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Absorption of the novel artemisinin derivatives artemisone and artemiside: Potential application of Pheroid™ technology

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ABSTRACT

Artemisinins have low aqueous solubility that results in poor and erratic absorption upon oral administration. The poor solubility and erratic absorption usually translate to low bioavailability. Artemisinin-based monotherapy and combination therapies are essential for the management and treatment of uncomplicated as well as cerebral malaria. Artemisone and artemiside are novel artemisinin derivatives that have very good antimalarial activities. Pheroid™ technology is a patented drug delivery system which has the ability to entrap, transport and deliver pharmacologically active compounds. Pharmacokinetic models were constructed for artemisone and artemiside in Pheroid™ vesicle formulations. The compounds were administered at a dose of 50.0 mg/kg bodyweight to C57 BL/6 mice via an oral gavage tube and blood samples were collected by means of tail-bleeding. Drug concentrations in the samples were determined using an LC/MS/MS method. There was 4.57 times more artemisone in the blood when the drug was entrapped in Pheroid™ vesicles in comparison to the drug only formulation ($p < 0.0001$). The absorption of artemiside was not dramatically enhanced by the Pheroid™ delivery system.

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1. Introduction

Malaria is an infectious disease caused by parasites of the *Plasmodium* genus. The parasites are primarily hosted by female *Anopheles* mosquitoes, which act as vectors that transmit the protozoan organisms to humans when feeding. There are four known species that infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. However, *P. falciparum* can be held liable for the majority of malaria infections (Hay et al., 2004; Gkrania-Klotsas and Lever, 2006; WHO, 2009).

Drug-resistant malaria is a huge threat and stringent measures must be taken to ensure the preservation of effectiveness of current antimalarial drugs. Drug-resistant malaria materializes with evolutionary, single or multiple, point-mutations in the *Plasmodium* genome rendering parasites insensitive to drugs (White, 2004). The emergence and spread of this phenomenon has greatly affected the control and treatment of malaria in endemic countries, specifically concerning *P. falciparum* infections (Hay et al., 2004; WHO, 2006). Combination drug therapy is currently the mainstay approach in preventing the development of further resistance to current antimalarials. Artemisinin-based combination therapy is the treatment of choice and it is of great importance that the efficacy of those therapeutic regimens is maintained (Dondorp et al., 2009). There is presently no other effective alternative to surmount the ever increasing problem of drug resistance. It is thus essential to focus all efforts on the research and development of novel antimalarial compounds and the effective delivery thereof (Baird, 2005; Mutabingwa, 2005).

Artemisinins are based on the natural product artemisinin that was first isolated in China in the early 1970s. Artemether, an artemisinin derivative, is known to be as effective as quinine for the treatment of severe *P. falciparum* malaria (Meshnick et al., 1996). Other artemisinin derivatives include dihydroartemisinin (DHA) and artesunate. One key advantage of these agents is the fact that they are active against all of the red blood cell stages of *P. falciparum* (Krishna et al., 2004). At this stage, there is limited resistance to these agents (Dondorp et al., 2009). Due to the short elimination half-life of artemisinin-based drugs it is recommended that they are used in combination with other drugs such as mefloquine, lumefantrine or amodiaquine as first-line therapies for the treatment of uncomplicated malaria (White, 2004). New artemisinin derivatives such as artemisone and artemiside are reported to be much more potent than the existing derivatives and it would be of great value to optimize

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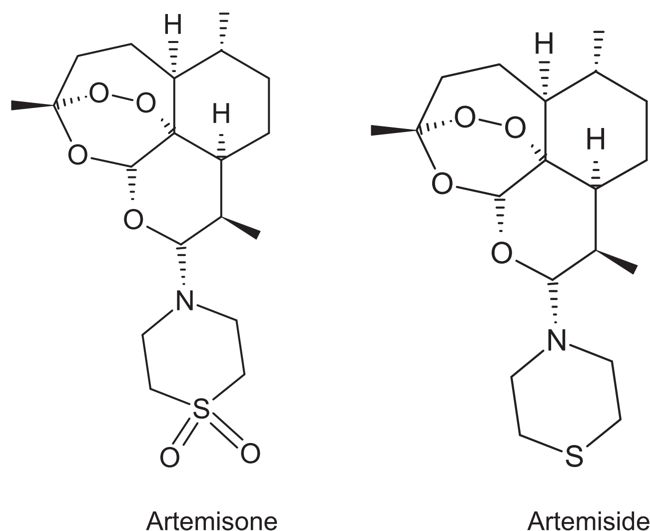

Fig. 1. Structures of artemisone and artemiside.

the delivery of these compounds by using novel drug delivery systems (Woodrow et al., 2005; Haynes et al., 2006).

