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Pharmacokinetic and Pharmacodynamic Aspects of Artelinic Acid in Rodents

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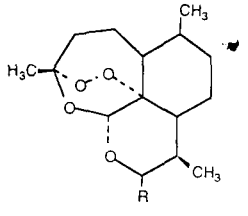
Abstract—The efficacy of artelinic acid and artemisinin, orally administered at 10 and 50 mg kg^{-1} day^{-1}, was compared in *Plasmodium berghei* infected mice. Subsequently, the pharmacokinetics of artelinic acid after intravenous, intramuscular, oral and rectal administration of a 20 mg kg^{-1} aqueous solution to rabbits were studied in a four-way randomized cross-over experiment. After intravenous administration, artelinic acid concentrations in blood plasma were high (C_0: 76 ± 15 mg L^{-1}), and the drug was rapidly eliminated from the central compartment, showing linear elimination kinetics with an elimination half-life of 15 ± 3 min. A large inter-subject variation appeared in the absorption rate and the extent of absorption (2–92%) over the 120 min interval after intramuscular administration. Also, a large inter-subject variation in individual rectal bioavailability (17–100%) was shown, which was dependent on the site of absorption in the rectum. The estimated oral bioavailability was low (4·6 ± 1·7%), probably due to a high first-pass effect and possible decomposition in the acidic gastric environment.

Artelinic acid is a water-soluble derivative of artemisinin (Fig. 1). Artemisinin is the parent compound of a new class of antimalarials and is isolated from the herb *Artemisia annua*. Artemisinin has a direct and rapid schizontocidal action on the erythrocytic stages of *Plasmodium* species with a minimum inhibitory concentration (MIC) of 10^{-7} M. Its antimalarial activity is superior compared with all other antimalarial drugs used today (Qinghaosu Antimalarial Co-ordinating Research Group 1979; Klayman 1985). Artemisinin is considered especially suited to the treatment of *Plasmodium falciparum* (severe malaria) and in cases of chloroquine resistance. The parent compound artemisinin is very poorly soluble in water as well as in oil. Therefore, several water soluble derivatives of artemisinin were synthesized recently (Fig. 1), enabling parenteral administration (Lin et al 1987).



R	Drug
=O	Artemisinin
-O-CH_2-(C_6H_4)-COOH	Artelinic acid
-O-(C=O)-(CH_2)$_2$-COOH	Artesunic acid

FIG. 1. Structural formula of artemisinin and its water soluble derivatives.

The availability of parenteral formulations is especially important for the treatment of cerebral malaria, when the rapid reversal of parasitaemia and restoration to consciousness is crucial and patients are unable to take oral antimalarial drugs. To overcome the instability of artesunate (an ester-based derivative) in aqueous solutions, the water-soluble carboxylate moiety in artelinic acid is coupled to the artemisinin skeleton by an ether linkage.

In-vivo experiments showed a superior efficacy of artelinic acid against *P. berghei* in mice in comparison with the parent compound artemisinin after intramuscular and oral administration. After subcutaneous injection, the antimalarial action of sodium artelinate was slightly less than that of artemisinin (Lin et al 1987; Van Vianen et al 1990). The objective of this study was to investigate the basic pharmacokinetic properties of artelinic acid, and to find out whether the difference in efficacy of artelinic acid and its parent compound artemisinin can be explained by a dissimilar pharmacokinetic behaviour. The pharmacokinetics of artemisinin have been discussed previously (Titulaer et al 1990, 1991).

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Materials and Methods

Materials

Artelinic acid was provided by ACF-Beheer B.V., Maarssen, The Netherlands. Other reagents and chemicals used were of analytical grade. Sodium artelinate was prepared according to Klayman (personal communication): 7 g (16·75 mmol) artelinic acid was suspended in 150 mL distilled water and cooled in an ice-bath. To the suspension was added, dropwise with stirring, an aqueous solution containing one molar equivalent of sodium hydroxide (16·75 mmol: 0·67 g in 10 mL distilled water). The suspension was stirred until the artelinic acid was all or nearly all dissolved. The solution was filtered and lyophilized. The product was purified by dissolving it in a minimum volume of absolute methanol, followed by reprecipitation with ethyl ether to give 6·5 g (yield 88%) of sodium artelinate.

