College of Pharmacy[1], Lanzhou University; Department of Pharmaceutics[2], The First Hospital of Lanzhou University, Lanzhou; College of Pharmacy[3], China Pharmaceutical University, Nanjing; Hefei Con-source Medicine Technology Corporation[4], Hefei, P.R. China

Dihydroartemisinin loaded nanostructured lipid carriers (DHA-NLC): Evaluation of pharmacokinetics and tissue distribution after intravenous administration to rats

Xiaoyun Zhang[1], Hua Qiao[2], Jianping Liu[3], Haijun Dong[4], Chenlin Shen[4], Jingman Ni[1], Yanbin Shi[1], Ying Xu[1]

Received March 25, 2010, accepted March 30, 2010

Jianping Liu, College of Pharmacy, China Pharmaceutical University, Nanjing 210009, P.R. China
Liujianpingljp@hotmail.com

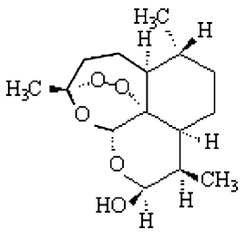
Pharmazie 65: 670–678 (2010) *doi: 10.1691/ph.2010.0082*

A simple and rapid LC-MS/MS method was established for the determination of dihydroartemisinin (DHA) in plasma and tissues of rats. Sample preparation was achieved by liquid–liquid extraction with aether and analysis was performed on LC-MS/MS in positive ion mode using electrospray ionization (ESI) as an interface. Target compounds were quantified in a single ion-monitoring (SIM) mode. DHA was monitored at m/z 267.1 and the internal standard finasteride at m/z 305.2. Chromatography was carried out using a Synergi fusion RP 80 column with a mixture of ethanol and 0.1% formic acid mixture (75:25) as the mobile phase. The pharmacokinetics and tissue distribution after intravenous administration of DHA in nanostructured lipid carrier (NLC) and in solution were then compared. The mean residence times (MRT) of the DHA-NLC was much longer than that of the DHA solution. In the tested organs, the AUC values of the DHA-NLC were higher than that of the DHA solution in liver, spleen, lung, brain and muscle, and lower than the DHA solution in heart and kidney. DHA-NLC prepared in this study is a promising sustained-release and drug-targeting system for antitumor drugs. It may also allow a reduction in dosage and a decrease in systemic toxicity.

1. Introduction

Dihydroartemisinin (DHA) is an artemisinin derivative with the C-10 lactone group replaced by a hemiacetal, and also the active metabolite of a number of artemisinin derivatives (O'Neill 2004). It is more effective than artemisinin against *P. falciparum* malaria parasites resistant to traditional antimalarial drugs (O'Neill and Posner 2004; White 2004; Longo et al. 2006). It is also indicated in the treatment of cerebral malaria. In recent years, DHA has also been shown to be effective against cancer cells (Efferth et al. 2004; Wu et al. 2005; Golenser et al. 2006; Ikuhiko et al. 2008). With the growing anti-tumor potential of DHA, there is an increasing demand for investigating its pharmacokinetics and tissue distribution. However, DHA is poorly water-soluble and has low bioavailability for oral administration due to slow drug dissolution and decompposion in stomach and intestine (Gabriëls and Plaizier 2004; Karbwang et al. 1997). Furthermore, half-life of DHA is very short (34–90 min) (Batty et al. 1996). It has been demonstrated that intravenous (IV) delivery of DHA results in the highest availability to the body as compared to all other routes and can lead to quick eradication of the malarial infection (Li et al. 1998). However, currently, no DHA product is available that enables IV delivery of DHA. Thus, the need of hour is to have an IV formulation of DHA. Lipid nanocarriers such as liposomes, nanoemulsions and nanoparticles have demonstrated a great potential in improved parenteral delivery of the hydrophobic agents since last two decades. The usefulness of liposomes and nanoemulsions for the improved delivery of antimalarial agents such as chloroquine (Owais et al. 1995) and primaquine (Dierling and Cui 2005) has been established. Recently, different polymeric, liposome based and lipid nanoparticle based approaches for the treatment of parasitic diseases have been reviewed (Date et al. 2007). In our lab (Zhang et al. 2010), the usefulness of second-generation lipid nanocarriers such as nanostructured lipid carriers (NLC) has been established for the parenteral delivery of DHA. NLC are lipid nanocarriers based on a mixture of biocompatible solid lipid and liquid lipid (oil) (Müller et al. 2002; Müller and Keck 2004; Alvarez et al. 2004; Schäfer-Korting et al. 2007; Blasi et al. 2007; Lin et al. 2007; Wissing et al. 2004). NLC have been regarded as an alternative to liposomes and nanoemulsions due to various advantages such as ease of manufacture, particulate nature, high drug loading and ability to sustain the release of the drug. Furthermore, the aqueous nature of NLC, their nanostructure and the biocompatibility of the excipients would enable IV delivery of the therapeutic agents without significant pain on injection. Additionally, the ability of NLC to sustain the delivery of therapeutic agents could be useful in combating the recrudescence which is commonly observed with monotherapy (Devries and Dien 1996). In view of this, NLC appeared to be a novel approach for improving the delivery of DHA. In the present investigation, DHA-NLC

have been formulated by using biocompatible excipients and were evaluated *in vivo* in comparison to a conventional solution formulation.



Since DHA do not have ultraviolet or fluorescent chromophores, analysis of the drug in biological fluids has been proven difficult. The pharmacokinetics of DHA have been previously characterized by HPLC using post column alkali decomposition (Batty et al. 1996) and electrochemical detection (ECD) (Bangchang et al. 1998; Navaratnam et al. 1997; Lai et al. 2007; Sandrenan et al. 1997). However, ECD methods are difficult to use routinely due to the very rigorous conditions that should be applied to prevent dissolved oxygen from entering the flowcell. In the literature, Liquid chromatography–mass spectroscopy (LC-MS), using different modes of ionization, has been described for the quantitation of DHA (Himanshu et al. 2005; Sabarinath et al. 2003; Souppart et al. 2002; Rajanikanth et al. 2003; Xing et al. 2007). A thermospray mass spectrometric method (Chi et al. 1999) is also described for the determination of DHA (as a metabolite of artemether) in human plasma. More recently a gas chromatography-mass spectrometric method (Mohamed et al. 1991) was described for the determination of DHA in human plasma. In the present work, a more sensitive, simple and rapid method with shorter analysis time (5 min/per sample) using LC-MS/MS has been developed for quantitation of DHA in rat plasma and tissues after intravenous administration of DHA-NLC and DHA solution at a dose of 10 mg/kg. The method was successfully applied to investigate the pharmacokinetics and the tissue distribution of DHA-NLC and DHA solution in rats.