Alternative drug delivery options, such as the Pheroid™ delivery system, may play a key role in ensuring effective delivery and enhanced absorption of these novel antimalarial compounds. Pheroid™ technology is a patented, novel, colloidal type drug delivery system. It primarily consists of the ethyl esters of essential fatty acids and nitrous oxide (N_2O)-water. It is postulated that the role of N_2O is to stabilize the moving autofluorescent particles surrounding the Pheroid™ vesicles and that it is essential for ensuring stability of the formulation and efficient delivery of test compounds. The stabilization can be microscopically observed through the tracking of the movement of the autofluorescent or fluorescently labeled particles representing the ethyl fatty acids. Additional studies are, however, still being conducted. The Pheroid™ delivery system is known to remain stable and structurally intact for a period of at least 24 months at room temperature.

The Pheroid™ delivery system is superior to most other delivery systems and is able to improve the delivery of dynamic complexes, reduce the time to onset of action, decrease the minimal effective drug concentration and enhance therapeutic efficacy. It is also able to indirectly decrease the cytotoxicity of therapeutic compounds and to infiltrate virtually all known barriers in the body. The system is further capable of targeting specific treatment areas, to transport genetic material to the cellular nucleus and to decrease drug resistance (Grobler et al., 2008). The aim of this study was to evaluate the possibility to enhance the absorption of artemisone and artemiside with the aid of Pheroid™-based formulations.

2. Materials and methods

2.1. Materials

Artemiside is the thiomorpholine precursor of artemisone (Fig. 1). This was prepared from thiomorpholine and DHA, and then converted into artemisone according to the methods described in the literature from a multigram-scale reaction. In short, Artemiside (12) and artemisone (14), were obtained from DHA α-trimethylsilyl ether (7) and trimethylsilyl bromide (TMSBr), followed by treatment of the intermediate bromide (8), formed *in situ*, with the amine nucleophile (Fig. 2, route a). The yield of the two compounds ranged between 40 and 60%. Oxidation of artemiside (12) produced artemisone (14) and also a sulfoxide (13). An easier route to follow involves the treatment of DHA (2) with a mixture of NaBr and then with TMSCl in toluene followed by the amine (Fig. 2, route b). Both artemiside (12) and artemisone (14) were isolated by crystallization of the crude product mixtures and are subsequently relatively accessible and produced as isomerically pure, air-stable, substances (Haynes et al., 2006). The aqueous solubility and octanol–water partition coefficients of artemisone and artemiside are given in Table 1. Vitamin F ethyl ester was obtained from Chemimpo (South Africa) and Cremaphor® EL was obtained from BASF (South Africa). Polyethylene glycol (PEG 400), D-α-tocopherol and butylated hydroxyanisole (BHA) were purchased from Chempure (South Africa). Analytical grade artemisinin and dimethyl sulfoxide (DMSO) were obtained from Merck (South Africa).

Table 1
Aqueous solubility and octanol–water partition coefficients.

	Artemiside	Artemisone
Solubility [mg/L]	<2	89
Log *P*	4.97	2.49
Log *P* (calculated)	3.98	2.08

Solubility = aqueous solubility at pH 7.2.
Log *P* = octanol–water partition coefficient for the neutral compound determined at pH 7.4, except artesunate which was determined by HPLC at pH 2.
Log *P* (calculated) = calculated log *P* value (Haynes et al., 2006).

2.2. Reference formulations

Oral reference formulations were prepared (0.0625 g drug in 10 ml of the final formulation) and administered at a dose of 50.0 mg/kg bodyweight for each compound to compare against the Pheroid™ vesicle formulations. The reference formulations were prepared immediately before administration by dissolving the appropriate amount of each drug in analytical grade DMSO. Purified water was then added until the desired volume was reached. The final DMSO to water ratio was 1:9 v/v. Both of the reference formulations formed a microsuspension after the addition of water to the DMSO-drug solution.

2.3. Pheroid™ formulations

Pheroid™ vesicles were prepared by heating and mixing vitamin F ethyl ester (66.2 g), Cremaphor® EL (27.6 g) and D-α-tocopherol (1.0 g). PEG 400 was then added (5.0 g) together with BHA (0.2 g) and mixed thoroughly. This mixture constituted the oil-phase of the Pheroid™ vesicle formulations. Nitrous oxide water was prepared by saturating purified water with N_2O under high pressure. The required amount of drug (0.0625 g) was added to 1.0 ml of the prepared oil-phase (at room temperature). This mixture was then agitated for a period of 2 min until all the drug particles had dissolved. The prepared nitrous oxide water was then added to the oil-phase to achieve a total volume of 10.0 ml (oil to water ratio, 1:9 v/v). The mixture was homogenised with a Heidolph Diax 600 homogeniser (Labotec, South Africa) at 8000 rpm for 1 min (Jonker et al., 2002). The size of the Pheroid™ vesicles was measured with a Malvern Mastersizer. The average size of the vesicles, prior to drug loading, was 3.81 μm and the median was 2.85 μm. Obscuration values of between 10 and 20% were used and the span was 1.94. All experimental formulations were kept in amber glass bottles.

