Klinische en farmacologische studies met artemether voor de behandeling van malaria
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Some pharmacokinetic and -dynamic comparisons of artemisinin derivatives in man
Abstract: Artemisinin, arteether, artesunate, artemether and dihydro-artemisinin were investigated in Caucasian and in Asian subjects primarily with respect to their pharmacokinetic behavior. A rigorously validated reversed-phase HPLC method combined with reductive electrochemical detection was used by us for the determination of these antimalarials in healthy individuals but also in patients with non-severe falciparum malaria. Single dose and multiple dosing regimens were investigated as well as the effects of food intake. All derivatives showed short elimination half-lives apart from arteether with an average $t_{1/2}$ of approximately 24 h. It appeared that absorption, i.m. for arteether, orally for artemether and orally or rectally for artemisinin is incomplete but still results in effective parasite killing concentrations. Effects of food intake are limited. However, rectal absorption is unpredictable. We found no arguments for the believe that the presumably very low bioavailability is caused by a first pass metabolism.

Higher concentrations during co-administration of either quinidine or omeprazole strongly suggest that a drug is metabolized by respectively CYP2D6 or CYP2C19. Such studies with artemether indicated that its metabolism is not subject to the genetic polymorphism for the enzymes CYP2D6 or CYP2C19 to a clinically important degree.

Key words: Artemisinanalog, HPLC, Electrochemical detection, Pharmacokinetics, Drug comparison, Dynamics
INTRODUCTION

Artemisinin is isolated from the herb *Artemisia annua*. For many years *Artemisia* extracts have been used in traditional Chinese Medicine for fever, hemorrhoids and many other diseases. Relatively recently it was found to possess anti-plasmodial activity and appeared to be successful in the treatment of human malaria. Emerging resistance and problematic side-effects of known antimalarial drugs have stimulated an intensive search for new therapies.

Artemisinin has a peculiar three-ring structure with a peroxide bridge in one of the rings. It has been shown that the anti-malarial activity depends on this peroxide configuration. By substitutions at the position where artemisinin has a double-bonded oxygen semi-synthetic derivatives such as artemether, the β-ethyl ether analog arteether and the salt artesunate have been developed, permitting parenteral administration. They now constitute a new class of potent antimalarial drugs. Some of these compounds are already used for many years in areas with endemic malaria, mainly in Asia. Previous studies in China and Vietnam have shown that artemisinin, which is widely used in China, quickly reduces parasitemia in acute falciparum malaria (Klayman, 1985; Hien and White, 1993; Sy et al., 1993).

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The drug is metabolized to dihydro-artemisinin, which may also have anti-malarial activity. Dosage regimens have up to the present been largely determined empirically. During a 1992 WHO conference relating to the pharmacological implications of the introduction and use of artemisia products in Vietnam, it was emphasized that efforts should be made to develop robust analytical methods, that monitoring should be made mandatory for pharmacological and idiosyncratic toxicity in ambulatory patients being treated with these analogues and that there was a need for the development and implementation of effective strategies to insure that control measures are used properly in order for these drugs to remain effective. Unfortunately, little pharmacokinetic data are available to aid the development of rational dosage regimens, because studies were hampered by the absence of a reliable technique for measuring the concentration of artemisinin (Zhao, 1987, de Vries and Tran, 1996). The development of selective analytical methods for the determination of artemisinin and its analogues and metabolites in biological fluids poses challenging problems. All are thermally labile, lack UV absorbance or fluorescent chromophores and do not possess functional groups for derivatization.

The aim of one of the studies was to investigate the effects of food intake on the pharmacokinetics of artemisinin. Food intake could, by its a influence on bioavailability, be a major determinant of the duration of action. Apart from an influence on bioavailability, food intake severe falciparum malaria, in order to be able to develop formulations that can be used for treatment in areas with endemic malaria.

For the enzymes CYP2D6 and CYP2C19, approximately 5% of the Caucasian population have a ‘poor metabolizer’ (PM) phenotype. However, these percentages differ for different ethnic groups (Brosen, 1990). We studied the possible inhibition of artemether metabolism by using quinidine (an inhibitor of CYP2D6) and omeprazole (an inhibitor of CYP2C19). Higher artemether concentrations during co-administration of either quinidine or omeprazole will be a very strong indication that artemether is metabolized by either CYP2D6 or CYP2C19.