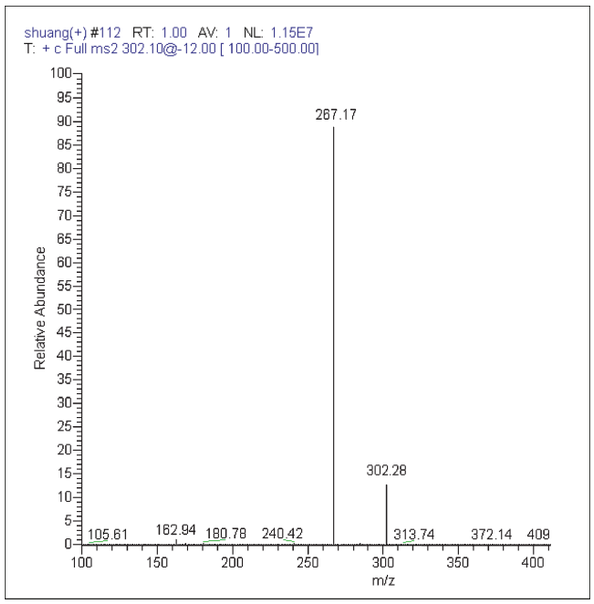
2. Investigations, results and discussion

2.1. Mass spectral analysis

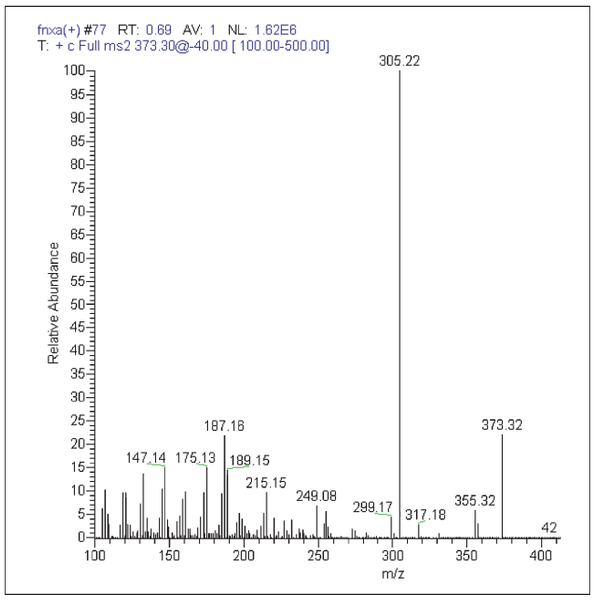
The atmospheric pressure chemical ionization (APCI) detection was firstly applied but no predominant ion with enough intensity was found for DHA. Therefore, a more intensive ionization was tested and electrospray ionization (ESI) was selected. However, when DHA was injected directly into the mass spectrometer along with the mobile phase with a positive ion interface, the protonated molecules $(MH)^+$ of DHA were not seen in abundance. A fragment of the DHA was observed at m/z 267.1 while for internal standard we were able to detect the fragment mass of m/z 305.2. The mass spectrometric parameters were optimized to obtain the best signal for the selected ions m/z 267.1 and m/z 305.3. The method was fully validated using those ions and parameters. The full scan mass spectra for DHA and finasteride is shown in Fig. 1 a and b, respectively.

2.2. Separation and relative retention time

Observed retention times were 3.95 and 4.16 min for DHA and IS, respectively, with a total run time of 5 min. Under these chromatographic conditions, blank plasma and tissues were tested for endogenous interference. No additional peaks due to endogenous substances were observed that would interfere with the detection of compounds of interest. Typical chromatograms are shown in Fig. 2a. b and c.



(a)

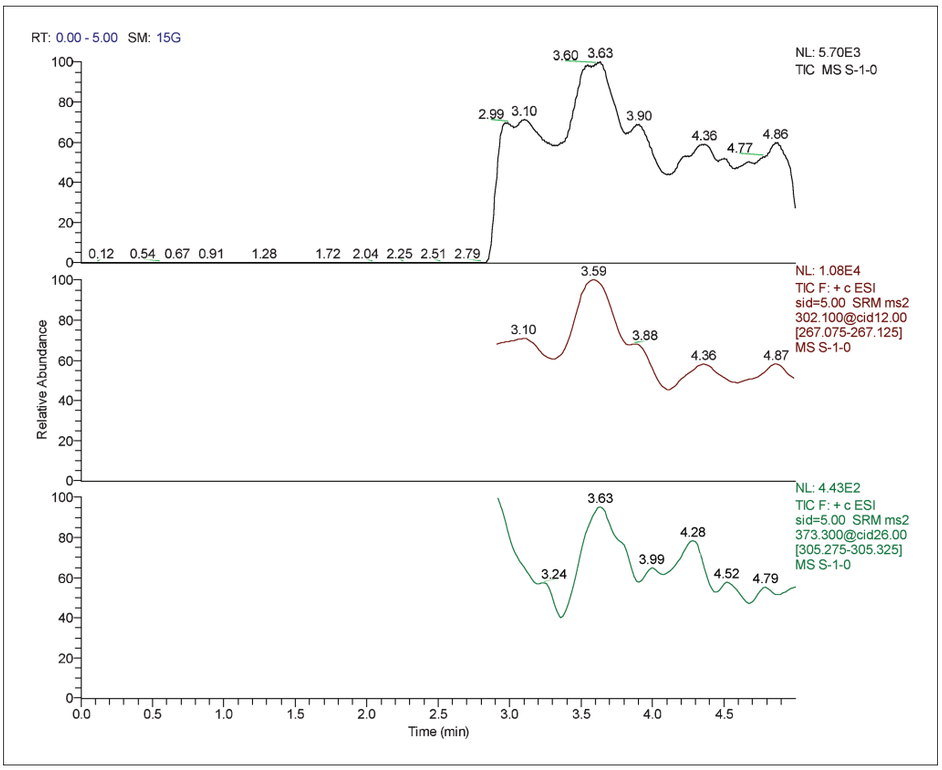


(b)