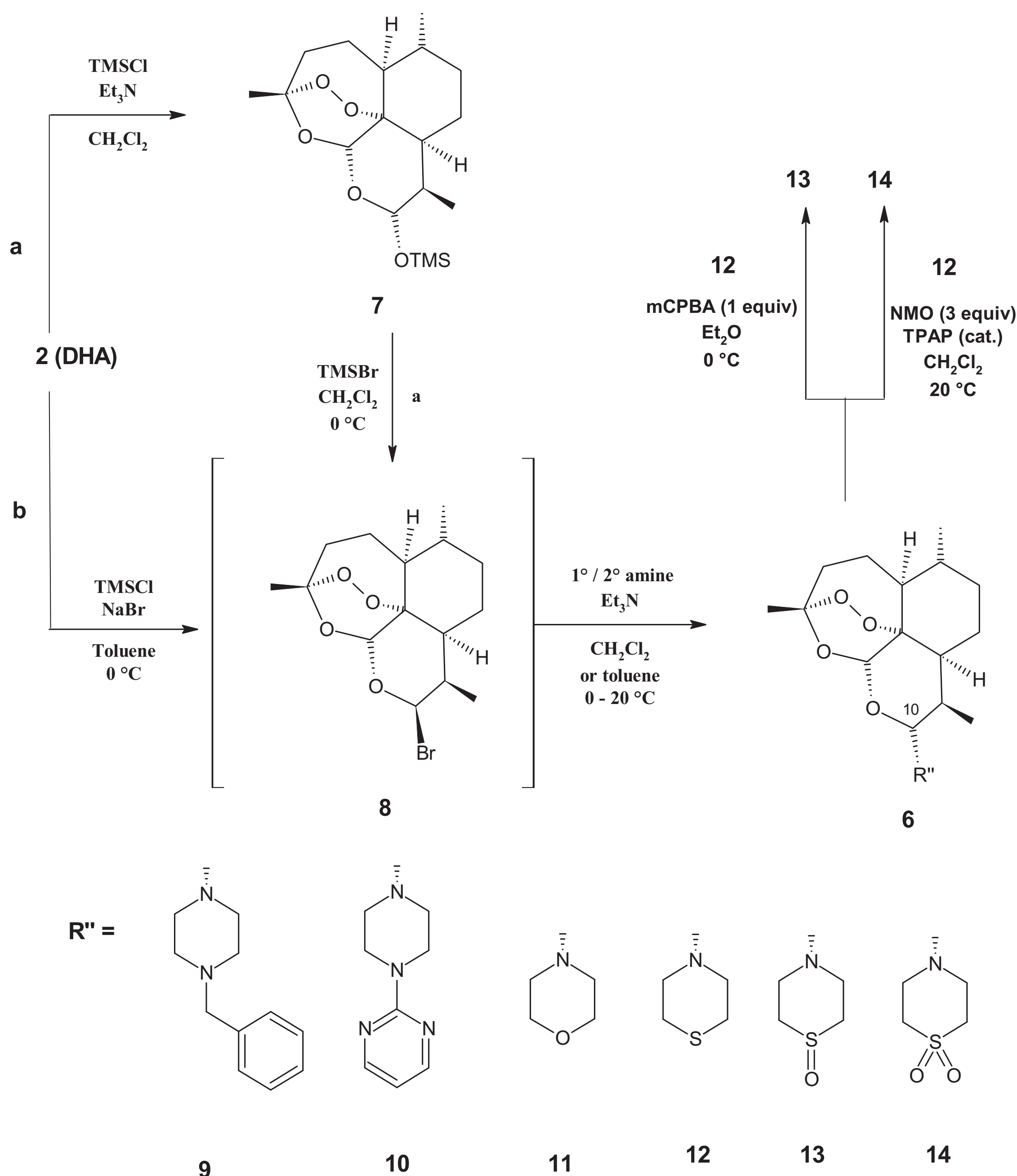

Fig. 2. Preparation of 10-alkylamino derivatives. Artemisone (14) is obtained by either treatment of (8) with thiomorpholine-5,5-dioxide or by oxidation of artemiside (12). Artemiside is obtained from (8) and thiomorpholine. Oxidation of artemiside also produces a sulfoxide (13) (Haynes et al., 2006).