Portions (0·75 mL) of 20% sodium artelinate solution in Water for Injection were sterilized by filtration (Acrodisc/

Gelman 0·2 μm filter) and stored in stoppered sterile glass vials at −20°C until use.

Stability study

The stability of sodium artelinate in 0·1 M HCl was studied at 37°C. Equal volumes of a sodium artelinate stock solution (5·50 mg in 50·0 mL bidistilled water, freshly prepared) and 0·2 M HCl were mixed. The mixture was placed in a water bath at 37°C and stirred during the experiment. After 2·5, 5, 7·5, 10, 15, 30, 45 and 60 min, 0·5 mL samples were taken and transferred to test tubes containing 0·5 mL 0·1 M NaOH. Subsequent extraction and HPLC analysis was carried out as described below.

Animals

Efficacy study. Five groups of three outbred Swiss mice (group 1: control; groups 2 and 3: 10 and 50 mg kg^{-1} artelinic acid; groups 4 and 5: 10 and 50 mg kg^{-1} artemisinin) were obtained from colonies of the animal facility of the University of Nijmegen, The Netherlands.

Pharmacokinetic study. Ten outbred ICO White New Zealand rabbits, 2·40 ± 0·12 kg (mean ± s.d.), were obtained from the Collective Animal Laboratory, GDL, Utrecht University, The Netherlands. From about 9 h before until 2 h after drug administration the rabbits received no food. During the period of blood sampling the animals were kept in isolation cages.

Methods

Efficacy study. P. berghei (strain K173) was maintained by weekly sub-inoculation (i.p.) of 10^5 parasitized red blood cells (erythrocytic stage) from infected donor mice into normal mice (Eling 1978). The mice for the efficacy study were also inoculated with 10^5 *P. berghei* on day 0. Groups of three mice were treated with no drug (control), 10 and 50 mg kg^{-1} of artemisinin or artelinic acid. Artemisinin (aqueous suspension) and artelinic acid (aqueous solution) were each orally administered once daily using a gastric tube. The control group received water through a gastric tube only. Parasitaemia (per cent of parasitized erythrocytes) was determined by microscopic examination of tail blood smears, taken at days 5 and 7 after infection after staining with May Grünwald/Giemsa's solution.

Pharmacokinetic study. Samples (0·2 mL) 20% aqueous sodium artelinate solution were administered in a four-way randomized cross-over study to the rabbits by the intravenous, intramuscular, oral and rectal routes. A wash-out period of one week was maintained between the test days. The solution was administered into the marginal ear vein and intramuscularly in the M gastrocnemius using 25G × 5/8″ needles (Terume Europe B.V., Leuven, Belgium), and rectally and orally using a gastric tube. Saline (1·5 mL rectally, 4 mL orally) was used to rinse the gastric tubes after administration.

Blood samples (0·5–1 mL) were collected by puncture of the marginal ear vein before, and at 5, 10, 20, 30, 45, 60, 90 and 120 min after administration. Artelinic acid concentrations in serum were measured using HPLC with UV detection (Titulaer & Vink-Blijleven 1993). Briefly, 250 μL of serum was extracted with 4·5 mL diethyl ether. After separating the phases by centrifugation, the aqueous layer was frozen in an acetone/dry-ice bath. The organic phase was decanted into clean, conically shaped glass tubes and evaporated to dryness. The residue was redissolved in 100 μL mobile phase. The separation was achieved on a Lichrosorb RP18 column, 10 cm × 3 mm i.d., particle size 5 μm (Chrompack B.V., Middelburg, The Netherlands), which was eluted with a mobile phase consisting of bidistilled water, acetonitrile and triethylamine (50:50:3 v/v/v) at a flow rate of 0·4 mL min^{-1}. The mobile phase was adjusted to pH 5·0 using concentrated orthophosphoric acid. UV detection was performed at 240 nm.