**MATERIALS AND METHODS**

**DRUG ASSAY**

For the determination of artemisinin and analogs a reversed-phase HPLC method using reductive electrochemical detection was set up as previously published by Melendez et al. (Melendez et al., 1991) with some rather important modifications. Instead of the BAS 200 liquid chromatograph, a Spectroflow 400 liquid chromatograph in combination with a Triathlon autoinjector coupled to an electrochemical detector (Decade) were used. The detector was operated in the reductive mode as a closed system under chromatography grade helium to exclude any access of oxygen. The Decade has a glassy carbon
also could influence the hepatic clearance by a stimulation of liver blood flow (de Vries et al., 1994). Enzymatic metabolism is probably the most important route of elimination for artemisinin and its derivatives. Another factor of great importance is that in areas where these compounds are likely to be used, facilities for intravenous drug administration are often not available; oral artemisinin is, therefore, likely to be administered as soon as possible to malaria patients in such areas.

Artemisinin suppositories have been studied in clinical trials in Vietnam and China. The studies showed that artemisinin suppositories reduce parasitemia as rapidly as intravenous administration of quinine to patients with acute and severe falciparum malaria. Because of the convenience of administration, proven efficacy and the lack of side effects, artemisinin suppositories have advantages for the early treatment of falciparum malaria and are expected to reduce morbidity and maybe also mortality associated with a high parasitemia. However, dose and dosage schedules of artemisinin suppositories have been decided on an arbitrary basis. It was, however, suggested that the absorption of artemisinin from suppositories was very poor and erratic (Zhao, 1987). We, therefore, designed a study to gain more insight into the pharmacokinetics of artemisinin suppositories in patients with non-electrode and a reference Ag/AgCl electrode. Regular electropolishing and wiping of the glassy carbon electrode was performed.

The glass materials that are used are silanised to minimize drug adsorption to glass. All chemicals and solvents used in the assay procedure are of analytical/chromatographic grade. Chromatographic separations were obtained with a Versapack column: CN 10 μm, (300 x 4.6 mm) 5 μm particle size, maintained at 30°C. The mobile phase consists of 60% acetoacetate buffer and 40% acetonitrile. The mobile phase was deoxygenated for at least 24 h before use and as this mobile phase is recirculated, the whole system will be deoxygenated. With minor changes in the mobile phase, the much less polar arteether or the pH sensitive artesunate can be determined in the same chromatogram together with artemisinin and dihydroartemisinin. Depending on the study, artemisinin or arteether were used as an internal standard. The chromatograms were recorded and analyzed with Kontron integrati on software.

Healthy Subjects

The pharmacokinetics of artemisinin were studied in 12 healthy male Vietnamese subjects. All subjects
recruited for the studies published here, healthy subjects as well as patients, had been subject to an informed consent procedure as published before (Duc et al., 1994). The following inclusion criteria were used: age between 19 and 39 years, normal weight and height and normal medical history and physical examination. It is worth mentioning that the mean weight of the Asian subjects we studied was ± 70% of otherwise comparable Caucasian populations.

To investigate the effects of food intake on the pharmacokinetics of artemisinin, 6 healthy male Vietnamese subjects were recruited. Inclusion and exclusion criteria were the same as for the previous study as were the blood sampling and other procedures.

Single and multiple dose regimens of artemether were studied in healthy Caucasian subjects. Seven healthy Caucasian subjects were studied for the possible influence of genetic polymorphism for the enzymes CYP2D6 or CYP2C19 on the metabolism of artemether and dihydro-artemisinin.

PATIENTS

The pharmacokinetics of a single oral dose of 500 mg artemisinin administered as capsules was studied in 8 adult male patients with uncomplicated falciparum malaria. Inclusion criteria encompassed a blood smear positive for *Plasmodium falciparum* and ranging from 1000-50,000/mm³ without mixed infection with other plasmodium species.

intervals. A complete physical examination was repeated 24 h after drug administration.

After dosing, 10 blood samples were taken at 1, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0 and 24 h after each dose. From an indwelling i.v. catheter, 5 ml blood was drawn into vacuum polystyrene tubes (Venoject II, Terumo) containing lithium heparinate. The blood samples were centrifuged, immediately, transferred into polypropylene tubes and stored at -20°C. They were transported to Amsterdam in frozen condition and stored there at -30°C until analysis.

