J. Pharm. Pharmacol. 1998, 50: 173–182
Received July 29, 1997
Accepted October 16, 1997

The Pharmacokinetics and Bioavailability of Dihydroartemisinin, Arteether, Artemether, Artesunic Acid and Artelinic Acid in Rats

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Abstract

The pharmacokinetics and bioavailability of dihydroartemisinin (DQHS), artemether (AM), arteether (AE), artesunic acid (AS) and artelinic acid (AL) have been investigated in rats after single intravenous, intramuscular and intragastric doses of 10 mg kg^{-1}. Plasma was separated from blood samples collected at different times after dosing and analysed for parent drug. Plasma samples from rats dosed with AM, AE, AS and AL were also analysed for DQHS which is known to be an active metabolite of these compounds.

Plasma levels of all parent compounds decreased biexponentially and were a reasonable fit to a two-compartment open model. The resulting pharmacokinetic parameter estimates were substantially different not only between drugs but also between routes of administration for the same drug. After intravenous injection the highest plasma level was obtained with AL, followed by DQHS, AM, AE and AS. This resulted in the lowest steady-state volume of distribution (0·39 L) for AL, increasing thereafter for DQHS (0·50 L), AM (0·67 L), AE (0·72 L) and AS (0·87 L). Clearance of AL (21–41 mL min^{-1} kg^{-1}) was slower than that of the other drugs for all three routes of administration (DQHS, 55–64 mL min^{-1} kg^{-1}; AM, 91–92 mL min^{-1} kg^{-1}; AS, 191–240 mL min^{-1} kg^{-1}; AE, 200–323 mL min^{-1} kg^{-1}). In addition the terminal half-life after intravenous dosing was longest for AL (1·35 h), followed by DQHS (0·95 h), AM (0·53 h), AE (0·45 h) and AS (0·35 h). Bioavailability after intramuscular injection was highest for AS (105%), followed by AL (95%) and DQHS (85%). The low bioavailability of AM (54%) and AE (34%) is probably the result of slow, prolonged absorption of the sesame-oil formulation from the injection site. After oral administration, low bioavailability (19–35%) was observed for all five drugs. In-vivo AM, AE, AS and AL were converted to DQHS to different extents; the ranking order of percentage of total dose converted to DQHS was AS (25·3–72·7), then AE (3·4–15·9), AM (3·7–12·4) and AL (1·0–4·3). The same ranking order was obtained for all formulations and routes of administration. The drug with the highest percentage conversion to DQHS was artesunic acid. Because DQHS has significant antimalarial activity, relatively low DQHS production could still contribute significantly to the antimalarial efficacy of these drugs.

This is the first time the pharmacokinetics, bioavailability and conversion to DQHS of these drugs have been directly compared after different routes of administration. The results show that of all the artemisinin drugs studied the plasma level was highest for artelinic acid; this reflects its lowest extent of conversion to DQHS and its slowest rate of elimination.

Dihydroartemisinin dihydroginghaosu, (DQHS) and its derivatives are members of a new class of antimalarial drug recommended for clinical development by the World Health Organization (Maurice & Pearce 1987). They are efficacious and act rapidly even against parasites resistant to other antimalarial drugs (Klayman 1985; Woerdenbag et

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al 1990; Yang et al 1995). DQHS is obtained by reduction of artemisinin with sodium borohydride (Qinghaosu, QHS) an endoperoxide-containing sesquiterpene lactone isolated by Chinese researchers (China Co-operative Research Group on Qinghaosu and its Derivatives as Antimalarials 1982a; Warburton 1984; Hoffman 1986) and characterized as the antimalarial principle of the plant *Artemisia annua*. In-vitro bioassay tests have shown DQHS to be 3·8–5·2 times more potent than artemisinin (China Co-operative Research Group on Qinghaosu and its Derivatives as Antimalarials 1982b; Gu et al 1984; Shmuklarsky et al 1993). However, because of its poor solubility in water or oils, DQHS has only been formulated as an oral preparation and has been used primarily as a semi-synthetic compound for derivatization to the oil-soluble drugs artemether (AM) and arteether (AE) and the water-soluble drugs artesunic acid (AS) and artelinic acid (AL) (Luo et al 1984; Lin et al 1987; Brossi et al 1988). The structures of the six compounds are shown in Figure 1.

Few pharmacokinetic data about DQHS or its derivatives are available for animals or man. DQHS tablets have been used by the Chinese since 1991; at doses of 1·1–2·2 mg kg^{-1}, peak serum levels of 130–710 ng mL^{-1} were obtained within 1·3 h (Zhao & Song 1993). When DQHS was given orally to rabbits or dogs at a dose of 20 mg kg^{-1}, peak plasma concentrations of 50 and 130 ng mL^{-1}, respectively, occurred between 1 and 2 h after dosing. DQHS oral half-lives were 1·0 and 2·1 h for rabbits and dogs, respectively (Zhao & Song 1990).