Pharmacokinetic analysis. Drug concentration-time curves obtained after intravenous and rectal administration were fitted to a one-compartment model with first-order elimination kinetics. Standard kinetic models and methods (PCNONLIN) were used to evaluate the data (Statistical Consultants Inc. 1986). Data from oral and intramuscular administrations were analysed using the following methods: the elimination rate constant k_{el} was determined, where possible, by semi-logarithmic regression analysis of the terminal linear phase of the curve. The areas under the concentration time curves (AUC) were calculated using the linear trapezoidal rule and extrapolation to infinity substituting the regression value for C_z at the last time-point. The fraction not absorbed was calculated according to the method of Wagner & Nelson (1963). The time to reach 50% absorption (relative to intravenous administration) ($t_{0·5}$) was calculated by regression analysis of the fraction absorbed plots. The bioavailabilities of the intramuscular, oral and rectal administrations were calculated by comparison of the areas under the curve with the area obtained after intravenous administration.

The drug concentration time curves are presented individually (Zuidema & Wyne 1989).

Histology study. At the completion of the experiments two rabbits were killed by intravenous injection of 4 mL 200 mg mL^{-1} sodium pentobarbitone solution. The sites of injection were visually inspected for signs of tissue change. Heart, liver, kidneys, spleen and parts of the gastrointestinal tract were also examined. Tissue samples from the injection sites (marginal ear vein, 4 and 16 days after administration; and M gastrocnemius, 6, 8 and 16 days after administration) were stored in 4% paraformaldehyde until histological preparation. After dehydration with ethanol (1 × 70, 1 × 96 and 2 × 100%), the samples were incorporated in glycol methacrylate (Technovit 7100, Kulzer, Wehrheim, Germany). After staining of the 5 μm slices (Microm HM 350) with haematoxylin (Gill No. 3) and eosin 0·5% the samples were incorporated in malinol.

Results

*Stability study.*The decomposition of artelinic acid in 0·1 M HCl at 37°C is depicted in Fig. 2.

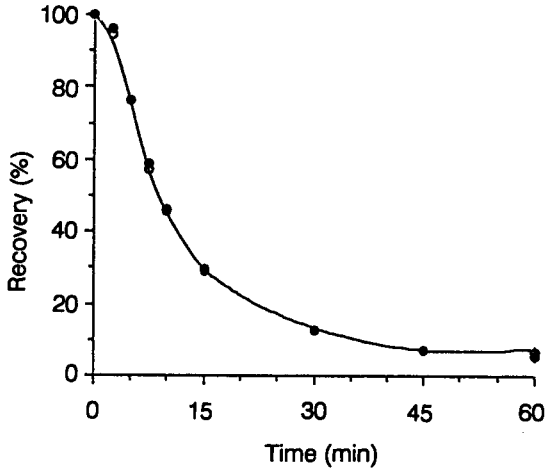

FIG. 2. Stability of sodium artelinate in 0·1 M HCl at 37°C (n = 2).

Efficacy study

The percentage parasitized erythrocytes on days 5 and 7 after infection are shown for both the artelinic acid and artemisinin therapy (Table 1). At a daily oral administration of 50 mg kg^{-1} artelinic acid almost no parasitaemia could be observed, while applying the same dose of artemisinin showed 6–10% infected erythrocytes at day 5. The lower dose of 10 mg kg^{-1} was less effective, but the difference between the two drugs was maintained. This difference in efficacy was even more pronounced at day 7 after inoculation: again parasitaemia in all mice receiving 50 mg kg^{-1} artelinic acid was suppressed, while mice receiving 50 mg kg^{-1} artemisinin showed parasitaemia (8–40%). The mice receiving the low dose of artemisinin all died with a high percentage of infected erythrocytes; two mice in the control group also died.

Table 1. Parasitaemia (%) in mice following inoculation with 5×10^5 *P. berghei*. Efficacy of artelinic acid and artemisinin.

		Day after inoculation	
Therapy (mg kg^{-1} day^{-1} p.o.)		5	7
Control		20, 25, 40	45[a]
Artelinic acid	10	< 1, 5, 6	6, 15, 15
	50	0, 0, 0	0, 0, < 1
Artemisinin	10	15, 20, 45	[b]
	50	6, 8, 10	8, 8, 40

[a] Two animals died by day 7. [b] Three animals died by day 7.