2.4. Drug administration

The absorption of artemisone and artemiside were evaluated in a mouse model. The drugs were tested in both a reference formulation and a Pheroid™ vesicle formulation. The animals utilized were male C57 BL/6 mice, weighing approximately 25 g each (Basco et al., 1999; Ojo-Amaize et al., 2007). The study and all procedures were approved by the Ethics Committee of the University of Cape Town, approval number 009/034. The drugs were administered via the oral route. Test animals were randomly allocated to each group. The exact procedure is described below.

2.4.1. Artemisone and artemiside

Reference group (p.o.) (N = 10): Artemisone/artemiside was administered orally at a dose of 50.0 mg/kg in DMSO/water (1:9 v/v). The total volume per administration was 200 µl. Blood samples (50 µl) were collected via tail-bleeding at 5, 10, 30, 60 and 120 min for artemisone and at 10, 30, 60, 120 and 180 min after drug administration for artemiside. The sample collection times vary between the two compounds because a pilot study indicated that artemiside had a longer half-life than artemisone. The five sample limit per test subject was also taken into consideration. The samples were centrifuged at 2000 rcf and

Table 2
ESI settings.

Curtain gas	20
Collision gas	5
Ionspray voltage (V)	5000
Source temperature (°C)	500
Gas 1 (psi)	50
Gas 2 (psi)	60

15 μl of plasma were collected from each sample and stored at −20 °C.

Pheroid™ group (p.o.) (N = 10): Artemisone/artemiside, entrapped in Pheroid™ vesicles, was administered orally at 50.0 mg/kg. The same protocol was used as described for the oral reference group.

2.5. Measurement of drug content

2.5.1. Instrumentation

A sensitive and selective LC/MS/MS method was developed to determine the drug concentrations in the collected plasma samples. An Agilent 1200 series HPLC system and an Applied Biosystems API 3200 triple quadrupole mass spectrometer were used.

2.5.2. Calibration standards

Stock solutions of artemisone and artemiside were prepared in ethanol at a concentration of 1 mg/ml. Mouse plasma (1240 μl) was spiked with the stock solution (10 μl) to obtain STD 1 at a concentration of 8 μg/ml. Serial dilution with mouse plasma resulted in STD 2 (4 μg/ml), STD 3 (2 μg/ml), STD 4 (1 μg/ml), STD 5 (0.5 μg/ml), STD 6 (0.25 μg/ml), STD 7 (0.125 μg/ml), STD 8 (0.0625 μg/ml), STD 9 (0.0313 μg/ml) and STD 10 (0.0156 μg/ml). Artemisinin was added to act as an internal standard (ISTD). The calibration standards were briefly vortexed, aliquotted into labeled polypropylene tubes and stored at −20 °C.

2.5.3. Mass spectrometry

The detection of artemisone, artemiside and artemisinin (ISTD) was performed on an AB Sciex API 3200 mass spectrometer (ESI in the positive ion mode, MRM). The settings on the apparatus are summarized in Tables 2 and 3.

2.5.4. Chromatography

Chromatography was performed on a Phenomenex Gemini-NX (5 μm, C18, 110A, 50 mm × 2 mm) analytical column using an Agilent 1200 series HPLC. For artemisone the mobile phase consisted of methanol and ammonium acetate (10 mM with 0.1% acetic acid) (60:40) and was delivered at 0.5 ml/min for 3 min. The organic phase was increased after 3 min to 95% for another 2 min to clean the column and was then brought back to 60% organic phase for 3 min to equilibrate the column. For artemiside the mobile phase consisted of methanol and ammonium acetate (10 mM with 0.1% acetic acid) (75:25) and was delivered at 0.5 ml/min for 3 min. The column was kept in a column compartment at 35 °C. An autosampler injected 10 μl (artemisone) and 5 μl (artemiside) into the HPLC column. The injection needle was rinsed with mobile phase before each injection for 10 s using the flush port wash station. The samples were cooled to 5 °C while awaiting injection.

Table 3
MS/MS settings.

Setting	Artemisone	ISTD	Artemiside
Q1 mass $[M+H]^+$	402.2	283.2	370.2
Q3 mass	163.2	151.1	163.2
Dwell time (ms)	150	150	150
Declustering potential (V)	46	26	26
Entrance potential (V)	2	9.5	5
Collision energy (V)	25	21	25
Collision cell exit potential (V)	4	4	4
Scan type	MRM	MRM	MRM
Polarity	Positive	Positive	Positive
Pause time (ms)	5	5	5

2.5.5. Liquid–liquid extraction

The extraction procedure was performed on ice using polypropylene test tubes. The plasma samples were thawed on ice and briefly vortexed. Twenty-five microlitres of a universal Britton Robinson buffer (pH 9) was aliquotted into clean polypropylene tubes and 15 μl of the plasma sample was added. The ISTD was spiked at an appropriate concentration into the universal buffer and 25 μl was subsequently added to the extraction tubes. 1-Chlorobutane (350 μl) was also added to each tube to function as an organic solvent. The samples were vortexed for 1.5 min and centrifuged at 7000 rcf for 5 min. The organic phase (300 μl) was transferred to clean polypropylene tubes and evaporated under vacuum in a rotor evaporation system at 30 °C for 45 min. Mobile phase (50 μl) was added to the dry samples. The samples were then vortexed for 30 s and transferred to 96 well polypropylene plates. Five or ten microlitres of each sample was then injected into the HPLC column and analyzed.