Benakis et al (1991) reported an elimination half-life of 28 h for arteether after an intramuscular dose of 25 mg kg^{-1} in beagles. Previous pharmacokinetic studies of artemether performed in rabbits using a TLC method for analysis of the parent compound (China Co-operative Research Group on Qinghaosu and its Derivatives as Antimalarials 1982c; Zeng et al 1984), showed that after intravenous administration of an artemether emulsion to rabbits the elimination half-life was approximately 40 min. In the same study, the bioavailability of artemether formulated as an oil solution and administered intramuscularly to rabbits was 37–50%. Other data from rats showed the elimination half-life to be 16 h after intramuscular injection of 80 mg kg^{-1} artemether (Zhou et al 1988). Pharmacokinetic studies of artemether in dogs have also been reported; an immunoassay technique was used to measure plasma concentrations (Zhao et al 1986). In these studies, after an intramuscular injection of 10 or 30 mg kg^{-1} artemether in peanut oil peak plasma concentrations were reached within 2–4 h and the elimination half-life was estimated to be 4·0–6·5 h.

The water-soluble derivatives of DQHS, artelinic acid and artesunic acid, have been found to be very effective against malaria in-vitro and seem to be of low toxicity in-vivo and in-vitro (Yang et al 1982; Lin et al 1987). Artesunic acid is available for clinical use in China and Southeast Asia as either

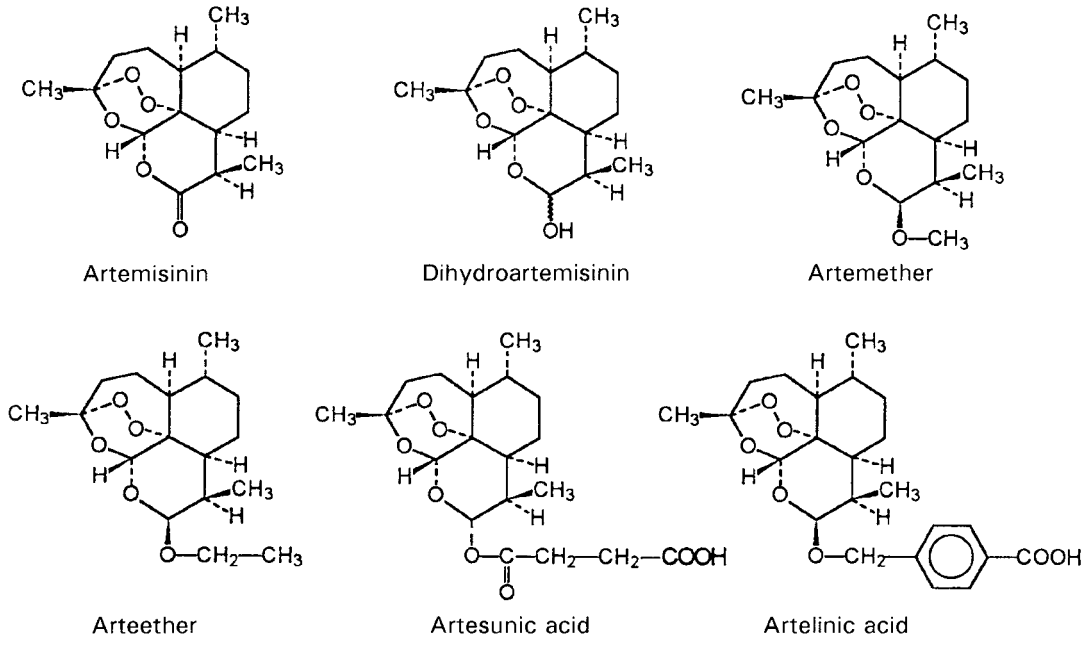

Figure 1. The structures of artemisinin and its derivatives dihydroartemisinin, artemether, arteether, artesunic acid and artelinatic acid.

intravenous or intramuscular formulation (Li et al 1982). Although AS is 5·2 times more potent than artemisinin, and less toxic than artemether and arteether, it has limited stability in solution (Zhou et al 1987; Panisko & Keystone 1990) and doses must be prepared immediately before administration. Pharmacokinetic data for man, rabbit, rat and dog suggest that artesunic acid is rapidly distributed and hydrolysed to DQHS by plasma esterase with an elimination half-life of 2–4 min in rabbits and 27 min in dogs (Edlund et al 1984; Zhao et al 1986; Yang et al 1986). Because of its rapid and extensive conversion to DQHS, artesunic acid could be considered a prodrug of DQHS (Titulaer et al 1991). Artelinic acid, as its sodium salt, has been shown to have approximately the same order of in-vitro antimalarial activity as artesunic acid, with the important advantage of being significantly more stable in aqueous solution (Klayman et al 1991). Pharmacokinetic data for rabbits showed that after intravenous injection the maximum plasma concentration (Cmax) for AL was high (76 ± 15 μg mL^{-1}), and that oral bioavailability was low ($4{\cdot}6 \pm 1{\cdot}7\%$) with the drug being rapidly eliminated (elimination half-life 15 ± 3 min) (Titulaer et al 1993).

Only limited information is currently available about the comparative pharmacokinetics and bioavailability of these compounds, and no direct comparison of their pharmacokinetics after different routes of administration has been reported. The purpose of these studies was: to use a new vehicle, cremophore EL, to pre-form the intravenous formulation of DQHS, arteether and artemether; to compare the pharmacokinetics and bioavailability of AE, AM, AL, AS and DQHS in rats after a single intravenous, intramuscular or intragastric administration of a dose of 10 mg kg^{-1}; and to examine the biotransformation of AE, AM, AL and AS to DQHS in-vivo.

