i$, initial volume of distribution; $V_d = CL/\lambda_2$, steady-state volume of distribution; AUC = dose/CL, area under curve; $t_{1/2}\lambda_1$, distribution half-life; $t_{1/2}\lambda_2$, elimination half-life; Fm, amount of artesunate metabolized to DHA.

In conclusion, the present work shows the relevance of the swine model to study the pharmacokinetics of artesunate after iv and im administrations. The pharmacokinetic parameters of artesunate and DHA were of the same order of magnitude as those achieved in humans with intra- and inter-individual variability consistent with that usually reported in humans for artemisinin-type compounds (between 50% and 70%). Moreover, we found that in pigs, as in humans, the im route is a suitable alternative to the iv route, at equivalent doses. Appropriate pharmacokinetic information, in particular concerning metabolism and drug interaction, is becoming increasingly important with the increased use of combination chemotherapy in the treatment of malaria. Also, the swine model may be a source of information to develop newer approaches for which the application of pharmacokinetic principles is fundamental.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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