For the food interaction study, 2 capsules of 250 mg artemisinin (ACF Pharmaceuticals) were administered after an overnight fast on two consecutive days, with and without food in a crossover design. The food consisted of a standard Vietnamese breakfast of warm rice soup with some vegetables and meat. Blood sampling and other procedures were the same as for the previous study. However, urine samples were also collected to estimate cumulative drug excretion. Three subjects received artemisinin without food on the first day, the other subjects were studied in the reversed order.

For the study of the pharmacokinetics of a single oral dose of 500 mg artemisinin (2 capsules of 250 mg artemisinin, ACF Pharmaceuticals) in Vietnamese malaria patients, the patients were admitted to the malaria unit of the ICRTM in Hanoi.

The artemisinin used in the single dose suppository study was formulated by Vinapha, Ho Chi Minh City, Vietnam, into suppositories containing 200 mg arte-
To gain more insight into the pharmacokinetics of artemisinin suppositories, a study was carried out in 8 Vietnamese male patients with non-severe falciparum malaria.

Concentrations of artesunate and dihydro-artesamine were followed in 6 Vietnamese patients with non-severe falciparum malaria after a multiple oral dose regimen with artesunate tablets. Inclusion and exclusion criteria, the work-up of the patients, blood sampling and the follow-up of all the Vietnamese malaria patients were the same as for the study with artemisinin capsules.

Multiple dose pharmacokinetics of artemether was investigated in 12 Chinese patients with uncomplicated *P. falciparum* infection.

**Experimental Design and Interventions**

For the single dose kinetic study in healthy Vietnamese subjects a blank blood sample was taken before a single oral dose of 2 capsules of 250 mg artemisinin (ACF Pharmaceuticals, Maarssen, The Netherlands) was administered after an overnight fast. Subjective symptoms and vital signs (blood pressure, pulse rate, respiration rate, temperature) were recorded regularly, starting at hourly intervals. Quantitative analysis of this formulation was performed to ensure compliance with The Netherlands standards for content and purity. All patients received 600 mg artemisinin rectally, i.e., 3 suppositories of 200 mg each. Patients did not defecate within 2 h.

In the multiple dose study with artesunate, patients received, on the first day, 2 oral doses of 150 mg artesunate (Mediplantex, Medicinal Plant Company no. 1, Hanoi) followed by a single 100 mg dose every day for 4 days. After the first dose, 14 blood samples were taken over a 6 day period. All the doses were preferably given on an empty stomach.

Blood sampling and other procedures in the Vietnamese malaria patients were similar to those described for the study with artemisinin capsules in healthy subjects. At the end of the study periods with artemisinin or artesunate, patients received a curative dose of mefloquine (Lariam, Roche) of 15 mg/kg.

In the multiple dose study with artemether, subjects received 80 mg of artemether orally at 0, 8, 24 and 48 h. Over that period, 21 blood samples were taken, i.e., 4–7 data points per dosing interval.

In a phase I study with arteether, multiple doses of 3.2 mg/kg arteether on day 1 and 0.8 mg/kg on days 2–5 (5
subjects) or 3.2 mg/kg on day 1 and 1.6 mg/kg on days 2–5 (5 subjects) were given. All drug administrations were i.m. For this purpose, arteether was formulated as a solution in sesame oil. All procedures have been published previously (Kager et al., 1994).

For the study of artemether dihydroartemisinin metabolism, the subjects received an oral dose of 100 mg artemether (Propharma NV, Geel, Belgium) 1 h after 50 mg quinidine and 1 h after 40 mg omeprazole. After dosing, 11 blood samples were taken.

MEASUREMENTS

Blood examinations in the Vietnamese malaria patients included: haemoglobin, hematocrit, white blood cell count, platelet count, blood urea nitrogen, creatinine, glucose, bilirubin, serum transaminases, alkaline phosphatase and hepatitis B surface antigen. These were performed at t = 0 and repeated at 48 h. Urine was examined for albumin, glucose, and sediment before the start of the study. An ECG was made before the start of the commenced and at 4, 24, 48 h. Subjective symptoms were recorded regularly, starting at hourly intervals. The following vital signs: temperature, blood pressure, pulse rate, respiratory rate and urine production were recorded at t = 0, 1, 2, 4, 8, 12, 24, 36, 48 h after drug administration. Physical examination was repeated twice daily until discharge. Parasite counts were checked twice daily until two negative consecutive examinations were obtained (Giemsa stain, thick film, number of parasites per 400

employed to describe arteether pharmacokinetics. All pharmacokinetic data were fitted to the appropriate equations using a nonlinear regression computer program.