Materials and Methods

Animals

Male, viral antigen-free, Sprague-Dawley rats, 7-8 weeks, 210–254 g, (Charles River, Kingston, NY) were housed in well ventilated cages and kept at room temperature (24 ± 2°C) and 40–60% humidity while on a regular 12-h light-dark lighting cycle for a minimum of 14 days before experiments. Standard pellet rodent chow and tap water were freely available. Animals were cared for in accordance with principles of The Guide for the Care and Use of Laboratory Animals (Department of Health, Education and Welfare, No. [NIH] 85–23). At all times during the study pain to animals was minimized by use of anaesthesia and analgesia. Rats in the intragastric dose group were fasted for 12 h before dosing but allowed free access to water.

Drug preparation and administration

The formulation of each drug and the routes of administration are shown in Table 1. The concentration of drug solution was 10 mg mL^{-1} for all formulations. For intravenous treatment single, short (<1 min) bolus doses of 10 mg kg^{-1} were injected into the tail vein of four rats. For intramuscular administration single 10-mg kg^{-1} doses were injected into the muscle of a rear leg of four rats using a 23-gauge needle. Before injection the hair was clipped and the area cleaned with 70% isopropanol solution. For intragastric dosing a single dose of 10 mg kg^{-1} was administered to four rats by gavage needle (Perfectum, TW, 20-gauge). The dose factor was 1 mL kg^{-1} for all treatments.

Extraction of plasma samples

Both liquid-liquid and solid-phase extraction methods were used for sample preparation. In all cases internal standard was added immediately before extraction. For analysis of artesunic acid and artelinic acid, plasma samples (0·1–1·0 mL) were vortex-mixed and transferred to 3-mL C_{18} solid-phase cartridges (J. T. Baker, Phillipsburg, NJ) previously conditioned by sequential washing with acetonitrile, methanol and distilled water (2·5 mL of each). After application of the plasma sample the cartridges were washed with H_3PO_4 (0·01%, pH 5) then acetonitrile–H_2O (1:19, pH 4·5) (2·5 mL of each) and vacuum was applied for at least 15 min to dry the cartridges completely. The compounds were eluted by washing the cartridges with methanol ($2 \times 0{\cdot}5$ mL) then ethyl acetate-*n*-butyl chloride (1:9, 0·5 mL). The eluents were evaporated to dryness under a stream of nitrogen at room temperature. Extraction of artemether and arteether from plasma samples was performed as previously described (Meléndez et al 1991). Each sample was reconstituted in EtOH–H_2O (1:1, 0·3 mL) for analysis by high-performance liquid chromatography with electrochemical detection (EC-HPLC). DQHS, artemether, arteether and artelinic acid are stable in rat plasma at −80°C for >6 months and artesunic acid is stable at −80°C for at least 2 weeks. For this reason, AS plasma samples were stored at −80°C and assayed within two weeks of collection.

EC-HPLC analysis

HPLC with reductive electrochemical detection was performed by the method of Meléndez et al

Table 1. Comparison of the main pharmacokinetic parameters of dihydroartemisinin, artemether, arteether, artesunic acid and artelinic acid in rats after single intravenous, intramuscular or intragastric doses.

