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In vivo evaluation of arteether liposomes

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Abstract

Arteether is a potent antimalarial agent that is available as oily solution intended for intramuscular injection. Liposomal formulation composed of dipalmitoylphosphatidylcholine (DPPC), dibehynoyl-phosphatidylcholine (DBPC), cholesterol and arteether in the molar ratio of 1:1:2:1 was chosen for in vivo evaluation. This composition was found to give stable liposomes compared with other formulations and it gave 67.56% trapping efficiency and particle size of 3.21 ± 0.76 μm. The liposomes were administered orally and intravenously to New Zealand rabbits at a dose of 50 mg/kg. The pharmacokinetic parameters following drug administration were determined in each case. Pharmacokinetic parameters after oral administration of liposomes were compared with those of oral aqueous suspension of micronized arteether. High bioavailability of arteether was evident in case of oral liposomes where faster rate and better absorption of arteether were observed compared with aqueous suspension. Oral liposomes gave higher C_{max} and shorter T_{max} as well as a higher value for AUC. Almost complete arteether absorption was observed for oral liposomes where relative bioavailability was 97.91% compared with 31.83% for the oral suspension. Intersubject variations were found to be relatively high in oral liposomes. The obtained values for mean residence time (MRT) and mean absorption time (MAT) indicated that arteether remains longer in gastrointestinal tract (GIT) with longer time period for absorption in case of suspension compared with liposomal formulation. In addition, arteether was successfully administered intravenously in liposomal formulations and showed longer elimination half-life with respect to other artemisinin derivatives. Thus an optimum oral liposomal formulation for arteether can be developed for fast and complete absorption of the drug from GIT. Furthermore, liposomal formulation of arteether could allow for intravenous administration of the drug in high-risk malaria patients with long duration of effect.

Keywords: Liposomes; Arteether; Oral administration; Intravenous; Pharmacokinetic

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1. Introduction

Arteether was introduced as a semisynthetic highly potent antimalarial agent (Brossi et al., 1988). Arteether is effective against the erythrocytic stage of chloroquine- and mefloquine-resistant strains of *Plasmodium falciparum* (Luo and Shen, 1987; Zeman and Sharma, 1991). The drug has been selected for clinical evaluation in high-risk patients particularly in case of cerebral malaria. Most frequently, arteether is given as an oily solution (sesame or other vegetable oils) intended for intramuscular injection. Arteether is a water insoluble drug (17 μg/ml at room temperature) and not suitable so far for intravenous or oral administration. Oral administration of arteether is expected to show low bioavailability due to slow drug dissolution as well as drug decomposition in stomach acid (Baker et al., 1993). The use of dissolving agents such as polyoxyethylated castor oil (Cremophore EL) in formulating poorly water soluble drugs for intravenous administration was reported to cause unacceptable side-effects (Carank and Sucker, 1986; Luke et al., 1987). A lipophilic carrier for intravenous and oral administration of such drugs was not always proven to have in vitro and in vivo stability.

Arteether has been formulated successfully in the form of liposomes in our laboratory and the produced liposomes were characterized using different types of phospholipids (Al-Angary et al., 1996). It was concluded that using phospholipids of long acyl chain length in presence of cholesterol can increase the trapping efficiency with a decrease in arteether release rate indicating stable liposomes.

In this study, a selected liposomal formulation with good properties was chosen to be given intravenously and orally to rabbits. The pharmacokinetic parameters of arteether obtained following oral and intravenous administration of the liposomal formulation as well as an oral administration of aqueous suspension were evaluated.

2. Materials and methods

2.1. Materials

L-α-Dipalmitoylphosphatidylcholine (DPPC), L-α-dibehynoyl- phosphatidylcholine (DBPC) and cholesterol (Chol.) were purchased from Sigma (St. Louis, MO). Arteether was synthesized from artemisinin, the active ingredient isolated from the herb *Artemisia annua* L. using a literature procedure. The purity of the prepared arteether was tested by thin layer chromatography (TLC) and differential scanning calorimetry (DSC). All solvents used for chromatographic determination of arteether in plasma were HPLC grade. All other reagents and solvents were of analytical grade.