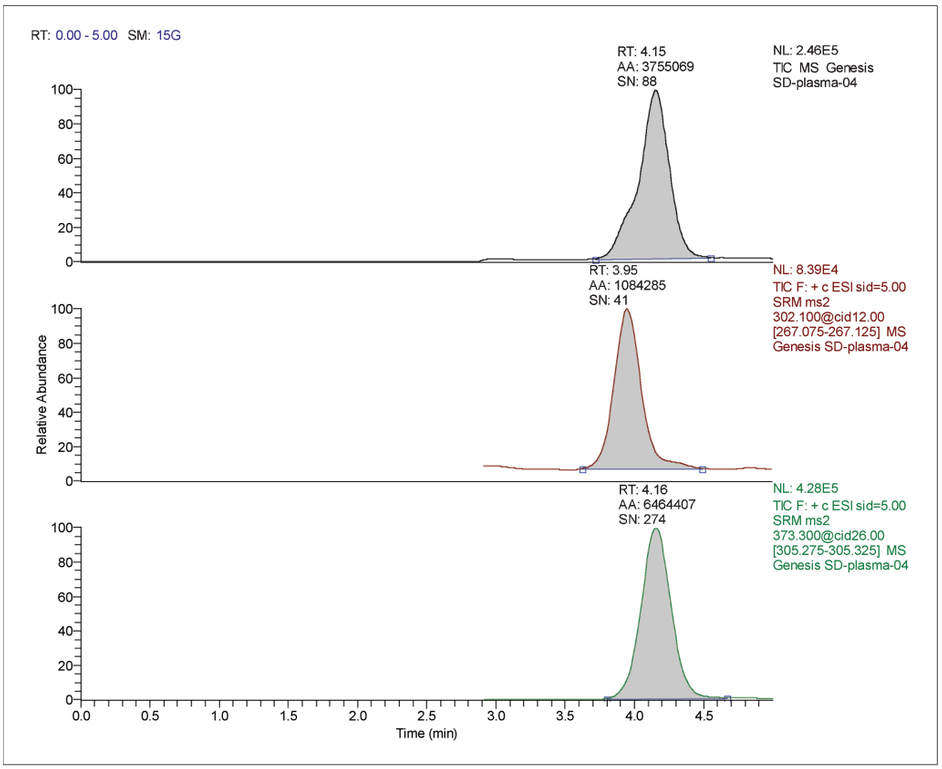
Fig. 1: Full mass spectra scan for dihydroartemisinin (a) and internal standard (b)

2.3. Linearity, limit of quantification and limit of detection

The standard curves of the DHA concentration (Y) to the peak-area ratio of DHA to IS (X) are listed in Table 1. The calibrations were linear over a certain range in all biosamples with a correlation coefficient (r) larger than 0.9953. The limit of quantification (LOQ) was defined as the lowest concentration at which both precision and accuracy were less than 20%. The current assay offered an LOQ of 2 ng/mL in plasma, 0.5 ng/g in tissues ($n = 6$), respectively. Usually, the limit of detection (LOD) could be determined at a signal to noise ratio (S/N) of 3. The LOD of current assay was 0.6 ng/mL in plasma and 0.15 ng/g in tissue.

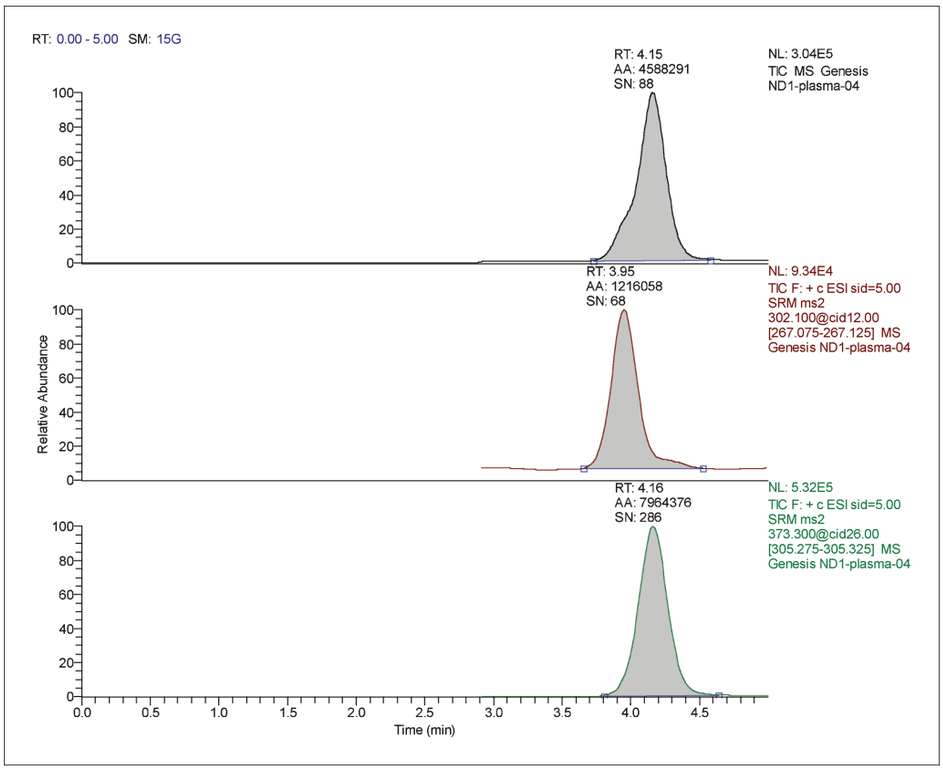


(a)



(b)

Fig. 2: Representative chromatograms of: (a) black plasma; (b) a black plasma sample spiked with dihydroartemisinin and IS (finasteride); (c) plasma sample after a single i.v. administration of dihydroartemisinin NLC at a dosage of 10 mg/kg. □: total ion current;□: dihydroartemisinin; □: finasteride



(c)

Fig. 2: (*Continued*)

.

Table 1: Calibration curves for DHA in plasma and tissues

Biosamaples	Calibration curves	Correlation coefficients (r)	Linear ranges (ng g^{-1})
Plasma	$Y = 0.342 + 512.85X$	0.9996	2∼4000 (ng mL^{-1})
Heart	$Y = 0.057 + 12.899X$	0.998	0.5∼200
Liver	$Y = 0.038 + 12.967X$	0.9961	0.5∼200
Spleen	$Y = 0.009 + 16.016X$	0.9953	0.5∼200
Lung	$Y = 0.045 + 17.995X$	0.9972	0.5∼200
Kidney	$Y = 0.086 + 15.742X$	0.9961	0.5∼200
Brain	$Y = 0.046 + 16.502X$	0.9969	0.5∼200
Muscle	$Y = 0.080 + 16.677X$	0.9985	0.5∼200

2.4. Precision and accuracy

The precision and accuracy of the method are summarized in Table 2. The validation of the sample preparation and LC-MS/MS procedure in plasma and different tissues demonstrated that the method is accurate and precise with coefficients of variation within- and between-batch below15% for all the samples.