Parameter	Dihydro-artemisinin	Artemether	Arteether	Artesunic acid	Artelinic acid
Molecular weight =	284·9	298·4	312·4	384·4	418·5
Dose (mg kg^{-1}) =	10	10	10	10	10
Dose (μM kg^{-1}) =	35·1	33·5	32·0	26·0	23·9
Intravenous formulation	Cremophore EL	1:3 Cremophore EL–saline	1:3 Cremophore EL–saline	0·9% Saline	0·9% Saline
Maximum plasma concentration (ng mL^{-1})	6683 ± 2405	6231 ± 1837	3724 ± 430	2106 ± 399	12706 ± 1010
Maximum plasma concentration (μM)	23·5 ± 13·0	20·9 ± 6·2	11·9 ± 1·4	5·5 ± 1·0	30·4 ± 2·2
Area under plasma concentration–time curve (ng h mL^{-1})	3184 ± 659	1857 ± 432	842 ± 154	738 ± 114	5481 ± 1754
Area under plasma concentration–time curve (μM h)	11·2 ± 2·3	6·2 ± 1·4	2·7 ± 0·5	1·9 ± 0·3	13·1 ± 3·8
Volume of distribution at steady–state (L)	0·50 ± 0·19	0·67 ± 0·11	0·72 ± 0·09	0·87 ± 0·35	0·39 ± 0·12
Clearance (mL min^{-1} kg^{-1})	55·4 ± 9·7	91·8 ± 21·0	200·0 ± 31·0	190·9 ± 30·3	32·1 ± 8·0
Distribution half-life (h)	0·22 ± 0·04	0·10 ± 0·01	0·10 ± 0·02	0·15 ± 0·02	0·19 ± 0·08
Elimination half-life (h)	0·95 ± 0·21	0·53 ± 0·14	0·45 ± 0·03	0·35 ± 0·08	1·35 ± 0·45
Mean residence time (h)	0·50 ± 0·15	0·38 ± 0·05	0·18 ± 0·02	0·29 ± 0·05	0·64 ± 0·21
Intramuscular formulation	1:9 Dimethyl-acetamide–oil	Sesame oil	Sesame oil	0·9% Saline	0·9% Saline
Maximum plasma concentration (ng mL^{-1})	1579 ± 443	692 ± 234	160·7 ± 12·4	1650 ± 446	8032 ± 1019
Maximum plasma concentration (μM)	5·5 ± 1·6	2·3 ± 0·8	0·5 ± 0·04	4·3 ± 1·2	19·2 ± 2·2
Time of maximum plasma concentration (min)	17·5 ± 2·89	28·8 ± 11·8	41·4 ± 14·4	15·0 ± 4·1	11·3 ± 2·5
Area under plasma concentration–time curve (ng h mL^{-1})	2719 ± 385	1007 ± 481	285·7 ± 80·5	773 ± 398	5023 ± 726
Area under plasma concentration–time curve (μM h)	9·5 ± 1·4	3·4 ± 1·6	0·9 ± 0·3	2·0 ± 1·0	12·0 ± 1·6
Volume of distribution at steady–state (L)	4·42 ± 2·42	6·01 ± 1·45	33·8 ± 10·0	1·77 ± 0·46	0·62 ± 0·13
Clearance (mL min^{-1} kg^{-1})	56·2 ± 3·34	91·0 ± 5·2	199·5 ± 3·3	215·9 ± 23·3	30·8 ± 0·8
Distribution half-life (h)	0·73 ± 0·13	0·23 ± 0·04	0·34 ± 0·07	0·15 ± 0·06	0·30 ± 0·02
Elimination half-life (h)	4·44 ± 0·27	1·78 ± 0·80	3·62 ± 0·91	0·54 ± 0·14	2·13 ± 0·18
Mean absorption time (h)	0·21 ± 0·04	0·14 ± 0·;04	0·25 ± 0·06	0·11 ± 0·07	0·02 ± 0·01
Mean residence time (h)	2·62 ± 0·46	2·13 ± 0·97	3·23 ± 1·24	0·56 ± 0·27	0·86 ± 0·13
Absorption, 0–8h (%)	85·4 ± 12·1	54·3 ± 25·9	34·0 ± 9·6	104·7 ± 53·9	91·6 ± 13·2
Intragastric formulation	1:9 Dimethyl-acetamide–oil	Sesame oil	Sesame oil	0·9% Saline	0·9% Saline
Maximum plasma concentration (ng mL^{-1})	769 ± 218	381 ± 113	324 ± 10	208 ± 25	3647 ± 2405
Maximum plasma concentration (μM)	2·7 ± 0·8	1·3 ± 0·4	1·0 ± 0·03	0·5 ± 1·0	8·7 ± 5·2
Time of maximum plasma concentration (min)	15·0 ± 4·1	28·8 ± 11·8	17·5 ± 2·9	30·0 ± 0	25·0 ± 5·8
Area under plasma concentration–time curve (ng h mL^{-1})	615 ± 56	366 ± 52	298 ± 68	217 ± 34	1650 ± 742
Area under plasma concentration–time curve (μM h)	2·2 ± 0·2	1·2 ± 0·2	1·0 ± 0·2	0·7 ± 0·1	3·9 ± 1·6
Volume of distribution at steady–state (L)	21·5 ± 4·3	16·1 ± 2·4	13·8 ± 1·7	14·4 ± 3·7	4·1 ± 3·4
Clearance (mL min^{-1} kg^{-1})	63·6 ± 10·5	90·6 ± 3·2	268·0 ± 3·5	240·1 ± 22·5	31·8 ± 1·6
Distribution half-life (h)	0·24 ± 0·03	0·19 ± 0·05	0·29 ± 0·16	0·26 ± 0·03	0·19 ± 0·02
Elimination half-life (h)	4·94 ± 0·73	2·04 ± 0·18	1·79 ± 0·47	1·34 ± 0·26	3·72 ± 1·53
Mean absorption time (h)	0·18 ± 0·03	0·24 ± 0·05	0·15 ± 0·10	0·25 ± 0·06	0·12 ± 0·01
Mean residence time (h)	5·29 ± 1·18	4·07 ± 2·3	1·61 ± 0·47	1·17 ± 0·19	1·94 ± 0·86
Bioavailability (%)	19·3 ± 1·8	19·7 ± 2·8	35·4 ± 8·1	29·5 ± 4·6	30·1 ± 13·5

Results are means ± s.d. (n = 4).

(1991) with minor modifications. Briefly, for simultaneous determination of AL and DQHS (artemisin as internal standard) a Waters μBondapack C-18 column (30 cm × 4·6 mm i.d.; Waters Associates, Milford, MA) was used with 36:64 acetonitrile–acetic acid (0·1 M, pH 3·75) as mobile phase. For determination of AS and DQHS (artemisinin as internal standard) a Waters μBondapack, CN column (30 cm × 4·6 mm i.d.) was used with 25:75 acetonitrile–acetic acid (0·1 M, pH 3·75) as mobile phase. For analysis of AE and DQHS (artemisinin as internal standard) a Waters μBondapack CN column (30 cm × 4·6 mm) was used with 20:80 acetonitrile–acetic acid (0·1 m, pH 5) as mobile phase. For determination of AM and DQHS (arteether as an internal standard) a Waters μBondapack CN column (30 cm × 4·6 mm) was used with 20:80 acetonitrile–acetic acid (0·1 M, pH 5) as mobile phase. All runs were isocratic with a flow rate of 1·5 mL min^{-1}. Data were acquired and analysed by use of a Waters 820 chromatography data system.