2.2. Preparation of arteether liposomes

Multilamellar liposomal vesicles (MLVs) were prepared following the film method. The required amounts of phospholipids mixture and cholesterol were weighed into a 100-ml glass flask and dissolved in the smallest possible volume of chloroform. The organic solvent was slowly evaporated at reduced pressure and temperature of 40°C until a thin film of drug lipis was deposited on the inner wall of the flask. The dried film was then hydrated in 0.9% sodium chloride (USP) by swirling at 50°C until all the lipid was dispersed. The dispersion was then vortexed for 1 min to produce multilamellar vesicles. The method of preparation as well as the in vitro evaluation of the produced liposomes were described with details in a previous study (Al-Angary et al., 1996). The liposomal formulation chosen for the in vivo study consisted of DPPC, DPBC, Chol. and arteether in the molar ratios of 1:1:2:1, respectively. This composition was found to give stable liposomes compared to other formulations with good trapping efficiency and suitable particle size for intravenous and oral administration to rabbits.

2.3. Animal experiments

A total of 16 adult male New Zealand rabbits weighing 3.38 ± 0.39 kg were used in this study. The rabbits were fasted with water allowed, for 36

h prior to initiation of the study. A total of six rabbits were administered the liposomal preparation containing 50 mg/kg of arteether directly into the stomach by oral intubation. Each of six other rabbits was orally administered an aqueous suspension of finely powdered arteether in the same dose (50 mg/kg). The aqueous suspension of finely powdered arteether was prepared by grinding arteether crystals using pestle and mortar into fine powder that passed through sieve with opening size of 63 μm (average particle size was 49.6 ± 13.59 μm as determined by light microscopy). Each dose was dispersed in 10 ml of distilled water by vortexing for 3 min and the dose was immediately administered to the corresponding animal. The remaining four rabbits each received an intravenous injection of the liposomal formulation containing arteether in a dose of 50 mg/kg. Blood samples were collected from the marginal ear vein, prior to and at selected time interval after drug administration using an implanted cannula into heparinized tubes. The samples were immediately centrifuged at 4000 rpm for 7 min and the plasma were separated and frozen at -20°C pending analysis. The research adhered to the principles of laboratory animal care that is followed by the laboratory animal care research center in King Saud University, Riyadh, Saudi Arabia.

2.4. Drug analysis

An aliquot of 0.5 ml of each plasma sample was treated exactly as described for arteether HPLC assay that was developed in our laboratory (Al-Angary et al., 1994). The concentration of arteether in each sample was determined using a constructed calibration curve.

2.5. Data analysis

The pharmacokinetic parameters for arteether after oral and intravenous administration were determined from the obtained plasma concentration-time data. Maximum plasma concentration (C_{max}) and the time to reach this maximum (T_{max}) were determined after oral administration of both liposomal and aqueous suspension formulations.

The total area under the curve (AUC) and the total area under the first moment curve (AUMC) up to last time point were estimated by the trapezoidal rule. The area of the tail was calculated using the plasma concentration at the last time point and the terminal elimination rate constant (K_{el}). The values of K_{el} and accordingly $t_{1/2}\beta$ were estimated from the least square regression analysis of the final segment of the curve.

The mean residence time (MRT) values were calculated as the ratio of $AUMC_{0-\infty}$ to $AUC_{0-\infty}$ in each case and the mean absorption time (MAT) as the difference between mean residence time of oral liposomes (MRT_{OL}) or oral suspension (MRT_{OS}) and the mean residence time of intravenous liposomes (MRT_{IV}) for arteether. The rate of absorption was also calculated as $C_{max}/AUC_{0-\infty}$.