Pharmacokinetic study

The statistics for the assay method used in the pharmacokinetic experiments are shown in Table 2. Fig. 3a shows the drug concentration-time profiles after intravenous administration. Table 3 summarizes the calculated pharmacokinetic parameters. Artelinic acid was rapidly eliminated after intravenous injection as indicated by the elimination half-life of 15 min. Semi-logarithmic plots of drug concentration vs time showed linear elimination kinetics over the observed concentration range. The absorption after rectal administration proceeded very rapidly (absorption half-life 2 ± 1 min (mean ± s.d., n = 10)). The peak concentration was reached after about 6 min (mean ± s.d.: 6 ± 3 min). The large differences in maximum blood level indicate a large inter-subject variation in bioavailability; the calculated bioavailability ranged from 17 to 100%.

Table 2. Statistics for artelinic acid HPLC assay.

	Mean	s.d.	n
Recovery	68·0	1·37%	3
Within-run variation	2·00	0·033 μg mL^{-1}	3
Between-run variation	2·01	0·116 μg mL^{-1}	5

Control sample: concentration = 2·0 μg mL^{-1} rabbit serum.

The concentration-time profiles after intramuscular and oral administration are depicted in Fig. 3. Fractions of 7 ± 2·2 (oral) and 9·5 ± 4·6 (i.m.) of the AUC were extrapolated to infinite time. The absorption after intramuscular administration, as indicated by the time to reach 50% absorption, proceeds much more slowly than after rectal administration. The extent of absorption at 120 min after intramuscular injection shows a large inter-subject variation; the fraction not absorbed at 120 min was 51 ± 31%, ranging from 2 to 98% (Fig. 4). After oral administration (Fig. 3d), the concentrations of artelinic acid in serum were low (< 2 mg L^{-1}). The bioavailability after oral administration, as estimated over the first two hours, was also very low (4·6 ± 1·7% (mean ± s.d.)).

Histology study

No disorders were found in the heart, lungs, gastrointestinal tract and the muscles at the injection sites, according to visual inspection. The spleen of one of the two rabbits was slightly enlarged. Microscopic examination of the histology preparations of the injection sites revealed no signs of abnormalities.

Table 3. Calculated pharmacokinetic parameters after administration of 20 mg kg^{-1} artelinate.

	Mean	s.d.
Intravenous (n = 10)		
k_{el}	0·049	0·013 (min^{-1})
$t_{\frac{1}{2}el}$	15	3 (min)
Vd	0·23	0·05 (L kg^{-1})
CL	11·0	2·6 (mL min^{-1} kg^{-1})
Rectal (n = 10)		
k_{el}	0·047	0·015 (min^{-1})
$t_{\frac{1}{2}el}$	18	12 (min)
k_{abs}	0·89	0·96 (min^{-1})
$t_{\frac{1}{2}abs}$	2·0	1·1 (min)
F	47	33 (%)
Oral (n = 9)		
$t_{\frac{1}{2}el}$	43	15 (min)
F	4·6	2·2 (%)
Intramuscular (n = 7)		
$t_{\frac{1}{2}el}$	56	31 (min)
$t_{fabs0·5}$[a]	145	108 (min)
F	44	29 (%)

[a] Time to reach 50% absorption, n = 10.