2.5. Recovery

The mean recoveries ($n = 5$) for DHA (5, 20, 200 and 3000 ng mL^{-1}) in plasma were $83.64 \pm 13.72\%$, $88.97 \pm 11.66\%$, $89.50 \pm 12.27\%$ and $86.31 \pm 11.95\%$, respectively. Mean recovery ($n = 5$) for internal standard was $99.86 \pm 5.36\%$.

2.6. Stability

Results of stability tests are shown in Table 3. DHA was shown to remain stable in plasma at room temperature for at least 4 h and can undergo at least three-freeze-thaw cycles. Long term stability of DHA in rat plasma was confirmed for at least 2 months at $-80\,^{\circ}$C.

2.7. Pharmacokinetics study

Plasma pharmacokinetic parameters of DHA-NLC formulation were compared to those of a DHA solution in rats. The plasma level of DHA was detectable up to approximately 12 h after intravenous administration of DHA-NLC or DHA solution. The mean plasma concentration-time profiles are shown in Fig. 3. The pharmacokinetic parameters and the compartment model were analyzed by software program DAS 2.0. The results show that the plasma concentration-time curves of both DHA-NLC and DHA solution fit the open two-compartment model. The relevant pharmacokinetic parameters are listed in Table 4. Following intravenous administration of the DHA solution, the mean measured peak plasma concentration achieved was 917.51 ng/mL and for DHA-NLC it was 289.28 ng/mL.

Table 2: Precision and accuracy data for DHA in plasma and tissues ($n = 6$)

Biosamples	Added concentration (ng mL^{-1})	Found concentration (ng mL^{-1})	Accuracy (%)	Precision (%)	
				Within-batch	Between-batch
Plasma	5	4.69	93.80	3.17	2.92
	20	19.55	97.73	6.42	6.91
	200	183.09	91.54	6.44	4.83
	3000	2672.97	89.10	5.79	6.11
Heart	1	1.00	100.25	9.52	7.74
	10	10.13	101.34	6.56	5.62
	150	142.59	95.06	7.01	4.57
Liver	1	1.06	106.39	2.32	9.15
	10	11.61	116.11	2.79	3.74
	150	155.70	103.80	4.02	5.41
Spleen	1	0.99	99.47	3.60	2.92
	10	10.00	99.95	7.28	5.87
	150	139.58	93.06	6.08	7.03
Lung	1	0.98	98.41	7.77	6.89
	10	10.00	99.96	3.06	5.48
	150	136.09	90.72	1.53	3.12
Kidney	1	0.96	95.64	9.82	8.41
	10	9.90	98.97	0.96	2.34
	150	142.60	95.07	2.12	3.81
Brain	1	1.10	110.06	3.78	5.20
	10	10.84	108.45	5.41	8.31
	150	150.75	100.50	5.53	6.52
Muscle	1	0.91	90.80	5.92	2.13
	10	9.35	93.46	5.39	5.44
	150	131.90	87.93	2.31	4.38

Table 3: Stabilities data of DHA in rat plasma ($n = 3$)

Storage conditions	Nominal concentration (ng mL^{-1})	Mean measured concentration (ng mL^{-1})	Mean recoveries	RSD (%)
Room temperature storage for 4 h	5	4.67	83.51	14.71
	20	18.32	89.73	8.44
	200	179.68	90.20	11.31
	3000	2849.55	81.75	9.27
-80 °C for 2 months	5	4.39	80.72	14.93
	20	17.97	91.38	6.82
	200	183.63	90.44	7.53
	3000	2692.51	79.61	8.92
-80 °C for 2 months (three freeze-thaw cycles)	5	4.57	78.37	12.35
	20	18.44	88.45	8.84
	200	189.64	87.39	7.59
	3000	2783.52	83.56	10.26

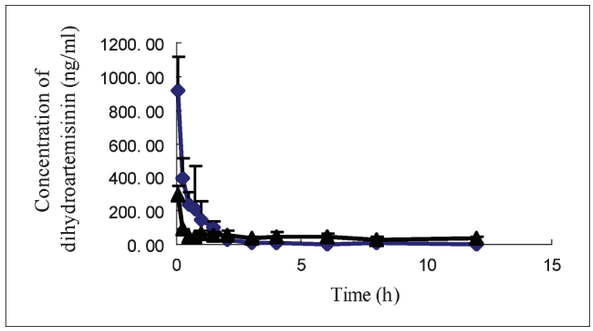


Fig. 3: Mean plasma concentration–time curves of dihydroartemisinin in rat after intravenous administration of dihydroartemisinin NLC and dihydroartemisinin solution at a dose equivalent to 10 mg/kg of dihydroartemisinin ($n = 3$). (▲) dihydroartemisinin solution and (▲) dihydroartemisinin NLC

After intravenous administration of the DHA-NLC, free DHA was available for solvation and might have solubilized in plasma. Subsequently, DHA from solution distributed rapidly compared to distribution of DHA entrapped in nanostructured lipid carriers. The initial plasma concentration (at 2 h) was lower for the DHA-NLC than for the DHA solution following intravenous administration, possibly because DHA was released slowly from NLC for an extended period of time and free drug was available for distribution only after its release. Therefore, DHA-NLC showed lower initial serum concentrations than in case of DHA solution. After 2 h, the plasma concentration was lower for the DHA solution than that for DHA-NLC because of its solubility in plasma ensuing rapid distribution, elimination and slower release of DHA from NLC leading to lower clearance.

Plasma kinetics of DHA-NLC and DHA solution shown in Table 4 with a higher AUC and lower rates of clearance may be due to slower distribution of DHA incorporated in NLC than from DHA solution. From Table 4, we can see that the

Table 4: Pharmacokinetic parameters of DHA-NLC and solution in rat plasma following intravenous adminitration ($n = 3$)

Parameters	Definitions	Units	DHA-NLC	DHA solution
A	Hybrid parameter	$ng\,mL^{-1}$	616.59*	1492.24
α	Distribution rate constant	h^{-1}	11.61	12.58
B	Hybrid parameter	$ng\,mL^{-1}$	54.25**	429.00
β	Elimination rate constant	h^{-1}	0.05**	1.08
$t_{1/2\alpha}$	Half-life for the distribution phase	h	0.06	0.06
$t_{1/2\beta}$	Half-life for the elimination phase	h	14.35**	0.65
V_c	Apparent volume of distribution	$(mg\,kg^{-1})/(ng\,mL^{-1})$	14.91*	5.21
CL_s	Total body clearance	$mg\,kg^{-1}/h\ (ng\,mL^{-1})$	7.23*	15.77
$AUC_{(0\text{-}t)}$	Area under the curve	$ng\,mL^{-1}\,h^{-1}$	555.21	552.08
$AUC_{(0\text{-}\infty)}$	Area under the curve	$ng\,mL^{-1}\,h^{-1}$	1382.45*	633.97
$MRT_{(0\text{-}\infty)}$	Mean residence time	h	25.99**	0.98
K_{10}	First-order elimination rate constant	h^{-1}	0.49**	3.03
K_{12}	Transport rate constant from central compartment to periphery compartment	h^{-1}	11.17	10.63
K_{21}	Transport rate constant from periphery compartment to central compartment	h^{-1}	0.98*	3.64
C_{max}	Concentration of maximum	$ng\,mL^{-1}$	289.28*	917.51

