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The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to volunteers

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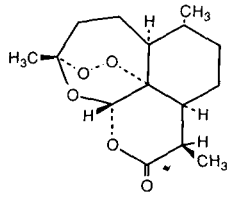
Abstract—The pharmacokinetics after oral, intramuscular and rectal administration of artemisinin, a new potent antimalarial drug, to healthy volunteers has been examined. The study was set-up as a four-way cross-over design with a wash-out period of one week between the test days. In ten volunteers artemisinin concentrations in serum were monitored using a reversed phase HPLC assay with UV detection after derivatization. After oral administration, artemisinin was rapidly but incompletely absorbed, the mean absorption time was 0·78 h and the bioavailability relative to the intramuscularly injected suspension in oil 32%. The mean residence time of the latter (10·6 h) was 3 times that of the oral formulation (3·4 h). This seems to enable a twice daily dosage regimen for the intramuscular oil injection, while the oral formulation necessitates a more frequent dosing interval. After intramuscular injection and rectal administration of an aqueous suspension, very low and variable artemisinin concentrations in serum were observed, probably indicating a poor and erratic absorption.

Artemisinin, a sesquiterpene lactone containing a peroxide bridge, is a leading compound of a new class of antimalarials. Since 1972, when it was isolated from the herb *Artemisia annua* by Chinese scientists, interest in this potent antimalarial has increased (Bruce-Chwatt 1982; Klayman 1985).



Artemisinin is a fast acting blood schizonticide with a minimum inhibitory concentration of 10^{-7} M. Parasite clearance is faster than with any other antimalarial drug (Qinghaosu antimalaria coordinating research group 1979). Although more than 2000 patients have been successfully treated with the drug (Qinghaosu antimalarial coordinating research group 1979; Jing-Bo Jiang et al 1982; Guoqiao L. et al 1982; Li Guoqiao et al 1985), reliable pharmacokinetic data in man are not available. A few pharmacokinetic studies in animals have been carried out (Niu Xinyi et al 1985; Zhao et al 1986) and Zhao (1987) described the application of an HPLC assay to human plasma and saliva samples. Although no pharmacokinetic evaluation was provided in that paper, artemisinin concentration time profiles after administration of a capsule and a suppository to one volunteer were published. A detailed review has been submitted for publication (Titulaer et al unpublished).

As artemisinin is a potent drug for the treatment of cerebral malaria, intramuscular and rectal routes of administration could be used in severe cases where patients are unable to take oral antimalaria drugs.

This study was designed to gain more insight into the pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to healthy volunteers, in order to rationally develop artemisinin formulations that can be used in the treatment of infections of *Plasmodium falciparum*.

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Materials and methods

Volunteers. Ten healthy male volunteers (21–30 years, mean ± s.d. 25 ± 2·9; 65–83·5 kg, mean ± s.d. 75·1 ± 7·1), not using any form of medication, were recruited by advertisement after approval of the study protocol by the medical ethics committee of the academic hospital of the University of Amsterdam. All volunteers gave their informed written consent and participated after a medical examination by an independent physician. During and at the end of the study the volunteers were asked to comment on the acceptability of the injections and possible side-effects.

Materials. Artemisinin was supplied by ACF-Chemie B.V., Maarssen, The Netherlands. Identity and purity were secured by a number of analytical methods.

The aqueous artemisinin suspension was prepared in a vehicle of 0·5% hydroxypropylmethylcellulose (400 mPa.s) and 0·9% NaCl for isotonicity. The pH of the vehicle was neutral. The vehicle was sterilized by autoclaving for 15 min at 121 °C. Under aseptic conditions 400 mg of ground artemisinin was put into sterile glass containers and sterilized by dry heating at 130 °C for 3 h. In preliminary experiments the stability of artemisinin at various temperatures and times was studied by differential scanning calorimetry. After sterilization, a representative set of samples was analysed by HPLC to confirm the artemisinin content and absence of decomposition products. The oily vehicle consisted of olive oil and was sterilized by dry heat for 2 h at 160 °C. Before administration, 4·0 mL vehicle was added to the artemisinin powder and the mixture was suspended by sonification.