Analysis of variance (ANOVA) was used to evaluate statistically significant differences in plasma arteether level at each sampling time. The values of C_{max}, and T_{max} as well as AUC were also subjected to ANOVA at a significant level ($p \leq 0.05$). Variations between rabbits in the pharmacokinetic parameters following administration of each dosage form were expressed by the coefficient of variation (CV, %).

3. Results and discussion

Based on the previous in vitro studies (Al-Angary et al., 1996), liposomes formulated with saturated long acyl chain phospholipids in the presence of cholesterol were chosen for in vivo studies. The chosen liposomal formulation consisted of DPPC:DBPC:Chol.:arteether in 1:1:2:1 molar ratios. The above formulation was characterized by high stability with small particle size and low particle size distribution (3.21 ± 0.76 μm) compared with other formulations. The liposomes also showed a good trapping efficiency (67.56%). Light microscopy of used samples revealed that liposomes were spherical, homogeneous in size and free from aggregates. Liposomes pellets were reconstituted just before administration to animals to ensure complete drug association with liposomes.

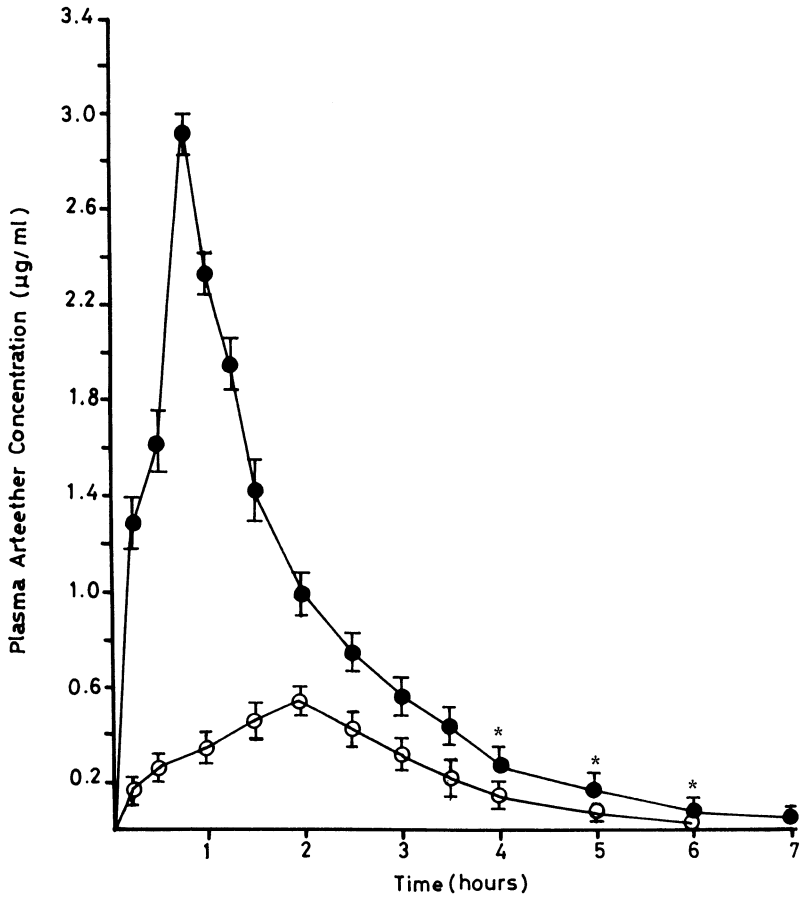

Fig. 1. Mean plasma concentration versus time profile of arteether (50 mg/kg) following oral administration of arteether liposomes ($n = 6$) (●) and aqueous suspension of finely powdered arteether ($n = 6$) (○) to rabbits. * Non-significant differences ($p > 0.05$) in plasma arteether concentration for two dosage forms at a specific time.