** $P < 0.01$. * $P < 0.05$.

$AUC_{(0\sim\infty)}$ increased from 633.97 ng/mL/h for the DHA solution to 1382.45 ng/mL/h for the DHA-NLC. However, the clearance decreased from 15.77 to 7.23 (mg/kg/h·(ng/mL)) accordingly. We also observe that the MRT value of the DHA-NLC (25.99 h) is higher than that of DHA solution (0.98 h). Increased MRT was due to the presence of Poloxamer188 on the surface of nanoparticles and the slow release of DHA from the NLC (Manjunath and Venkateswarlu 2005). This observation was in agreement with some previous studies, for example, MRT of camptothecin SLNs increased around 18 times compared to solution of camptothecin administered intravenously (Yang et al. 1999).

As is shown in Table 4, the distribution half-life of DHA-NLC (0.06 h) was equal to that of DHA solution (0.06 h), suggesting that DHA-NLC was taken up by tissues the same way as DHA solution. The volume of distribution of DHA-NLC was 14.91 ((mg/kg)/(ng/mL)) and this was considerably larger than that (5.21) of the DHA solution. Furthermore, CLs of DHA solution was higher than that of DHA-NLC, which suggested that NLC is an effective sustained-release drug delivery system.

2.8. Tissue distribution study

The concentrations of DHA were determined in various tissues of rats such as heart, liver, spleen, lung, kidney, brain and muscle and the data of tissue distributions of DHA-NLC and DHA solution after intravenous adminitration at different time points are

Table 5: Distribution of DHA-NLC and DHA solution in tissues after a single intravenous administration to rats

	Concentration ($ng\,g^{-1}$, $\overline{X} \pm SD$, $n = 3$)			
	DHA solution	DHA-NLC	DHA solution	DHA-NLC
Organ	15 min		30 min	
Heart	282.29 ± 19.61	172.12 ± 19.91	188.1 ± 55.70	177.56 ± 21.79
Liver	988.77 ± 52.41	877.98 ± 65.86	753.41 ± 53.69	902.37 ± 9.78
Spleen	10.34 ± 3.57	29.22 ± 3.32	28.98 ± 12.46	71.54 ± 6.68
Lung	35.49 ± 3.95	178.19 ± 20.46	119.50 ± 21.45	207.15 ± 34.30
Kidney	18.16 ± 1.00	2.49 ± 4.48	62.01 ± 5.78	24.88 ± 8.23
Brain	5.66 ± 2.59	36.15 ± 10.53	54.20 ± 5.68	141.36 ± 24.89
Muscle	3.50 ± 1.38	24.31 ± 7.38	12.19 ± 0.11	52.67 ± 2.01
Organ	60 min		120 min	
	DHA solution	DHA-NLC	DHA solution	DHA-NLC
Heart	141.22 ± 38.48	119.37 ± 18.36	133.64 ± 3.30	81.99 ± 8.00
Liver	663.99 ± 16.96	692.92 ± 18.13	393.88 ± 18.80	335.84 ± 16.69
Spleen	15.97 ± 1.29	39.00 ± 8.72	5.52 ± 2.61	36.48 ± 8.59
Lung	35.22 ± 8.08	90.78 ± 13.85	13.51 ± 8.59	41.74 ± 20.55
Kidney	36.02 ± 5.78	15.55 ± 7.88	22.33 ± 4.59	18.76 ± 6.11
Brain	26.67 ± 3.21	69.54 ± 5.14	23.79 ± 6.01	29.31 ± 7.64
Muscle	26.63 ± 1.65	52.75 ± 2.01	23.41 ± 2.59	47.25 ± 3.39
Organ	240 min		360 min	
	DHA solution	DHA-NLC	DHA solution	DHA-NLC
Heart	44.13 ± 3.46	69.78 ± 5.89	17.93 ± 7.68	28.73 ± 7.29
Liver	167.97 ± 2.42	96.63 ± 13.67	81.10 ± 8.67	17.07 ± 13.39
Spleen	5.13 ± 0.49	17.60 ± 8.68	4.21 ± 3.36	4.39 ± 3.58
Lung	17.09 ± 3.64	39.53 ± 5.24	14.35 ± 7.97	23.00 ± 2.70
Kidney	17.24 ± 6.19	11.95 ± 6.33	11.05 ± 5.67	6.65 ± 3.76
Brain	6.65 ± 1.85	18.95 ± 7.11	5.62 ± 1.56	10.52 ± 1.88
Muscle	22.26 ± 2.89	22.63 ± 5.99	15.94 ± 1.59	20.21 ± 1.45

Table 6: Pharmacokinetic parameters of DHA-NLC and DHA solution in various organs of rat after intravenous adminitration ($n = 3$)

Organs	DHA formulations	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	K_{10} (h^{-1})	$AUC_{(0\text{-}t)}$ (ng/g/h)	$AUC_{(0\text{-}\infty)}$ (ng/g/h)	V_c ((mg/kg)/(ng/g))	CL_s (mg/kg/h/(ng/g))
Heart	DHA-NLC	0.53**	3.45	0.34**	531.91	694.57	6.32**	14.90
	DHA solution	0.06	2.01	41.11	630.72	774.00	43.85	13.07
Liver	DHA-NLC	0.66	7.10**	134.22**	2302.87	2764.82	4.64	3.62
	DHA solution	0.88	1.29	0.46	2107.15	2399.35	9.05	4.17
Spleen	DHA-NLC	2.58	16.61**	0.28	411.48*	556.03*	184.74*	51.49*
	DHA solution	1.62	2.58	0.34	172.41	219.03	469.68	161.87
Lung	DHA-NLC	0.72*	12.44	219.03	411.48*	556.03*	38.90**	18.01*
	DHA solution	1.56	17.87	172.41	172.41	219.03	130.76	46.88
Kidney	DHA-NLC	8.41*	18.51	0.14	74.71	118.92	813.83*	121.07*
	DHA solution	2.85	10.88	0.22	141.50	197.00	241.65	54.04
Brain	DHA-NLC	1.85	2.86	0.36	232.59	268.08*	106.23*	38.75
	DHA solution	1.89	2.96	0.27	101.16	119.55	309.80	83.65
Muscle	DHA-NLC	5.92	36.32	0.14**	202.18	328.23	214.66*	30.89
	DHA solution	5.92	31.51	0.06	102.30	292.22	621.40	40.08

** $P < 0.01$. * $P < 0.05$

summarized in Table 5. At 30 min after administration both of DHA-NLC and DHA solution to rats, the highest level of DHA was observed in tested tissues except for the heart. The concentration of DHA in the liver was dramatically higher than that in other tissues tested. Pharmacokinetic parameters of DHA-NLC and DHA solution in various organs of rats are listed in Table 6. The results showed that AUC values of DHA-NLC were higher than that of the DHA solution in liver, spleen, lung, brain and muscle; and lower than that of the DHA solution in heart and kidney. Compared to the DHA solution, targeting efficiency was increased in liver, spleen, lung and brain, but decreased in heart and kidney when DHA-NLC was administered intravenously.