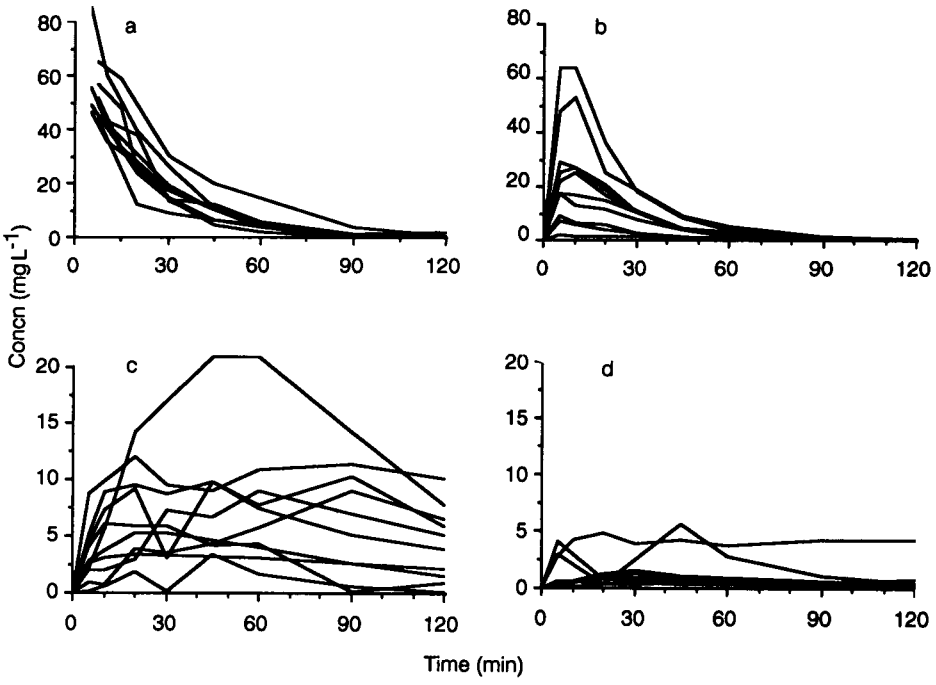

FIG. 3. Artelinic acid concentration-time profiles after administration of 20 mg kg^{-1} aqueous solution (n = 10). (a) Intravenous, (b) rectal, (c) intramuscular, (d) oral administration.

Discussion

After intravenous administration, artelinic acid concentrations in serum could be described by a one-compartment open model with first-order elimination kinetics. The elimination rate is much higher than would be expected from the renal clearance capacity (normal creatinine clearance in the rabbit is approximately 3 mL min^{-1} kg^{-1} (Kozma et al 1974; Contrepois et al 1987), calculated artelinate clearance: $11 \pm 2{\cdot}6$ mL min^{-1} kg^{-1}) and suggests artelinate to be a high-clearance drug. High-clearance drugs often show a low bioavailability due to a considerable first-pass effect. The volume of distribution (Vd) is small compared with that of artemisinin and many other antimalarials in man (Table 4). The corresponding high concentrations in serum might partly explain the high efficacy as found in this study and demonstrated in Table 1.

Table 4. Volume of distribution (Vd) of antimalarial drugs.

Drug	Vd (L kg^{-1})	Subjects
Artemisinin	23[a]	Healthy*
Quinine	2·2	Healthy†
Quinine	1·7	Uncomplicated malaria†
Quinine	1·2	Cerebral malaria†
Quinidine	2·5–3·5	Healthy†
Chloroquine	116–285	Healthy††
Mefloquine	13·5–29·1	Healthy††
Mefloquine	6	Uncomplicated malaria†
Primaquine	149–257[b]	Healthy††
Pyrimethamine	2·83–2·93	Healthy††
Halofanthrine	100–570	Healthy††

[a] Estimated value, [b] unit in L instead of L kg^{-1}. * Titulaer et al (1990); † White (1985), †† White (1987)

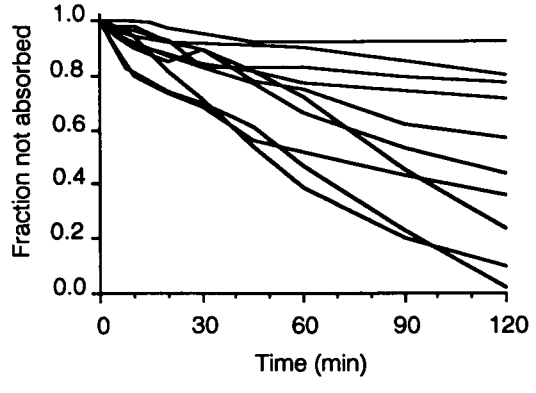

FIG. 4. Fraction not absorbed after intramuscular injection of 20 mg kg^{-1} sodium artelinate aqueous solution (n = 10).

The large inter-subject variation in bioavailability after rectal administration may be caused by the fact that with high-clearance drugs the first-pass effect is partially avoided by the rectal route. In three cases which showed a high rectal bioavailability, it was observed on inserting the rectal cannula that the upper part of the rectum was blocked by the presence of faeces. This would limit the site of absorption to the lower part of the rectum.