Recoveries varied between 81 and 87% for artesunic acid and artelinic acid, and between 89 and 94% for artemether, arteether, and DQHS. The parent compounds and DQHS eluted within 20 min. The limit of quantitation was 5 ng mL^{-1} plasma for AM, AE, AS and DQHS and 10 ng mL^{-1} for AL. The inter- and intra-day coefficients of variation for accuracy and precision were within ±10% for all five drugs.

Data analysis

The plasma concentrations of the compounds were calculated by comparing the peak-height ratio of the peaks of interest with those of a standard curve run with each set of experimental samples. The concentration–time data for DQHS, AM, AE, AS and AL were fit to a two-compartment open model using a non-linear, extended least-squares fitting procedure (Rstrip and Minsq, version 5; MicroMath Scientific Software, Salt Lake City, UT). The area under the plasma concentration–time curve (AUC) was determined by the linear trapezoidal rule with extrapolation to infinity based on the concentration of the last time point divided by the terminal rate-constant. For intragastric and intramuscular dosing extrapolations to time-zero concentrations were forced through zero. Clearance (CL) was calculated by dividing the dose by AUC for intravenous injection. Mean residence time (MRT) was determined by dividing the area under the first moment curve (AUMC) by the AUC. The mean absorption time (MAT) was calculated by subtracting the $MRT_{intravenous}$ from $MRT_{intragastric}$. The volume of distribution at steady state (Vss) was calculated as the product of CL and $MRT_{intravenous}$. The oral and intramuscular bioavailability (F) were calculated by dividing the $AUC_{intragastric}$ or $AUC_{intramuscular}$ by $AUC_{intravenous}$ for each drug, and percentage conversion of AM, AE, AS and AL to DQHS (DQHSm) were calculated by use of the formula $AUC_{DQHSm}/AUC_{DQHSm} + AUC_{Parent\ drug}$ (Back et al 1990; Li & Huempel 1992).

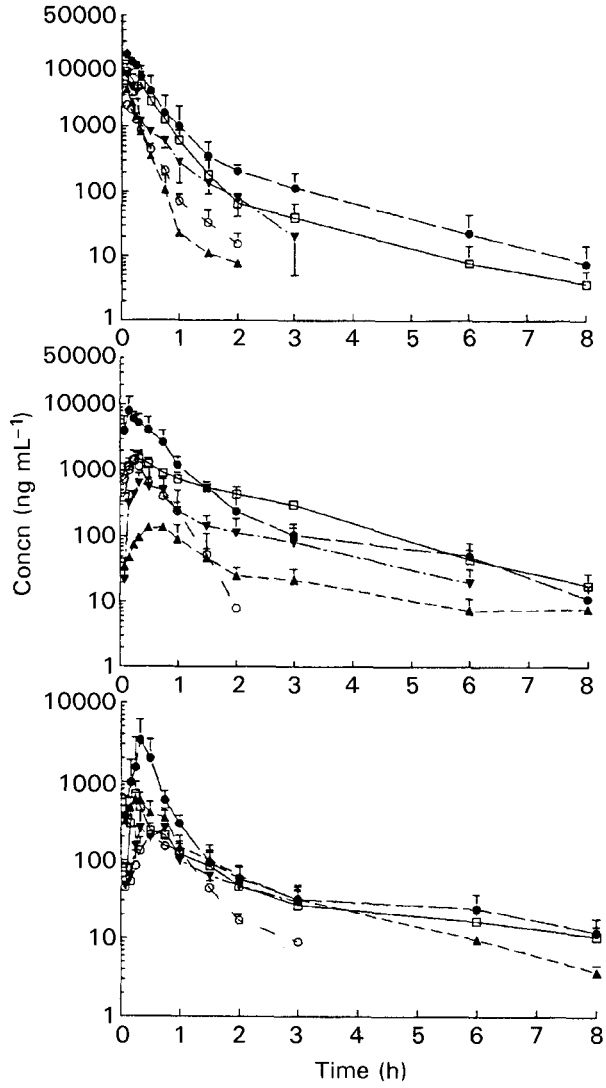

Figure 2. Profiles of mean plasma concentration against time for DQHS (□), AM (▼), AE (▲), AS (○) and AL (●) after intravenous (top), intramuscular (middle) and intragastric (bottom) administration of 10 mg kg^{-1} to rats (n = 4).