It is clear that the AUC for DHA-NLC was lower than that for DHA solution in the heart, which indicated that targeting efficiency in heart of the DHA-NLC was lower. As a result DHA-NLC can decrease cardiac accumulation toxicity as well as side effects of NLC on the heart.

The AUC in the spleen which is rich in reticuloendothelial cells system (RES), was much higher for DHA-NLC than for DHA solution. The comparison of other parameters of the spleen showed that DHA-NLC resulted in higher $t_{1/2\alpha}$ and $t_{1/2\beta}$ but lower K_{10}, V_c and CL_s values than DHA solution. These results indicated that encapsulation of DHA in NLC might promote the phagocytosis of the molecule by mononuclear phagocytic cells.

The AUC of DHA from NLC in lung was higher than that of DHA from solution, indicating that incorporation of DHA in NLC increases lung uptake of the molecule. Furthermore, the $t_{1/2\beta}$ (12.44 h) in the lung for DHA from NLC was substantially shorter than that for DHA from solution (17.87 h), suggesting that DHA-NLC was cleared from the lung more rapidly than DHA solution. Encapsulation of DHA within NLC may therefore significantly reduce accumulation of DHA in the lung.

The pharmacokinetic parameters in the kidney indicated that both DHA-NLC and DHA solution were primarily cleared from the kidney. In this tissue, the CL_s of DHA-NLC was 2.24 times higher than that of DHA solution whilst the V_c value of DHA-NLC was 3.37 times higher than that of DHA solution. Thus, DHA-NLC is more rapidly eliminated from the kidney, DHA solution has a longer residence time in the kidney. Targeting efficiency to the kidney of DHA-NLC was lower. DHA-NLC can decrease renal accumulation and reduce the toxicity and side effects of the drug on the kidney.

DHA could be detected in the brain after intravenous injection of both DHA-NLC and DHA solution, suggesting that DHA could penetrate the blood brain barrier. DHA-NLC gave a slightly higher brain AUC but markedly lower brain V_c, in comparison to DHA solution. Hence, NLC encapsulation of DHA may be able to enhance dmg penetration into the brain.

The distribution of DHA in the muscle is high. Similar values of CL_s in the muscle were obtained for DHA-NLC and DHA solution. The AUC of DHA-NLC was slightly greater than that of DHA solution in the muscle, but the $t_{1/2\alpha}$ and $t_{1/2\beta}$ values for both DHA-NLC and DHA solution were equivalent, suggesting that NLC encapsulation of DHA did not alter DHA pharmacokinetics in the muscle.

3. Experimental

3.1. Material

Dihydroartemisinin (DHA, 99.0% purity) was purchased from Shaanxi Sciphar Biotechnology Co., Ltd., China. Finasteride was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and used as internal standard (IS). Glycerol monostearate (MS) was purchased from Sinopharm Chemical Reagent Co., Ltd., China. Miglyol® 812 (caprylic/capric triacylglycerols, Sasol, Germany) and Lutrol® F 68 (Poloxamer 188, BASF, Germany) were kindly donated from Beijing Fengli Jingqiu Commerce and Trade Co., Ltd., China. Tween 80 (CRODA, Great Britain) was purchased from Shanghai Chemical Reagent Co., Ltd., China. Methanol and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Analytical grade formic acid was purchased from DIMA, America. Ethanol, acetone and other chemicals were of analytical reagent grade. Ultra-pure water was purified by Milli-Q Plus water system (Millipore Corporation, Bedford, MA, USA).

3.2. Preparation of DHA-NLC

DHA-NLC was prepared by solvent diffusion method (Zhang et al. 2010). MS and Miglyol® 812 were used as solid lipid material and liquid lipid material, respectively. Briefly, MS, Miglyol® 812 and drug were dissolved in 5 ml mixed organic solvent of ethanol and acetone (1:1, v/v) in a water bath at 55 °C. The resultant organic solution was quickly dispersed into 20 ml aqueous solution of Tween80 (1% (W/V)) and Poloxamer188 (1% (W/V)) at room temperature (25 °C) under mechanical agitation (DC-40, Hangzhou Electrical Engineering Instruments, China) with 3000 rpm for 30 min until NLC suspensions were obtained.

3.3. Animals

Male Wistar rats (provided by Lanzhou University Animals Center, body weight 330–350 g) were used for pharmacokinetics and tissues distribution studies. The rats were fasted overnight with free access to water prior to the beginning of the study. All protocols and procedures were approved by the Lanzhou University Animal Care and Use Committee.

3.4. Preparation of standard and quality control samples

Standard stock solutions of DHA and finasteride were prepared in methanol. DHA and finasteride were weighed on a SARTORIUS AG electronic analytical balance (Beijing, China). Appropriate amounts of drugs were dissolved using methanol in volumetric flasks to make a 1 mg/mL stock solution of each. A series of standard solutions with a concentration in the range of 0.02∼40.00 μg/mL were prepared by serial dilution of a stock solution with methanol. All solutions were stored at -80 °C and brought to room temperature before use. To prepare the standard calibration samples, DHA standards at the concentration of 2, 5, 10, 30, 70, 200, 500, 1500 and 4000 ng/mL in plasma or 0.5, 1.5, 3.5, 10, 25, 75 and 200 ng/g in tissue homogenates were prepared by adding 10 μL of DHA stock solution to 100 μL plasma or different tissue homogenates of normal rats. Quality control (QC) samples, which were used for validation, were prepared in the same way as the standard calibration samples. The solution of internal standard (2 μg/mL) was also prepared in methanol. The stock solution was stored in a ULT 2586-5-A14 freezer (Revco scientific, Asheville NC, USA) at −80 °C.