Fig. 1 shows the mean plasma concentration versus time profile of arteether following oral administration of arteether liposomes and aqueous suspension of arteether at a dose of 50 mg/kg to rabbits in each case. The determined pharmacokinetic parameters for arteether are shown in Table 1, following administration of the dosage forms. Oral liposomal administration gave shorter T_{max} and a much higher C_{max} compared with the aqueous suspension of arteether. The values of T_{max} and C_{max} were 47.5 ± 6.12 min and 3.03 ± 0.46 μg/ml, respectively for liposomes, and 117 ± 6.71 min and 0.552 ± 0.041 μg/ml, respectively, for the aqueous suspension. The above data show a highly significant faster absorption of arteether from the liposomal formulation compared with the aqueous suspension. This was also supported by calculating the absorption rate of arteether defined as $C_{max}/AUC_{0-\infty}$,, which was 0.635 ± 0.066 h^{-1} and 0.356 ± 0.043 h^{-1} for arteether in liposomes and aqueous suspension, respectively. Between rabbits variations of the two dosage forms were described by the coefficient of variance (CV%) of the pharmacokinetic parameters (Table 1). The CV% of the aqueous suspension for C_{max}, T_{max} and $AUC_{0-\infty}$ were markedly lower than those of liposomes. This could be attributed to the dependence of drug absorption, in case of aqueous suspension, on the constant low rate of drug dissolution with little differences between animals. On the other hand, absorption of arteether from liposomal formulation is con-

Table 1
Pharmacokinetic parameters for arteether after administration of three dosage forms: oral aqueous suspension (OS), oral liposomes (OL) and intravenous liposomes (IVL)

Parameter	OS	OL	IVL	CV%		
				OS	OL	IVL
C_{max} (μg/ml)	0.552 ± 0.041	3.03 ± 0.47	–	7.36	15.34	–
T_{max} (min)	117.0 ± 6.71	47.5 ± 6.12	–	5.73	12.89	–
$AUC_{o-\infty}$ (μg·h/l)	1.5449 ± 0.127	4.768 ± 0.507*	4.8699 ± 0.0722*	8.54	10.74	1.48
$t_{1/2}\beta$ (h)	1.143 ± 0.128*	1.083 ± 0.131*	1.11 ± 0.049*	11.20	12.10	4.41
MRT (h)	2.533 ± 0.069	1.9113 ± 0.096*	1.7571 ± 0.064*	2.76	5.02	3.64
MAT (h)	0.7766	0.1542	–	–	–	–
$C_{max}/AUC_{0-\infty}$ (h^{-1})	0.3677 ± 0.041	0.635 ± 0.067	–	11.03	10.58	–

* Treatments are statistically non-significant from each other ($p > 0.05$).

trolled by independent factors such as drug dissolution from liposomes and intrinsic absorption of free and/or encapsulated drug through the gastrointestinal tract (GIT) wall, and this could be highly different from one rabbit to another.

Generally, oral liposomes showed higher bioavailability where faster rate and higher extent of absorption (higher AUC) were observed compared with aqueous suspension of arteether.

The low bioavailability of arteether from the aqueous suspension could be due to lower dissolution rate of drug particles, while in case of liposomes, dissolution of the drug could be faster in addition to the possibility of fast absorption of phospholipid carrier containing the drug. The absolute bioavailability which is defined as AUC_{oral}/AUC_{IV} was 97.91% for oral liposomes compared with 31.83% for oral suspension. These results show that not only fast absorption of arteether from GIT but also almost complete absorption from the liposomal formulation is evident.