All reagents and solvents used for the artemisinin assay were of analytical grade.

Methods. Study design. The ten volunteers received artemisinin as an aqueous suspension by the oral, intramuscular and rectal routes, and intramuscularly as a suspension in oil in a randomized four-way cross-over design. To study the effects caused by the route of administration rather than by formulation differences, the same aqueous suspension was used for the oral, rectal and intramuscular routes. For each administration the dose was 400 mg artemisinin. Between each administration a wash out period of one week was maintained.

The oral aqueous suspension was administered with 100 mL of water after over-night fasting. A breakfast, consisting of 2 or 3 sandwiches, milk, tea or coffee, was taken 45 min after administration.

The rectal aqueous suspension was supplied as a micro-clysma using a syringe with rectal cannula, while the volunteer lay on his stomach. This position was kept for 10 min after administration.

The intramuscular suspensions were given in the upper outer quadrant of the buttock, the gluteus maximus, using 18 G, 1·5 inch needles (Terumo Europe NV, Leuven, Belgium). Care was

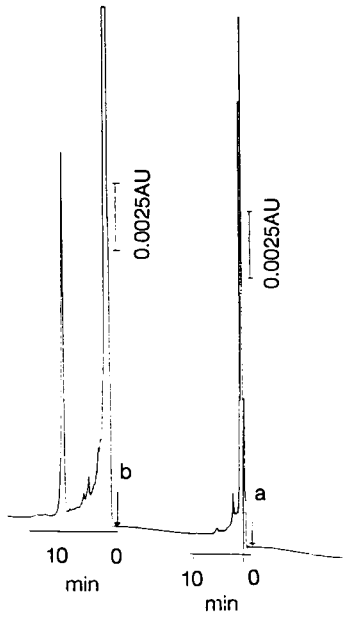

FIG. 1. Chromatograms of artemisinin analysis. (a) Blank serum. (b) Spiked serum standard, 1 μg mL^{-1}. Details of the HPLC assay are given in the text.

taken to place the injection actually intramuscularly, as important pharmacokinetic differences resulting from intra-adipose instead of intramuscular injection have been reported (Zuidema et al 1988).

Blood samples were collected by means of a cannula during the first 7 h, the other samples were obtained by venipuncture in the vein of the forearm. Blood samples were collected before treatment and at t = 15, 30 and 45 min and 1, 2, 3, 5, 7 h (all administrations). Additional samples were collected at 12 and 24 h (rectal administration), or 12, 24, 36 and 48 h (i.m. suspension in oil). After a period of at least 1 h, the samples were centrifuged and the serum separated and collected. The serum samples were stored at −20 °C before analysis.

HPLC assay. Artemisinin concentrations were measured using a reversed phase HPLC method with UV detection after derivitization, as described by Zhao Sishan (1987) with a modification in the extraction procedure. The HPLC system consisted of a Wisp 710B autosampler, a Model 6000A pump (both from Waters Ass., Milford, MA, USA), a Model 783A UV detector (Applied BioSystems Inc., Ramsey, NJ, USA), a model BD41 flatbed recorder (Kip & Sons, Delft, The Netherlands) and a Model SP4100 integrator (Spectra-Physics Inc., San José, CA, USA). The 10 cm Lichrosorb C18 5 μm column (Chrompack B. V., Middelburg, The Netherlands) was eluted with 55% 0·01 M phosphate buffer, adjusted to pH 4·5 with 85% orthophosphoric acid, and 45% methanol at a flowrate of 0·8 mL min^{-1}.