3.5. Method validation

The procedures and criteria used for validation of the method were based on the FDA guidance document on bioanalytical method validation. The degree of interference by endogenous substances was assessed by inspection of chromatograms derived from processed blank and rat samples.

The accuracy and intra- and inter-batch precisions of the method was evaluated for QC samples from six replicates and multiple batch analyses of QC over the linear concentration range of the method. Intra-batch accuracy and precision was determined on a single day from six independent preparations of QCs. Additional inter-batch precision and accuracy was determined by performing the intra-batch accuracy on three separate days and calculating the accuracy and precision for all QC samples at each concentration. The intra- and inter-batch precisions were expressed by relative standard deviation (RSD).

The recovery of DHA was tested in duplicate at four concentration levels (5, 20, 200 and 3000 ng/mL) by comparing the peak areas from extracted plasma sample with those found by direct injection of standard solutions at the same concentration.

Stability of DHA was evaluated using QC samples after storage at -80 °C for 2 months, after three-freeze-thaw cycles, and for unprocessed samples after 4 h at room temperature by running the QC samples along with freshly prepared standards.

3.6. Drug administration and sampling

DHA was dissolved in a mixture of Tween 80, dimethyl sulphoxide, ethanol and 0.9% sodium chloride solution (25:40:20:15 w/w/w/w) by ultrasonic processing in ultrasonic cleaner (SB5200DTD, China) to obtain DHA solution with DHA concentration of 10 mg/mL. For pharmacokinetics study, rats were randomly divided into two groups with three rats in each group. Group 1 was treated with DHA solution while group 2 with the DHA-NLC. Each preparation was injected through the tail vein at the DHA dose of 10 mg/kg. After intravenous injection, the rats were anaesthetized with ether and blood samples were collected by retro-orbital venous plexus puncture at the specific schedule of 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 12 h, respectively. Plasma was obtained by centrifugation at 4500 rpm for 10 min and stored at -80 °C before analysis. Blank plasma was collected by the same method before rats were administrated.

For the tissue distribution study, 36 rats were assigned randomly to two groups and received DHA solution and DHA-NLC via a tail vein injection at a single dose of 10 mg/kg, respectively. For each preparation and each sampling time point, 3 rats were killed. Plasma, heart, liver, spleen, lung, kidney, brain and muscle were obtained 0.25, 0.5, 1, 2, 4 and 6 h after intravenous administration, respectively. Tissue samples were put into a normal saline solution to remove the blood or content, blotted on filter paper, and then weighed and homogenized in saline solution (0.5 g/mL). The obtained tissue homogenates were used for analysis.

3.7. Sample extraction procedure

To 100 μL of the above plasma or tissue homogenates, 10 μL of internal standard (finasteride) and 1 mL (for plasma) or 3 mL (for tissue) of ether were added. The resulting mixtures were thoroughly vortex-mixed for 3 min followed by centrifugation at 14,000 rpm for 10 min. The supernatant was collected and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was then reconstituted with 100 μL mobile phase and centrifuged at 14,000 rpm for 5 min, and an aliquot (10 μL) of the supernatant was injected into the LC-MS/MS system for analysis.

3.8. Chromatographic and MS conditions

The chromatography was carried out on a Finnigan Surveyor MS Pump Plus LC-MS/MS mass spectrometer (TSQ QUANTUM Discovery MAX, Thermo, USA) composed of a vacuum degasser (Finnigan Surveyor), a quarternary pump (Finnigan Surveyor), a thermostated autosampler (Finnigan Surveyor Autosampler Plus) and a thermostated column compartment (Finnigan Surveyor). The injection was made with a Finnigan Surveyor automatic injector.

The mass selective detector was coupled with an electrospray ionization interface (ESI) and on-line nitrogen generation system (Thermo, USA). The analysis was achieved using an Xcalibur1.4 Data Processing System. The mobile phase used for the analysis consisted of formic acid (0.1%): methanol (25:75, v/v), filtered and degassed completely with vacuum for approximately 15 min before use to prevent bubbles or impurities entering the system. The mobile phase was delivered at a flow rate of 0.25 mL/min. The analysis was carried out using a Synergi fusion RP 80 HPLC column, 4 μm, 150 mm × 2.0 mm (Phenomenex, USA) using a guard column (Phenomenex, USA) with C-12 max-RP cartridges.

3.9. Optimization of the MS parameters

An ESI was used operating in the positive ion mode. The optimized conditions for DHA and finasteride were as follows: Spray Voltage was set at 4.8kV; Capillary Temperature was 320; Skimmer offset was set at -5V; Sheath Gas Pressure was 30Arb; Aux Gas Pressure was 3Arb; Collision Pressure was 1.5 Arb. Target compounds were quantified in a single ion-monitoring (SIM) mode. DHA was monitored at m/z 267.1 while finasteride at m/z 305.2.

3.10. Data analysis

The pharmacokinetic parameters of DHA in rats were calculated by the pharmacokinetic software DAS 2.0 (Drug and statistics 2.0, the Committee of the Mathematic Pharmacology, the Chinese Society of Pharmacology, Hefei, China). Statistical comparisons between groups were made using single-factor ANOVA and multiple comparisons were performed using t-test for independent groups assuming equal variances within each group.

Acknowledgement: We gratefully acknowledge the analysis technological support of Hefei Con-source Medicine Technology Corporation of China.

References

Alvarez-Román R, Naik A, Kalia YN, Guy RH, Fessi H (2004) Skin penetration and distribution of polymeric nanoparticles. J Control Release 99: 53–62.

Bangchang KN, Congpuong K, Hung LN, Molunto P, Karbwang J (1998) Simple high-performance liquid chromatographic method with electrochemical detection for the simultaneous determination of artesunate and dihydroartemisinin in biological fluids. J Chromatogr B 708: 201–207.

Batty KT, Davis TM, Thu LT, Binh TQ, Anh TK, Ilett KF (1996) Selective high-performance liquid chromatographic determination of artesunate and α- and β- dihydroartemisinin in patients with falciparum malaria. J Chromatogr B 677: 345–350.

Blasi P, Giovagnoli S, Schoubben A, Ricci M, Rossi C (2007) Solid lipid nanoparticles for targeted brain drug delivery. Adv Drug Deliv Rev 59: 454–477.

Chi HT, Ramu K, Baker JK, Hufford CD, Lee IS, Lin ZY, McChesney JD (1991) Identification of the *in vivo* metabolites of the antimalarial arteether by thermospray high-performance liquid chromatography/mass spectrometry. Biol Mass Spectrom 20: 609–628.

Date AA, Joshi MD, Patravale VB (2007) Parasitic diseases: liposomes and polymeric nanoparticles versus lipid nanoparticles. Adv Drug Deliv Rev 59: 505–521.