2.6. Statistical evaluation

The recovered experimental data was evaluated in terms of the drug plasma concentration versus time. The following parameters were calculated:

- the peak drug plasma concentration (C_{max}) (Cp°) in ng/ml;
- time to peak plasma concentration (T_{max});
- apparent elimination half-life ($T_{1/2}$);
- area under the plasma concentration–time curve between time zero and the time of last sample collection ($AUC_{0\text{-last}}$) in ng h/ml and the
- area under the plasma concentration–time curve from time zero to infinity ($AUC_{0\text{-inf}}$) in ng h/ml.
- results are reported as mean ± SEM.

Noncompartmental analysis was used to calculate the parameters for artemisone and artemiside (WinNonlin version 5.2, Pharsight Corporation, CA, USA). Linear interpolation was used to determine the area under the concentration time curve. $AUC_{0\text{-last}}$ is defined as the AUC computed from time zero to the time of the last *Y*-value above the lower limit of quantitation of the assay. All values below this limit were treated as "missing". $AUC_{0\text{-inf}}$ was calculated by extrapolating the concentration time curve from time zero to infinity, using the last three concentration time points to estimate the elimination rate constant (λz). This constant was also used to determine the observed elimination half-life ($T_{1/2}$) of the compounds. The summary statistics and Mann–Whitney non-parametric test were performed using Prism version 4 (GraphPad Software Inc., CA, USA).

The relative absorption was determined by using the calculated arithmetic mean of the area under the curve ($AUC_{0\text{-last}}$) values of both the oral reference formulation and the oral Pheroid™ vesicle formulation data. Eq. (1) was used to calculate the relative absorption:

$$\text{Relative absorption (RA)} = \frac{[AUC]_A}{[AUC]_B} \quad (1)$$

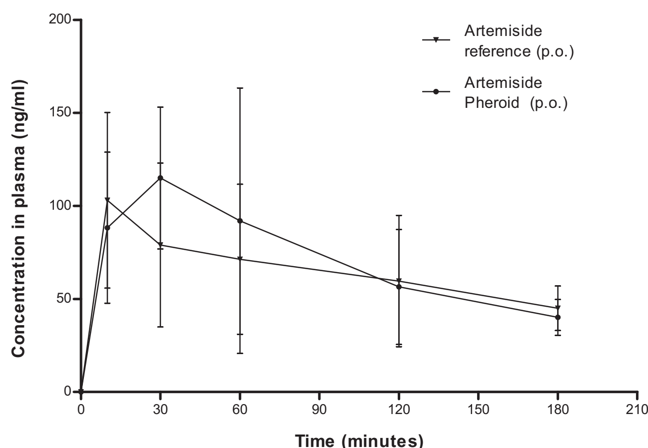

Fig. 3. Mean concentration versus time graph overlay of the oral artemisone reference and Pheroid^{TM} vesicle formulations.

3. Results

Calibration standards for artemisone and artemiside were analyzed in duplicate during each study sample batch. The calibration range was between 31.3 ng/ml and 4000 ng/ml for artemisone, and between 15.6 ng/ml and 4000 ng/ml for artemiside. Therefore, the limit of quantification for artemisone was 31.3 ng/ml, and 15.6 ng/ml for artemiside. Quadratic (1/*x*, weighting) regressions were used to construct calibration curves. The accuracies (%Nom) for the artemisone calibration standards were between 94.1% and 103.3% and the precision (%CV) for the artemisone calibration standards was between 0.1% and 15.1% for the entire calibration range. The accuracies for the artemiside calibration standards were between 88.3% and 113.4% and the precision for the artemiside calibration standards was between 1.2% and 11.6% for the entire calibration range. No peaks were observed in the double blank samples (without analytes and internal standard), and no peaks were observed for the analytes in the blank sample (without analytes, but with internal standard). The methods performed well during all the sample batches and reproducible calibration curves could be constructed.

3.1. Oral artemisone formulations

The parameters of the oral reference and the oral Pheroid^{TM} vesicle group were calculated and are given in Table 4. The results are graphically presented in the form of an overlay of the mean concentration versus time graphs in Fig. 3.