A volume of 1·0 mL serum, or spiked serum as standards, was pipetted into a clean reagent tube. After addition of 4·0 mL n-chlorobutane and vortexing for 30 s, the phases were separated by centrifugation at 5000 rev min^{-1} for 15 min. The organic phase was transferred to a clean glass conical tube by freezing the aqueous layer with an acetone–dry ice mixture and decanting the chlorobutane. After evaporation by means of a gentle stream of nitrogen at 40°C, the residue was redissolved in 100 μL methanol. After addition of 400 μL of 0·2% NaOH solution and mixing, the stoppered tubes were placed in a waterbath at 45°C for 30 min. After quick cooling the samples were acidified by adding 50 μL 0·1 M acetic acid in methanol. The injection volume was 200 μL. Artemisinin concentrations were calculated by measuring peak areas and applying linear regression to a calibration curve of at least 6 points. Each run was checked with additional spiked samples unknown to the analyst.

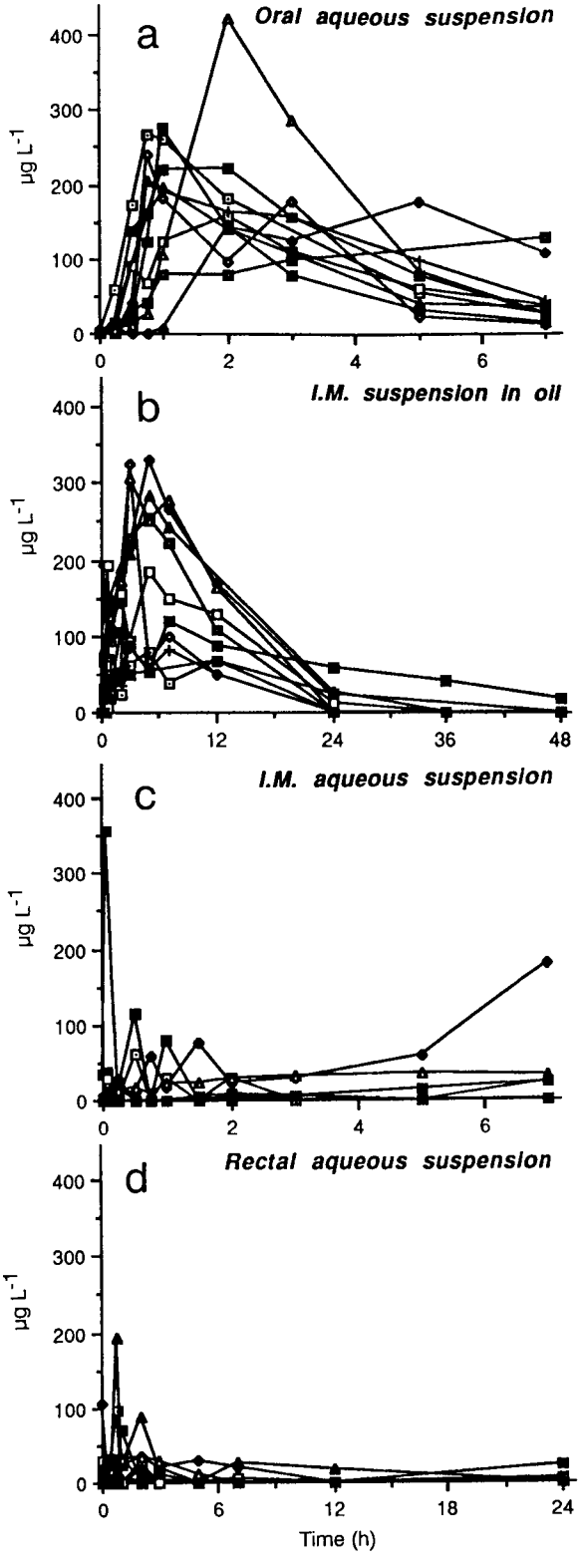

FIG. 2. Plots of artemisinin concentrations in serum versus time (n = 10) after administration of 400 mg artemisinin: (a) oral administration, (b) i.m. suspension in oil, (c) i.m. aqueous suspension, (d) rectal administration.

Table 1. Pharmacokinetic parameters after oral and i.m. administration (400 mg artemisinin).