Results

Pharmacokinetics

The plasma concentration–time curves after intravenous administration of the five artemisinin drugs are presented in Figure 2 (top); the intravenous results from DQHS, artemether and arteether are the first reports of the use of the cremophore for-

mulation. It is apparent that plasma drug concentrations could be quantitated for up to 8 h, and the disposition of all five compounds was rapid and biphasic. Estimates of the pharmacokinetic parameters after intravenous dosing of the five drugs at 10 mg kg^{-1} are presented in Table 1. The maximum plasma concentration was observed at the first sampling time, 5 min, for all drugs. It is apparent that for artelinic acid the plasma level was highest, the elimination half-life and volume of distribution were lowest and the total clearance was slowest. Because a 10-mg kg^{-1} dose of artemether or DQHS are equivalent to 140 or 147%, respectively, of a 10-mg kg^{-1} dose of artelinic acid, the equivalent molecular weight was used to derive the results. When Cmax is recalculated on a μM basis, Cmax for artenilic acid was still higher than those for DQHS and artemether (Table 1). Because of the big differences between the molecular weights of the drugs, the AUC parameter was also estimated using μM concentrations for direct comparison of drugs.

After intramuscular injection, the plasma concentration of all five drugs peaked within 45 min and then declined in a biphasic manner. The data were fit to a two-compartment open model, as is shown in Figure 2 (middle). Examination of the Cmax values in Table 1 shows that the plasma level was again highest for artelinic acid and lowest for arteether, irrespective of whether calculations were performed with ng mL^{-1} or μM quantities. The absorption of the five drugs was rapid, as is evidenced by the values of MAT (Table 1). The distribution and elimination phases for DQHS, artemether and arteether after intramuscular administration were considerably slower than those calculated after intravenous injection, owing to prolonged absorption of the sesame oil formulations.

The plasma concentration–time curves after intragastric administration of the five drugs are shown in Figure 2 (bottom). The pharmacokinetic parameters calculated after intragastric and intramuscular dosing are also presented in Table 1. The maximum plasma concentrations of the five drugs were attained within 30 min and, as was observed with the intravenous and intramuscular data, Cmax was highest for artelinic acid (Table 1). Absorption, distribution and elimination of the five drugs were rapid after intragastric administration. Compared with intravenous and intramuscular administration the Vss of the five compounds were much higher after intragastric administration, indicating either lower absorption or greater biotransformation of the drugs after intragastric dosing.

Absorption and bioavailability

Different formulations were used for the drugs in these studies (Table 1). The lipid-soluble ethers, artemether and arteether were prepared in sesame oil whereas artesunic acid and artelinic acid were formulated as their sodium salts (5% $NaHCO_3$) in saline. In this way comparisons of bioavailability could be made within each group of drugs (water-soluble artesunic and artelinic, and lipid-soluble arteether and artemether). DQHS for intramuscular injection was formulated in dimethylacetamide (DMAC)–sesame oil and in cremophore for intravenous dosing.

Low bioavailability was observed for all drugs after intragastric administration (Table 1). The bioavailabilities of artelinic acid and artesunic acid formulated in saline were similar, but the bioavailability of arteether was almost twice that of artemether. Use of the DMAC–sesame oil formulation for DQHS resulted in oral bioavailability similar to that of artemether.

Conversion of AM, AE, AS and AL to DQHS

Changes of DQHS plasma concentrations with time, and percentage conversion of AM, AE, AS and AL into DQHS are shown in Figure 3 and Table 2, respectively. Not surprisingly, artesunic acid led to the highest plasma concentrations of DQHS and the highest percentage conversion after all three routes of administration. Although percentage conversion to DQHS was lowest for artelinic acid, the peak plasma level of DQHS was quite similar to those of artemether and arteether for all three types of administration. These data demonstrate that DQHS is a major metabolite of AM, AE, AS and AL in the rats after intravenous, intramuscular or intragastric administration. The dose of DQHS required to produce plasma levels equivalent to those of DQHS formed as a metabolite after administration by all three routes of treatment of each of the drug was calculated as previously described (Li & Huempel 1992) using data obtained after administration of DQHS alone.

Discussion

Because of formulation difficulties, the intravenous pharmacokinetics of arteether, artemether and DQHS have not been studied extensively. Therefore, comparative information about the disposition and bioavailability of these compounds after different routes of administration is limited or incomplete, or both. Cremophore, a new intravenous vehicle approved by FDA in 1992 (Rogers 1993) was used in these studies to generate a complete set of pharmacokinetic data which was