The incorporation of artemisone in a Pheroid^{TM} vesicle formulation produced promising results. The T_{max} of artemisone, when incorporated in Pheroid^{TM} vesicles, was increased dramatically. When comparing the T_{max} of the two data sets, the reference formulation rendered a T_{max} of just 7 min (0.12 h) while a time delay was observed with the Pheroid^{TM} vesicle formulation for which the T_{max} was approximately 30 min (0.55 h) ($p < 0.0001$). The T_{max} of artemisone, when incorporated in the Pheroid^{TM} vesicle formulation, was delayed by a time-factor of 4 in comparison to that of the reference formulation. The C_{max} was greatly increased by the Pheroid^{TM} vesicle formulation, the reference formulation produced a C_{max} of 809.5 ng/ml while the Pheroid^{TM} vesicle formulation gave a C_{max} of 1550.0 ng/ml ($p < 0.005$). That amounts to an increase in artemisone's C_{max} by approximately 90% with the aid of the Pheroid^{TM} delivery system. The incorporation of artemisone in a Pheroid^{TM} vesicle formulation also had a major impact on the $T_{1/2}$ of the drug. The reference formulation had a $T_{1/2}$ of approximately 20 min ($T_{1/2}$ = 0.36 h) while the Pheroid^{TM} vesicle formulation extended the $T_{1/2}$ to more than 60 min ($T_{1/2}$ = 1.10 h) ($p < 0.005$). The difference amounts to an increase of more than 3-times that of the reference formulation.

The area under the concentration time curves (AUC) was determined. There was a significant difference between the two values (reference, $AUC_{0\text{-last}}$ = 458.7 ng h/ml and Pheroid^{TM}, $AUC_{0\text{-last}}$ = 2219.0 ng h/ml) ($p < 0.0001$). The $AUC_{0\text{-inf}}$ of the reference formulation was calculated as 604.6 ng h/ml and 3094.0 ng h/ml for the Pheroid^{TM} vesicle formulation ($p < 0.0001$). The relative absorption (RA) of the Pheroid^{TM} vesicle formulation was RA = 4.57 in comparison to the reference formulation which was represented by RA = 1.00. These calculated values imply that the Pheroid^{TM} vesicle formulation was absorbed 4.57 times better than the reference formulation, or when converted to percentage values, reasoning that the reference is represented by 100%, the Pheroid vesicle formulation was 357% better absorbed.

3.2. Oral artemiside formulations

The parameters of the oral reference formulation and the oral Pheroid^{TM} vesicle formulation are presented in Table 4. The results are graphically presented in the form of an overlay of the mean concentration versus time graphs in Fig. 4.

The incorporation of artemiside in a Pheroid^{TM} vesicle formulation produced less promising results. The T_{max} of artemiside, when incorporated in a Pheroid^{TM} vesicle formulation, was not extended as was the case with artemisone. When comparing the T_{max} values of the two data sets, the reference formulation had a T_{max} of approximately 30 min (0.55 h) while the Pheroid^{TM} vesicle formulation delayed the T_{max} to approximately 35 min (0.57 h) ($p > 0.05$). The C_{max} was also not greatly increased by the Pheroid^{TM} vesicle formulation, the reference formulation had a C_{max} of 116.4 ng/ml while the Pheroid^{TM} vesicle formulation had a C_{max} of 137.7 ng/ml ($p > 0.05$). The difference amounts to an increase in artemiside's C_{max} by approximately 18% with the aid of the Pheroid^{TM} delivery system. The data generated from this study did not lend itself to the accurate determination of either the $T_{1/2}$ or the $AUC_{0\text{-inf}}$. The reason for this may be due to the manner in which the elimination rate constant (λz) was calculated. A least squares regression was calculated using the last few points of the concentration curve, assuming that the elimination phase had been reached in the mouse. The slope of that regression then represents λz. Due to the fact that so few subjects were sampled, and due to some of the detected concentrations which did not return to zero, the estimation for certain subjects were exaggerated. The elimination rate constant for certain subjects could not be calculated due to that occurrence. For

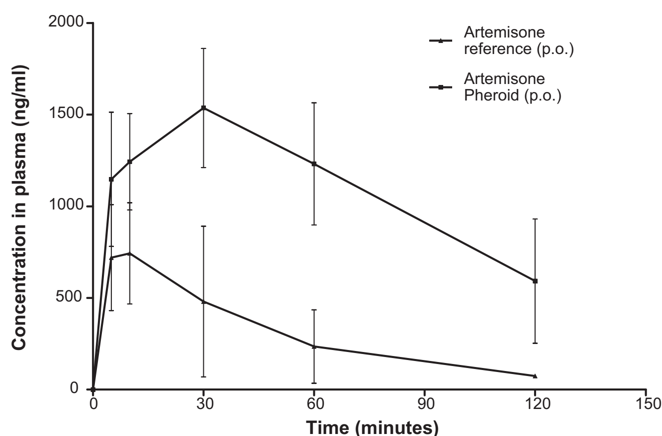

Fig. 4. Mean concentration versus time graph overlay of the oral artemiside reference and Pheroid^{TM} vesicle formulations.