STATISTICS

Pair-wise comparisons were made with the Wilcoxon signed-ranks test for matched pairs. For comparisons of unpaired data the two sample t-test was used. Possible interactions of artemether and dihydro-artemisinin were evaluated with the one-way analysis of variance.

RESULTS AND DISCUSSION

DYNAMICS

Studies in patients with malaria – in which dynamic effects of different artemisinin analogs on cure rates, fever clearance rates, parasite clearance rates or on parameters for toxicity were compared – have so far, although different dosages were used, not revealed any clinically significant differences. Although physicochemical and kinetic distinctions could be of importance also in the studies summarized here, no differences with respect to efficacy or tolerance were observed. However, it should be emphatically stated that these studies were not designed for the detection of such dynamic differences.

DRUG ASSAY
leukocytes). Blood sampling and other procedures were as described for the study with artemisinin capsules in healthy subjects.

**DATA ANALYSIS**

Pharmacokinetic parameters were estimated according to standard methodologies (Gibaldi and Perrier, 1982). The elimination constant $K_{el}$ was mostly calculated by log linear regression of the last 4 concentration time points. The area under the curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity. Clearance/F (Cl/F) was then calculated as dose/AUC. Distribution volume/F (Vd/F) was calculated as CL/F/$K_{el}$. A simple deconvolution approach was used to calculate absorption rates between successive plasma concentrations and the cumulative amount of drug absorbed. The concentration time curves of artemisinin, dihydro-artemisinin and artemether were also fitted to a one compartment model with a lagtime, in which $K_{el}$ was entered as a constant. For the absorption phase, either a zero order model or a first order absorption model was used. A tri-exponential equation describing a two-compartment open model with first order absorption was

For the study of possible pharmacokinetic dissimilarities, our internal standard assays for the various artemisinin analogs went through a within laboratory validation procedure for concentrations between 15–250 ng/ml which showed an intra-assay variability between 7–9.5%. Sensitivities in the 5–10 ng/ml range were achieved. Our assay was introduced on a routine basis after 4 cross checks with artemether, 2 with 10 spiked samples and 2 with 10 samples after in vivo drug administration, with 2 other laboratories. In these cross checks, evaluations of the dihydro-artemisinin determinations were also incorporated. Concordance between the results for concentrations above the detection limits of our laboratory and the other laboratory that used the same equipment and procedures was displayed by correlation coefficients between 0.983–0.998.

**SINGLE ORAL DOSE OF ARTEMISININ IN HEALTHY VIETNAMESE SUBJECTS**

In this study, the concentration time curves were fitted to a one compartment first order elimination model with a lagtime. Pharmacokinetic calculations gave the following results: a mean (±SD) Vd/F of 19.4 ± 6.9 l/kg, a mean
absorption half-life of 0.58 ± 0.54 h with a mean (± SD) calculated maximum concentration of 391 ± 147 ng/ml, occurring at 1.81 ± 0.73 h after drug intake. Elimination was rapid with a mean (± SD) half-life of 2.59 ± 0.55 h. No information about the actual Vd or Cl was obtained because of the uncertainty with respect to the probably very low bioavailability and the lack of an i.v. formulation of artemisinin. It was concluded that absorption of orally administered artemisinin is incomplete, although a first-pass effect could not be excluded. However, the rather uniform elimination could be an argument against such first-pass metabolism. In any case, effective parasite killing concentrations were obtained with this dose in healthy subjects.

**FOOD INTERACTION STUDY IN HEALTHY VIETNAMESE SUBJECTS**

The mean (± SD) amount of unchanged artemisinin excreted in urine was very low: 0.071 ± 0.053 mg with food and 0.033 ± 0.023 mg without food. No statistically significant differences were observed in the parameters of the two experimental conditions. There was, however, a rather wide interindividual difference in the influence of food on the pharmacokinetic parameters. Because we did not find differences in the pharmacokinetics of artemisinin after food (which can be expected to influence liver blood flow), while on the basis of its low urinary excretion it seems very likely that artemisinin is eliminated by liver metabolism, liver blood flow is probably an elimination half-life of 3.1 ± 2.1 h. The deconvolution method revealed that two different absorption profiles could exist: one pattern was apparently first order, and the other zero order. Most curves showed a lagtime. The large Vd/F ratio is in agreement with a very low value for F, i.e. a very low bioavailability. In this study, it was shown that absorption of artemisinin from suppositories was very poor and erratic. However, despite a low bioavailability the time for 90% parasite clearance to occur was 36 h, also fever clearance time was 36 h and with these 600 mg artemisinin suppositories plasma concentrations remained above the in vitro MIC of *P. falciparum* for 12 h. These results suggest that oral artemisinin is dosed too high.