	C_{max}	t_{max}	AUC	MAT	t^{1}_{2abs}	t^{1}_{2el}	k_{el}	MRT
	$\mu g\ L^{-1}$	h	$\mu g\ h\ L^{-1}$	h	h	h	h^{-1}	h
Oral								
Min	159	0·75	574	0·49	0·14	1·0	0·24	2·6
Max	440	2	1018	1·79	0·93	2·9	0·67	3·9
Mean	260	1	819	0·78	0·54	1·9	0·41	3·4
s.d.	94	0·5	190	0·41	0·29	0·6	0·14	0·7
n	10	10	8	8	8	8	8	8
I.m.								
Min	91	0·75	993	1·10	0·76	4·16	0·07	7·2
Max	331	7	3945	3·56	2·47	15·96	0·17	25·5
Mean	209	3·4	2419	2·30	1·59	7·44	0·11	10·6
s.d.	97	2·0	1055	0·20	0·51	3·83	0·04	5·8
n	10	10	10	8	8	8	8	8

The modified extraction procedure resulted in a cleaner sample. A representative chromatogram is shown in Fig. 1. Although the recovery was lowered by the modified procedure (71% at c = 1·1 $\mu g\ mL^{-1}$), the precision was improved (CV = 3·5% at c = 1.1 $\mu g\ mL^{-1}$ and 4·9% at c = 0·13 $\mu g\ mL^{-1}$, n = 6). The limit of detection was comparable to the method of Zhao (1987) (2·5 ng mL^{-1}).

Pharmacokinetic analysis. The elimination rate constant k_{el} was determined where possible by a half logarithmic regression analysis of the terminal linear phase of the curve.

The absorption rate constant was calculated by the subtraction method. Half-lives were calculated from the rate constants according to the relation $t^{1}_{2} = 0{\cdot}693^{-1} \times k$. The time at which maximum concentrations occur (t_{max}) was read from the curve. The areas under the concentration time curves (AUC) were calculated using the linear trapezoidal rule and extrapolation to infinity. Relative bioavailability was calculated by comparison of the areas under the curve.

The area under the moment curve (AUMC), mean residence time (MRT), mean absorption time (MAT) and mean elimination time (MET) were calculated using the methods of the statistical moment analysis.

The drug concentration time curves are presented individually in order to avoid false interpretation or obscuring individual characteristics (Zuidema & Wynne 1989).

Results

Oral administration. The concentration time curves after oral administration are shown in Fig. 2a. Two curves could not be extrapolated to infinity and have been deleted for the calculation of the related pharmacokinetic parameters.

The estimated pharmacokinetic parameters are summarized in Table 1. From the semi-logarithmic representation of most of the curves a linear kinetic behaviour appeared within the concentration range studied. Artemisinin is quickly absorbed after oral administration as can be derived from the mean MAT of 0·78 h and the mean absorption half-life of 0·54 h. The relative bioavailability compared with the intramuscularly administered suspension in oil was 32·2 ± 7·5% (n = 8).

Intramuscular suspension in oil. After i.m. administration of the suspension in oil, the concentration time curves are less consistent than after the oral administration. Some curves show rather low peak concentration but last longer, while others exhibit higher peak concentrations and a rather short duration, but intermediate profiles do occur also. In Fig. 2b the individual curves are shown.

The estimated pharmacokinetic parameters are summarized in Table 1. None of the volunteers experienced pain on injection or reported local side effects.

Intramuscular and rectal aqueous suspension. As can be derived from Fig. 2c, d artemisinin is poorly and erratically absorbed after intramuscular or rectal administration of the aqueous suspension. Compared with the oral administration and the i.m. suspension in oil the artemisinin concentrations in serum are low and variable.

Discussion

In this study a rapid but incomplete absorption of artemisinin after oral administration has been observed. The bioavailability, relative to the intramuscular administration of 32% is in accordance with the observed absolute bioavailability of 37% in rats (Niu Xinyi et al 1985).