Table 4
Summary of the pharmacokinetic parameters.

Formulation: 50.0 mg/kg p.o. and 5.0 mg/kg IV	Mean ± SEM				
	T_{max} (h)	C_{max}/Cp° (ng/ml)	$T_{1/2}$ (h)	$AUC_{0\text{-last}}$ (ng h/ml)	$AUC_{0\text{-inf}}$ (ng h/ml)
Artemisone (reference, p.o.)	0.12 ± 0.01	809.50 ± 98.12	0.36 ± 0.06	485.70 ± 106.30	604.60 ± 143.80
Artemisone (Pheroid™, p.o.)	0.55 ± 0.05	1550.00 ± 105.40	1.10 ± 0.26	2219.00 ± 122.70	3094.00 ± 392.10
Artemiside (reference, p.o.)	0.55 ± 0.19	116.40 ± 14.03	7.52 ± 4.84	174.20 ± 27.47	749.80 ± 387.40
Artemiside (Pheroid™, p.o.)	0.57 ± 0.09	137.70 ± 18.44	6.50 ± 4.46	211.20 ± 30.80	554.20 ± 212.4

example, the concentrations detected in the last three samples collected from subject 2 (Pheroid™ vesicle formulation) were 36.0, 36.0 and 34.0 ng/ml respectively. The values would not translate to the calculation of a useful $T_{1/2}$ or $AUC_{0\text{-inf}}$ value and consequently resulted in grossly inflated estimations. Nevertheless, the incorporation of artemiside in Pheroid™ vesicles did not have a major impact on the calculated $T_{1/2}$ of the drug, the reference formulation had a calculated $T_{1/2}$ of approximately 450 min ($T_{1/2}$ = 7.52 h) while the Pheroid™ vesicle formulation had a $T_{1/2}$ of approximately 400 min ($T_{1/2}$ = 6.49 h) ($p > 0.05$). The $AUC_{0\text{-inf}}$ of the reference formulation was calculated as 749.80 ng h/ml and 554.20 ng h/ml for the Pheroid™ vesicle formulation ($p > 0.05$).

The relative absorption of the Pheroid™ vesicle formulation was RA = 1.21 in comparison to the reference formulation which was represented by RA = 1.00. These calculated values imply that the Pheroid™ vesicle formulation was not significantly more absorbed when compared to the reference formulation. A summary of the results is given in Table 5.

4. Discussion

The results acquired with artemisone are very promising. The concentration versus time graphs obtained from the reference formulation and the Pheroid™ vesicle formulation data, in terms of the basic configuration and the well defined data points obtained during both the absorption and elimination phases of the drug, are robust. The C_{max} and T_{max} are also well defined in these graphs. The calculated C_{max} and T_{max} values correlate very well with the apparent corresponding points on the concentration versus time curve of both the reference and Pheroid™ formulations.

The reference formulation produced significantly lower C_{max} and T_{max} values than the Pheroid™ formulation. The difference may be due to the less favourable absorption characteristics of the reference formulation. The reference formulation appeared to form a micro-suspension after the addition of water to the DMSO-drug solution. It was previously reported that most artemisinins are known for their low aqueous solubility and resultant poor and erratic absorption upon oral administration (Wong and Yuen, 2001). It is possible that the Pheroid™ system was able to improve the solubility of artemisone and subsequently enhance the otherwise more erratic absorption characteristics associated with this drug class by encapsulating the drug in a lipophilic layer. This was achieved by first dissolving artemisone in the oil-phase of the Pheroid™ system and then adding the appropriate amount of nitrous oxide water to promote the formation of a lipophilic Pheroid™/drug complex (Velkov et al., 2005).

Table 5
Summary of the absorption results.

		Relative absorption (RA)
Artemisone	Reference	1.00
	Pheroid™	4.57
Artemiside	Reference	1.00
	Pheroid™	1.21

Various studies concerning the interaction of lipophilic compounds with intestinal fatty acid-binding protein (I-FABP) suggest that the binding of these lipophilic entities (Pheroid™ vesicles) to I-FABP may lead to an increase in the cytosolic solubility of those entities (Velkov et al., 2005). Thus increased cytosolic solubility may also subsequently facilitate the transport of the Pheroid™ vesicles from the intestinal lumen across the enterocytes to sites of drug distribution. This occurrence, in conjunction with the improved solubility of artemisone in the Pheroid™ vesicles, may explain the observed increase in the C_{max} value. The increase in C_{max} and T_{max} values explain why the $T_{1/2}$ had more than doubled and the absorption increased with the use of Pheroid™ technology. This did not hold true for the reference formulation which was not able to completely dissolve the drug nor was it able to provide the same, well defined, lipophilic characteristics as the Pheroid™ system to promote an interaction with I-FABP.