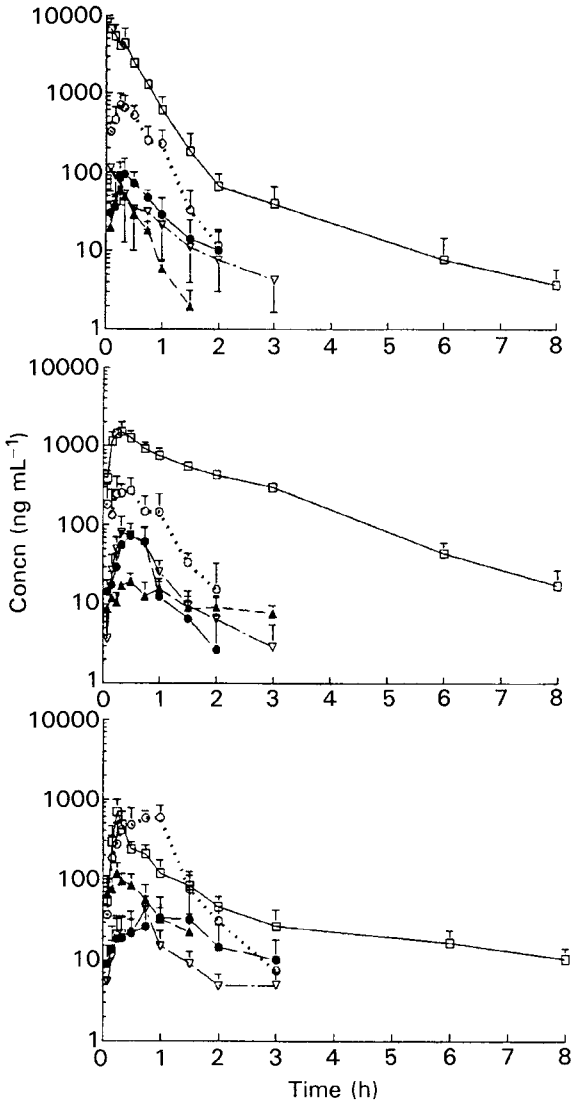

Figure 3. Profiles of mean plasma concentration against time for DQHS (□), and DQHS as a metabolite converted from AM (▼), AE (▲), AS (○) and AL (●) after intravenous (top), intramuscular (middle) and intragastric (bottom) administration of 10 mg kg^{-1} to rats (n = 4).

then used to compare the disposition and bioavailability of these important antimalarial compounds. This report contains the first direct comparison of the pharmacokinetics of these compounds and the first comparative study of the conversion of arteether, artemether, artelinic acid and artesunic acid to their active metabolite DQHS.

A rapid and biphasic decline in plasma concentration was observed for all five artemisinin analogues after intravenous, intramuscular or intragastric administration. After intravenous injection the small Vss indicated little distribution of these drugs into peripheral tissues (Table 1). The maximum plasma concentrations of all the drugs were observed 5 min after intravenous dosing, indicating rapid and complete mixing. From the data in Table 1 it is apparent that the highest plasma level, the longest elimination half-life and the slowest total clearance were obtained for artelinic acid. When Cmax and AUC were calculated on a μM basis, because of the different molecular weights of the drugs, Cmax and AUC for artelinic acid were still higher than for the other four drugs. As a result Vss was lowest for artelinic acid and increased in the order DQHS, artemether, arteether and artesunic acid. The pattern was the same for all routes of administration except intramuscular, after which AUC for artelinic acid was 10-fold greater than for arteether. The clearance of artelinic acid was the slowest for all three routes. After intravenous dosing the terminal half-life was longest for artelinic acid, followed by DQHS, artemether, arteether and artesunic acid.

The plasma concentrations of artelinic acid were not only higher than those of the other four compounds after intravenous injection, but also after intramuscular and intragastric administration (2–18 times), even when calculated on a μM basis (Table 1). This somewhat surprising result (the μM kg^{-1} dose of artelinic acid was approximately 40% lower than those of DQHS, arteether and artemether) is probably a consequence of the slow biotransformation and hydrophilic nature of artelinic acid which would tend to reduce Vss and intrinsic CL. Artesunic acid Cmax after intravenous dosing was lowest irrespective of whether ng mL^{-1} or μM concentrations were used (see Table 1). For this compound distribution and elimination were extremely rapid, which is reflected in the largest Vss and the fastest plasma clearance. The pharmaco-Kinetic parameter estimates for the other three drugs (DQHS, artemether and arteether) fell between the values of artelinic acid and artesunic acid.

If the intramuscular bioavailability of the sesame oil formulations of artemether and arteether are compared with those of DQHS, artelinic acid and artesunic acid it is apparent that artemether and arteether are absorbed to a much lesser extent than the water-soluble drugs. This incomplete absorption is most probably because of prolonged and highly variable uptake from the injection site. If the intravenous dose is used as reference, the bioavailabilities of artelinic acid and artesunic acid were similar after intramuscular administration, indicating that absorption of artelinic acid and artesunic acid from rat muscle is essentially complete (> 91·6%). In contrast, the intramuscular bioavailabilities of artemether and arteether were very low, indicating incomplete absorption of these drugs at least during the first 8 h after intramuscular administration. The bioavailability of DQHS, on

Table 2. Areas under plasma–concentration-time curves and conversion rates of dihydroartemisinin as a metabolite, and estimation of dihydroartemisinin dose from the concentration of dihydroartemisinin as a metabolite after intravenous, intramuscular and intragastric administration of 10 mg kg^{-1} artemether, arteether, artesunic acid and artelinic acid to rats.