**MULTIPLE ORAL DOSE STUDY WITH ARTUSENATE IN VIETNAMESE MALARIA PATIENTS**

After oral administration of artusenate, only dihydroartemisinin was detected in plasma. These results confirm the findings published by others (Benakis et al., 1993; Batty et al., 1996).

**MULTIPLE ORAL DOSE STUDY WITH ARTEMETHER IN CHINESE MALARIA PATIENTS**

The data were fitted to an open one-compartment multiple dose model. In general, good fits and correlation coefficients (mean $r = 0.94$, SD = 0.003) were obtained for the fitting procedure indicating that the model was
not a rate-limiting factor (de Vries et al., 1994). Artemisinin is, therefore, probably a so-called low-clearance drug.

**Single Oral Dose of Artemisinin in Vietnamese Malaria Patients**

Also in patients with non-sever falciparum malaria, effective parasite killing concentrations were reached after a single oral dose of artemisinin. The pharmacokinetic parameters after single oral doses of artemisinin as found in these malaria patients without concomitant disease showed no substantial differences from those found in healthy Vietnamese subjects. Dihydroartemisinin could be demonstrated in these malaria patients but the levels were generally lower than those of artemisinin itself.

**Single Dose Artemisinin Suppository Study in Vietnamese Malaria Patients**

In this study, the following kinetic parameter estimates (mean ± SD) were found: \( V_{d/f} \) 80.8 ± 61.9 l/kg, absorption time for, respectively, 50% and 90% of the dose 3.4 ± 1.8 h and 6.5 ± 3.0 h, calculated maximum concentration 105.3 ± 60.1 ng/ml occurring at 7.2 ± 3.9 h, and acceptable for the estimation of PK-parameters. \( C_{max} \) (mean, ± SD) for the first dose was 169 ± 104 ng/ml. For \( t_{1/2} \) alpha we found (mean, ± SD) 1.28 ± 0.55 h. The PK-parameter estimates for the active metabolite dihydroartemisinin were similar as those for artemether. It was concluded that artemether reaches relatively high peak-levels in a short time and that also this analog is rapidly eliminated. The parameters \( V_{d/f} \), AUC and \( C_{max} \) of artemether showed remarkable inter- but also intra-subject variability. We feel that the most reasonable explanation for this variability is variation of the amount of artemether that is absorbed from different tablets.

**Phase I Study with Arteether in Healthy Caucasian Subjects**

For calculation of the pharmacokinetic parameters concentrations were fitted to an open-two compartment multiple dose model without a lagtime. The kinetic parameters (mean, ± SD) found for the two multiple dose regimens were: \( t_{1/2} \) alpha 3.40 ± 4.60 h, \( t_{1/2} \) beta 21.33 ± 9.34 h, \( V_{d/f} \) 1560.1 ± 1100.4 l and \( C_{l/f} \) 52.6 ± 43.6 l/h. The distribution and elimination rates as found by us corresponded with those published for the dog (Benakis et al.,
1991). Of course the most striking observation with respect to the kinetic behavior of artemether is the very long elimination half-life which is at least 10-fold longer than those of artemisinin and artemether. This could be explained by the fact that artemether is considerably less polar than the two other compounds. An important argument in support of the conclusion that indeed we are dealing with very slow elimination is the fact that we saw that during the 3.2, 1.6, 1.6, 1.6 mg/kg dose regimen, artemether accumulated and that a good description of this accumulation curve was obtained with an open-two compartment multiple dose model.

**STUDY OF ARTEMETHER AND DIHYDRO-ARTEMISININ METABOLISM IN HEALTHY CAUCASIAN SUBJECTS**

Artemether and dihydro-artemisinin concentrations were fitted to a open-one compartment model. The following parameters were calculated: AUC, clearance, Vd and half-life. No significant differences for these parameters were found after administration of quinidine or omeprazole. These results make it unlikely that artemether metabolism or the metabolism of dihydro-artemisin is subject to a genetic polymorphism for the enzymes CYP2D6 or CYP2C19 to a clinically important degree and that inter-ethnic differences could be expected on the basis of the polymorphism of these enzymes.


REFERENCES