The results obtained with artemiside, when compared to artemisone, did not indicate any increase in absorption. Artemiside is more hydrophobic than artemisone, and the reference formulation also formed a precipitate with the addition of water to the DMSO-drug solution. The precipitate also had a more flaky appearance than the artemisone reference. The obtained data is the result of the poor solubility characteristics of the drug. The poor solubility probably caused erratic absorption which in turn led to irregularities in the concentration versus time curve which had relatively large SEM-values. Fig. 4 (reference graph) did not show a representative drug concentration value during the absorption phase. This may be attributed to the fact that the first samples were collected at 10 min instead of 5 min. Since no previous studies have been conducted on this drug in a mouse model which could otherwise be used as a reference, the sample intervals were chosen based on the relatively short $T_{1/2}$ history of the drug class. Another consideration was the small blood volume of the test animals. Only 5 samples could be collected from each animal. Thereafter, the reduction in blood volume may start to have a concentrating effect of the drug in the plasma due to the decrease in the blood volume as a result of the sample collection. The sample intervals were chosen to ensure the best possible utilization of the limited amount of samples. For future studies, the first samples should be collected at 2–5 min post administration rather than at 10 min. However, the T_{max} and C_{max} values in the reference formulation curve (Fig. 4) are still in relative concordance with the calculated values.

The drug concentration versus time curve of the artemiside Pheroid™ vesicle formulation was more favourable in terms of the basic configuration with definitive data points obtained during both the absorption and elimination phases of the drug. The C_{max} and T_{max} were both well defined on the corresponding graph. The calculated C_{max} and T_{max} values correlated well with the corresponding points on the concentration versus time curve of the Pheroid™ formulation. Artemiside was encapsulated by first dissolving the drug in the oil-phase of the Pheroid™ system and then adding the appropriate amount of nitrous oxide water to promote the formation of a lipophilic Pheroid™/drug complex (Velkov et al., 2005). Although a small improvement was observed in the results with the aid of this formulation in terms of the C_{max} and T_{max} over that of the reference there was not a very apparent increase in the absorption of

artemiside when administered in conjunction with Pheroid™ technology. The Pheroid™ vesicle formulation, however, took longer to reach its C_{max} in comparison to the reference formulation which made it possible to record definite data points during the absorption phase of the curve. This may probably be explained by the usually rapid conversion of most artemisinin derivatives to DHA or other metabolites when it is not protected against this rapid conversion processes (Haynes et al., 2006). It is very likely that the lipophilic attributes of the Pheroid™ system were able to partially protect the drug against this rapid metabolic conversion. This in turn would explain the increase in the T_{max} and C_{max} of artemiside, the first samples were collected at 10 min and represented the T_{max} for the reference formulation while the same samples of the Pheroid™ vesicle formulation represented a point during the absorption phase, thus suggesting a slight delay in absorption.

When comparing the drug absorption results of artemisone to that of artemiside the differences become very apparent. Both compounds were orally administered at a dose of 50.0 mg/kg and yet the artemisone Pheroid™ vesicle formulation provided a peak plasma concentration of 1500.0 ng/ml in contrast to the 137.0 ng/ml of the artemiside Pheroid™ vesicle formulation. This may be due to the differences in the metabolic conversion and degradation of these drugs. Most artemisinin class drugs are very quickly converted into DHA or other metabolites after oral administration while artemisone, in contrast to other artemisinins, is not converted into DHA. Artemisone is metabolized to the products M1–M5 (Haynes et al., 2006). The differences in metabolic products and the varied rapidity of the conversion rates may shed some light on the differences encountered in the results of the two compounds. However, further studies are required to fully elucidate the results.

5. Conclusion

Artemisone was proven to be relatively well absorbed. The results provide compelling evidence in favour of the ability of the Pheroid™ delivery system to further enhance the absorption of artemisone. The experiments indicated a very dramatic improvement in the C_{max} and $T_{1/2}$ of the drug and a time delay in T_{max} when administered in a Pheroid™ vesicle formulation. This effectively translates to a scenario where the drug concentration could be significantly decreased and still achieve therapeutic drug plasma concentrations. The combination of artemisone and Pheroid™ technology should prove to be an essential component in antimalarial combination therapy regimens in the very near future. The Pheroid™ delivery system did not produce such promising results with artemiside. Only a marginal increase was observed in the T_{max} and C_{max} values with no added benefit to the $T_{1/2}$ of the drug. The contrast between artemisone and artemiside may be attributed to varying solubility characteristics and to different metabolic pathways of the drugs.

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