This low bioavailability could also be derived from former pharmacokinetic experiments in laboratory animals. Experiments in dogs revealed that after oral administration of 10 mg kg^{-1} artemisinin concentrations were too low to be determined with a RIA assay, but i.m. administration of an oil suspension (10 mg kg^{-1}) showed peak concentrations of about 200 ng mL^{-1} (Zhao et al 1986). Pharmacokinetic studies in rats, described by Niu Xinyi et al (1985) showed that artemisinin concentrations in blood after oral administration of doses much higher than those used in this study (300 mg kg^{-1} vs 5 mg kg^{-1}), were too low to measure, but artemisinin concentrations were detectable in serum after administration of 900 mg kg^{-1} orally.

Artemisinin appeared to be stable in in-vitro stomach and ileum preparations, but a high metabolic clearance was demonstrated by in-vitro experiments with rat liver slices (Niu Xinyi et al 1985). Thus the incomplete absorption can at least partially be ascribed to a high first pass clearance in the liver.

It is generally accepted that a constant and sufficiently high concentration of an antibiotic or chemotherapeutic drug is needed to cure infective diseases and to prevent drug resistance. With artemisinin however, no detailed studies on the relation between the antiplasmodial activity and the concentration profile in blood exist. The short MRT after oral administration probably necessitates a frequent dosing regimen.

The MRT of the oily intramuscular formulation is longer and seems to allow a twice daily dose regimen. In the literature a 100% bioavailability for the intramuscular oily injection in rats has been mentioned (Niu Xinyi et al 1985).

After intramuscular administration of the suspension in oil, the elimination half-life is about 3·5 times that after oral administration, and although the sampling scheme provided no samples after 7 h after the oral administration, extrapolation indicates that artemisinin concentrations in serum are detectable much longer after intramuscular administration than after oral administration. This apparent discrepancy in elimination half-lives is explained by the phenomenon that at low absorption rates and rapid elimination rates the absorption rate interferes with the apparent elimination rate. The relatively large variability in peak concentration and duration is seen more often with intramuscular injections. The shape (area) of the i.m. depot and/or the activity of the volunteer (blood-flow through the muscle) may affect the release rate.

The bioavailability of the aqueous intramuscular formulation seems to be much lower than the oily intramuscular formulation. A value of 49·7% is mentioned in rat studies (Niu Xinyi et al 1985). However, the observation time is not long enough to measure the release reliably. An explanation might be that the aqueous suspension behaves like an intramuscular depot with very slow release characteristics.

Factors affecting drug release from intramuscularly administered suspensions include solubility, presence of solvent and aggregation status. Artemisinin is poorly soluble in oil as well as in water. At the site of injection, the aqueous solvent is more rapidly cleared by spreading and distribution than oily solvents, which may cause aggregation of artemisinin particles and subsequent lowering of the release rate. Such a slow release is probably not clinically useful.

A similar phenomenon is probably the cause of the poor absorption of artemisinin from the rectal aqueous suspension. In this case the depot is removed daily by defaecation, therefore another approach for a rapid rectal administration is necessary.

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Influence of the method of Fluosol-DA administration on antipyrine metabolism in the rat

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Abstract—Antipyrine disposition has been determined in the rat following administration of Fluosol-DA by an intravenous infusion without blood removal or a haemodilution procedure, and compared with data from sham haemodiluted rats (blood removed and returned) and control rats which only received antipyrine. Antipyrine total body and renal clearance and the formation clearance of two of its metabolites were affected differently at 48 h by the pretreatments. The haemodilution procedure enhanced, the sham haemodilution reduced, and the intravenous infusion had no effect on the phenobarbitone inducible cytochrome P450 isoenzyme activity.

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Animal studies have shown that Fluosol administration alters the microsomal and non-microsomal mediated metabolism of drugs (Shrewsbury & White 1990a). This was not unexpected as the perfluorochemical (PFC) emulsion particles of Fluosol are taken up by the reticuloendothelial system, and reach a maximum hepatic content in two days (Lutz et al 1982; Lowe & Bollands 1985).

Two methods of administering Fluosol have been predominantly used in animal studies: infusion (administering Fluosol without removing blood); and haemodilution (administering Fluosol while removing blood). This investigation was undertaken to determine if the method used to administer Fluosol affected the microsomal metabolism of antipyrine, which is extensively metabolized by the microsomal mixed function