Devries PJ, Dien TK (1996) Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. Drugs 52: 818–836.

Dierling AM, Cui Z (2005) Targeting primaquine into liver using chylomicron emulsions for potential vivax malaria therapy. Int J Pharm 303: 143–152.

Efferth T, Benakis A, Romero MR, Tomicic M, Rauh R, Steinbach D, Häfer R, Stamminger T, Oesch F, Kaina B, Marschall M (2004) Enhancement of cytotoxicity of artemisinins toward cancer cells by ferrous iron. Free Radic Biol Med 37: 998–1009.

Gabriëls M, Plaizier-Vercammen J (2004) Design of a dissolution system for the evaluation of the release rate characteristics of artemether and dihydroartemisinin from tablets. Int J Pharm 274: 245–260.

Golenser J, Waknine JH, Krugliak M, Hunt NH, Grau GE (2006) Current perspectives on the mechanism of action of artemisinins. Int J Parasitol 36: 1427–1441.

Himanshu N, Daryl JM, Kirsch LE, Lawrence F (2005) Development and validation of a high-performance liquid chromatography–mass spectroscopy assay for determination of artesunate and dihydroartemisinin in human plasma. J Chromatogr B 816: 233–242.

Ikuhiko N, Henry L, Narendra P, Singh TS (2008) Anticancer properties of artemisinin derivatives and their targeted delivery by transferrin conjugation. Int J Pharm 354: 28–33.

Karbwang J, Na-Bangchang K, Congpuong K, Molunto P, Thanavibul A (1997) Pharmacokinetics and bioavailability of oral and intramuscular artemether. Eur J Clin Pharmacol 52: 307–310.

Lai CS, Nair NK, Mansor SM, Olliaro PL, Navaratnam V (2007) An analytical method with a single extraction procedure and two separate high performance liquid chromatographic systems for the determination of artesunate, dihydroartemisinin and mefloquine in human plasma for application in clinical pharmacological studies of the drug combination, J Chromatogr B 857: 308–314.

Li G, Peggins JO, Fleckenstein LL, Masonic K, Heiffer MH, Brewer TG (1998) Pharmacokinetics and bioavailability of dihydroartemisinin, arteether, artemether, artesunic acid and artelinic acid in rats. J Pharm Pharmacol 50: 173–182.

Lin XH, Li XW, Zheng LQ, Yu L, Zhang QQ, Liu WC (2007) Preparation and characterization of monocaprate nanostructured lipid carriers. Colloids and Surfaces A: Physicochem Eng Aspects 311: 106–111.

Longo M, Zanoncelli S, Manera D, Brughera M, Colombo P, Lansen J, Mazué G, Gomes M, Taylor WR, Olliaro P. (2006) Effects of the antimalarial drug dihydroartemisinin (DHA) on rat embryos *in vitro*. Reprod Toxicol 21: 83–93.

Manjunath K, Venkateswarlu V (2005) Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. J Control Release 107: 215–228.

Mohamed SS, Khalid SA, Ward SA, Wan TSM, Tang HPO, Zheng M, Haynes RK, Edwards G (1999) Simultaneous determination of artemether and its major metabolite dihydroartemisinin in plasma by gas chromatography-mass spectrometry-selected ion monitoring. J Chromatogr B 731: 251–260.

Müller RH, Keck CM (2004) Challenges and solutions for the delivery of biotech drugs- a review of drug nanocrystal technology and lipid nanoparticles. J Biotechnol 113: 151–170.

Müller RH, Radtke M, Wissing SA (2002) Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm 242: 121–128.

Navaratnam V, Mordi MN, Mansor SM (1997) Simultaneous determination of artesunic acid and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies. J Chromatogr B 692: 157–162.

O'Neill PM (2004) Medicinal chemistry: a worthy adversary for malaria. Nature 430: 838–839.

O'Neill PM, Posner GH (2004) A medicinal chemistry perspective on artemisinin and related endoperoxides. J Med Chem 47: 2945–2964.

Owais M, Varshney GC, Choudhury A, Chandra S, Gupta CM (1995) Chloroquine encapsulated in malaria-infected erythrocyte-specific antibody-bearing liposomes effectively controls chloroquine -resistant Plasmodium berghei infections in mice. Antimicrob Agents Chemother 39: 180–184.

Rajanikanth M, Madhusudanan KP, Gupta RC (2003) A HPLC-MS method for simultaneous estimation of alpha,beta-arteether and its metabolite dihydroartemisinin in rat plasma for application to pharmacokinetic study. Biomed Chromatogr 17: 440–446.

Sabarinath S, Rajanikanth M, Madhusudanan KP, Gupta RC (2003) A sensitive and selective liquid chromatographic/electrospray ionization tandem mass spectrometric assay for the simultaneous quantification of alpha-, beta-arteether and its metabolite dihydroartemisinin in plasma, useful for pharmacokinetic studies. J Mass Spectrom 38: 732–742.

Sandrenan N, Godbillon A, Netter C, Donker M, Valkenburg CV (1997) Determination of artemether and its metabolite: dihydroartemisinin in plasma by high-performance liquid chromatography and electrochemical detection in the reductive mode. J Chromatogr B 691: 145–153.

Schäfer-Korting M, Mehnert W, Korting HC (2007) Lipid nanoparticles for improved topical application of drugs for skin diseases. Adv Drug Deliv Rev 59: 427–443.

Souppart C, Gauducheau N, Sandrenan N, Richard F (2002) Development and validation of a high-performance liquid chromatography–mass spectrometry assay for the determination of artemether and its metabolite dihydroartemisinin in human plasma. J Chromatogr B 774: 195–203.

White NJ (2004) Antimalarial drug resistance. J Clin Invest 113: 1084–1092.

Wissing SA, Kayser O, Muller RH (2004) Solid lipid nanoparticles for parenteral drug delivery. Adv Drug Deliv Rev 56: 1257–1272.

Wu GD, Zhou HJ, Wu XH (2005) Apoptosis of human umbilical vein endothelial cells induced by artesunate. Vascul Pharmacol 41: 205–212.

Xing J, Yan HX, Wang RL, Zhang LF, Zhang SQ (2007) Liquid chromatography-tandem mass spectrometry assay for the quantitation of β-dihydroartemisinin in rat plasma. J Chromatogr B 852: 202–207.

Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ (1999) Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J Control Release 59: 299–307.

Zhang XY, Liu JP, Qiao H, Liu H, Ni JM, Zhang WL, Shi YB (2010) Formulation optimization of dihydroartemisinin nanostructured lipid carrier using response surface methodology. Powder Technol. 197: 120–128.