The high variability in pharmacokinetic profile resulting from intramuscular injections is known (Gibaldi 1977; Zuidema et al 1988). Many factors affect the release from the intramuscular injection site. With water-soluble drugs, ionization and subsequent absorption or binding to tissue

Table 5. Comparison of artemisinin and artelinate maximum blood levels.

	Intravenous	Rectal	Intramuscular	Oral
Artemisinin[a,b]	—	0·056±0·058	0·075±0·011	0·260±0·094
Artelinate[c]	76±15	26±19·7	9±4·9	1·9±1·7

All concentrations, expressed as mean±s.d. are in mg L^{-1}, but not corrected for dose. [a]Man; dose: 5 mg kg^{-1}. [b]No intravenous administration was done. [c]Rabbits; dose: 20 mg kg^{-1}.

components may play a role (Sund & Schou 1964). Also drug lipophilicity influences the release rate in intramuscular injections (Kadir et al 1990). In the case of solvent depletion at the site of injection, crystallization or particle aggregation may occur, hence introducing rate limiting factors related to those of intramuscular suspension injections (Hirano et al 1981). During the observed time period of 120 min the absorption after intramuscular injection was incomplete for most individuals and ranged from 8 to nearly 100%, while the plots of fraction not absorbed indicated a zero-order absorption process. This might be explained by the assumption that the highly hydrophilic artelinate is transported into the blood compartment (intercellular) via the vehicle rather than by transcellular diffusion. Drug metabolism in the muscle tissue as an explanation for incomplete absorption is less likely, although there are no data available in the literature of artelinic acid on this subject. Also the injection technique (either intramuscular or intermuscular) might be a factor, although great attention was paid to experimental techniques (Groothuis et al 1980; Marshall & Palmer 1980). Further detailed experiments are needed to study the underlying mechanisms and long-term effects.

Following oral administration the absorption rate and extent of absorption was unexpectedly low, but could not exactly be calculated due to the rapid elimination, and the bioavailability could only be roughly estimated. Values might be low due to the short observation time of 2 h. Nevertheless, the estimated bioavailability did not exceed 7%. It is assumed that, like the parent compound artemisinin, which showed an oral availability in man of about 32% (Titulaer et al 1990), artelinic acid is subject to extensive first-pass metabolism.

Considering the different experimental design in the previously described artemisinin study (Titulaer et al 1990) and the experiments described here (artemisinin: 5 mg kg^{-1} in man, artelinic acid 20 mg kg^{-1} in rabbits), and despite the substantial variability after intramuscular and rectal administration and the very poor oral bioavailability, artelinic acid peak concentrations in plasma are higher for all routes of administration when compared with artemisinin (Table 5). This might suggest a large difference in distribution volume. These observations are in accordance with the aforementioned difference in efficacy. In addition, the rapid decomposition by aqueous mineral acids, as reported for artelinic acid and arteether (both ether derivatives of artemisinin) by Idowu et al (1989a, b), may be of significance during its residence in the stomach. Further evidence was found in the stability study with 0·1 M HCl at 37°C. As shown in Fig. 2, only a minor amount (about 6%) is still present unchanged after 1 h in the acidic environment. The mechanisms which, despite the observed short half-life and poor oral bioavailability, lead to the remarkable efficacy are, for the most part, not yet understood. Biotransformation of artelinic acid into active metabolites, or a very rapid action directly after the absorption in the portal vein, might both be factors.

Although, with respect to artemisinin and its derivatives, no detailed studies on the relation between the antiplasmoidal activity and the concentration time profile exist, it is generally accepted that a constant and sufficiently high concentration of chemotherapeutic drugs is needed to cure infective diseases and to prevent drug resistance. Controlled-release formulations extending the half-life of artelinic acid in blood plasma might be of great value in therapy. To obviate the decomposition of artelinic acid in an acidic environment, oral formulations might be improved using enteric coatings.

It may be concluded that artelinic acid is rapidly absorbed, distributed and eliminated after intravenous and rectal administration. The release rate from the intramuscular depot is variable, showing a considerable fraction of the drug not absorbed after 2 h. The site of absorption in the rectum appears to affect the bioavailability after rectal administration, indicating a potential value for alternative rectal formulations with high availability.

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