Arteether was also successfully administered intravenously to rabbits in liposomal formulation. The plasma drug concentration curve versus time is shown in Fig. 2. The curve fitted a two compartment open model resembling that of qinghaosu (artimisinin) and artemether obtained after intravenous administration of the drugs in rats and rabbits (China Cooperative Research Group, 1982). Table 1 shows that arteether gave $t_{1/2}\beta$ of 1.14 h as terminal elimination half-life (68.6 min), compared with 30.13 min for artimisinin and 39.6 min for artemether, following intravenous injection in rats and rabbits in the form of emulsion (China Cooperative Research Group, 1982), indicating a longer elimination half-life for arteether in this case. Intravenous administration of the water soluble sodium artesunate was reported to have a very short duration of action and suffered from great instability. The plasma level of sodium artesunate in rats was shown to fit a one compartment open model with $t_{1/2}$ of 15.6 min (China Cooperative Research Group, 1982). It appears that arteether in liposomal formulation could be given intravenously with long duration of action due to high drug and liposomal stability.

MRT and MAT (Cutler, 1978; Yamaoka et al., 1978) were shown to be useful parameters for predicting behavior of drug after oral administration in its dosage form (Kawashima et al., 1993; Aoyagi et al., 1990). The mean residence time after oral administration of arteether suspension MRT_{OS} and liposomes MRT_{OL} to rabbits was found to be 2.534 ± 0.069 and 1.911 ± 0.096 h, respectively. While the mean residence time for intravenous administration MRT_{IV} was 1.757 ± 0.064 h. On the other hand, MAT was 0.776 h for the oral suspension and 0.154 h for oral liposomes. These results show that MRT value for oral suspension is significantly greater than for liposomes ($p < 0.05$) indicating that the drug remains longer in GIT in case of suspension due to slow dissolution. The MAT value of the suspension is about five times greater than of liposomes,

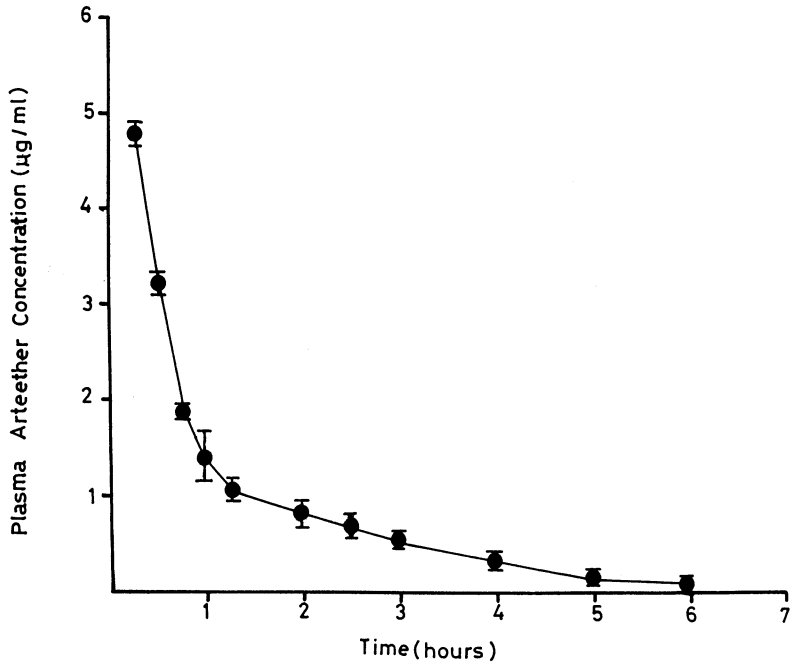

Fig. 2. Mean plasma concentration versus time profile of arteether (50 mg/kg) following intravenous administration of arteether liposomes to rabbits ($n = 4$).

and clearly shows a longer time period for absorption of arteether from the suspension with longer residence time in GIT according to the moment analysis parameters.

In conclusion, arteether liposomes were successfully prepared for oral and intravenous administration. Oral administration of liposomes showed high bioavailability of arteether compared with poor bioavailability of the aqueous suspension as demonstrated by pharmacokinetic parameters. These liposomal formulations can allow the use of the potent antimalarial arteether effectively in case of high-risk malarial patients.

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