Parameter	Artemether	Arteether	Artesunic acid	Artelinic acid
Intravenous administration				
Area under plasma concentration–time curve of dihydroartemisinin as a metabolite (ng h mL^{-1})	65·1 ± 33·8	29·1 ± 2·2	474 ± 183	63·2 ± 40·0
Conversion rate of diihydroartemisinin as a metabolite (%)	3·7 ± 2·4	3·4 ± 0·6	38·2 ± 7·4	1·4 ± 1·1
Estimate of dihydroartemisinin dose (mg kg^{-1})	0·20 ± 0·13	0·09 ± 0·02	1·49 ± 0·51	0·20 ± 0·09
Intramuscular administration				
Area under plasma concentration–time curve of dihydroartemisinin as a metabolite (ng h mL^{-1})	70·8 ± 25·6	43·4 ± 11·5	236 ± 79	44·1 ± 25·6
Conversion rate of dihydroartemisinin as a metabolite (%)	9·0 ± 1·6	14·2 ± 4·2	25·3 ± 7·1	1·0 ± 0·58
Estimate of dihydroartemisinin dose (mg kg^{-1})	0·26 ± 0·08	0·16 ± 0·04	0·87 ± 0·27	0·16 ± 0·09
Intragastric administration				
Area under plasma concentration–time curve of dihydroartemisinin as a metabolite (ng h mL^{-1})	37·7 ± 8·0	49·6 ± 17·8	595 ± 145	61·9 ± 54·5
Conversion rate of dihydroartemisinin as a metabolite (%)	12·4 ± 3·6	15·9 ± 2·2	72·7 ± 5·8	4·3 ± 3·0
Estimate of dihydroartemisinin dose (mg kg^{-1})	0·61 ± 0·13	0·81 ± 0·25	9·67 ± 2·24	1·01 ± 0·68

Conversion rate was calculated from $AUC_{DQHSm}/AUC_{DQHSm} + AUC_{Parent\ drug}$. DQHS dose was estimated from $AUC_{DQHSm}/AUC_{DQHS} \times 10$mg DQHS dose. Results are means ± s.d. (n = 4).

the other hand, was midway between those of the other pairs of drugs, implying that although it was absorbed more completely than the oil-soluble analogues its absorption was still less than the absorption of the water-soluble drugs.

If percentage conversion to DQHS (see Table 2) for the intravenous and intragastric routes is compared, initial indications are that arteether and artemether undergo significant first-pass metabolism after oral administration. It is interesting to note that after oral administration of artemether to man, DQHS plasma levels exceeded those of the parent drug at all times (Teja-Isavadharm et al 1996) supporting the above conclusion that artemether undergoes first-pass metabolism in rats.

DQHS was formed in-vivo after administration of AM, AE, AS and AL by all three routes, and plasma concentrations of DQHS formed as a metabolite could be quantitated for up to 3 h (Figure 3). When the in-vivo conversion of AE, AM, AS and AL to DQHS was compared, major differences were found. The drug with the greatest percentage conversion over all three routes of administration was AS, followed by AE, AM and AL. Differences between the metabolism or tissue distribution of the lipid- and water-soluble analogues could provide possible explanations of this observation. Artesunic acid is hydrolysed by ubiquitous plasma esterase, rather than by hepatic enzymes, and is rapidly converted to DQHS. Artelinic acid is the sodium salt of the β-hydroxymethylbenzoate ether of DQHS. The benzoate group would increase the steric hindrance of the molecule making the ether linkage less accessible

to oxidative enzymes in the liver. It would also have an electron-withdrawing effect on the ether bond further increasing its metabolic stability. Arteether and artemether are so lipid-soluble that their conversion to DQHS is probably limited by their distribution into peripheral tissues.

The high in-vivo conversion of artesunic acid to DQHS observed in these studies confirms the observations of others (Edlund et al 1984; Zhao et al 1986; Yang et al 1986) that artesunic acid is rapidly and predominantly hydrolysed to DQHS and is essentially a prodrug. This might also explain why artesunic acid and DQHS have the same in-vitro anti-malarial potency against both chloroquine-resistant and chloroquine-sensitive strains of *P. berghei* (China Co-operative Research Group on Qinghaosu and its Derivatives as Antimalarials 1982b). Although the DQHS levels obtained after dosing with artemether, arteether or artelinic acid were less than 2·6% those after intravenous or intramuscular dosing with DQHS, and less than 10% those after intragastric dosing with DQHS, the high antimalarial activity of DQHS probably contributes significantly to the overall antimalarial activity of these drugs in-vivo.

To summarize, the artemisinin compounds studied were rapidly distributed and eliminated after intravenous, intramuscular and intragastric administration to rats. After intramuscular treatment the prolonged absorption of artemether and arteether from the muscle was probably because of a depot effect of the sesame oil formulation. Compared with the other three compounds (AM, AE and AS) and as evidenced by the lower DQHS levels attained after dosing, the biotransformation of artelinic acid to DQHS seems to be slow in the rat. This was reflected in the plasma level being highest and the elimination slowest for artelinic acid. In contrast with artelinic acid, the plasma level was lowest and elimination fastest for artesunic acid, because of its rapid and extensive conversion to DQHS. Despite the low DQHS levels observed after dosing with AE, AM and AL, DQHS could still contribute a significant portion of their overall antimalarial activity. Compared with the oil-soluble drugs (AM and AE), AL and AS have the advantages of being administered by either intravenous, intramuscular or oral routes. Compared with the water-soluble drug artesunic acid, artelinic acid has a longer elimination half-life and a lower percentage conversion to DQHS, which might explain its lower toxicity. In conclusion, artelinic acid is an efficacious treatment for multi-drug-resistant malaria; it is less toxic than the lipid-soluble arteether and artemether and more stable than the water-soluble ester artesunic acid.

Acknowledgements

This work was performed while Qi-Gui Li held a National Research Council (Walter Reed Army Institute of Research) Research Associateship. Work in the laboratory was supported by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR).

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