

## PHARMACOKINETIC STUDY OF ARTEMISININ AFTER ORAL INTAKE OF A TRADITIONAL PREPARATION OF *ARTEMISIA ANNUA* L. (ANNUAL WORMWOOD)

KARIN RÄTH, KATJA TAXIS, GITTA WALZ, CHRISTOPH H. GLEITER, SHU-MING LI, AND LUTZ HEIDE  
*Pharmazeutische Biologie, Pharmazeutisches Institut, Universität Tübingen, Tübingen, Germany; Abteilung für Klinische Pharmakologie, Universitätsklinikum Tübingen, Tübingen, Germany*

**Abstract.** *Artemisia annua* L. (annual wormwood) contains the antimalarial artemisinin. Aqueous preparations of the dried herb are included in the pharmacopoeia of the People's Republic of China for treatment of fever and malaria. Fourteen healthy male volunteers received one liter of tea prepared from nine grams of *Artemisia annua* leaves. Blood samples were taken and artemisinin was detected by reversed phase high-performance liquid chromatography. The mean  $\pm$  SD maximum plasma concentration of artemisinin was  $240 \pm 75$  ng/mL and the mean  $\pm$  SD area under the plasma concentration-time curve was  $336 \pm 71$  ng/mL  $\times$  hr. Artemisinin was absorbed faster from herbal tea preparations than from oral solid dosage forms, but bioavailability was similar. One liter of an aqueous preparation of nine grams of *Artemisia annua* contained 94.5 milligrams of artemisinin (approximately 19% of the usually recommended daily dose). Artemisinin plasma concentrations after intake of this herbal tea are sufficient for clinical effects, but insufficient to recommend such preparations as equivalent substitutes for modern artemisinin drugs in malaria therapy.

### INTRODUCTION

Malaria is the world's most important parasitic infection, causing more than a million deaths and 500 million cases annually. Despite tremendous efforts for the control of malaria, the global morbidity and mortality has not principally changed over the last 50 years.<sup>1</sup> The key problem is the failure to get the existing, effective tools against malaria to be applied in those areas where they can be of most benefit.<sup>2–4</sup> If safe and effective antimalarial preparations could be produced by simple means from locally grown medicinal plants, such preparations may offer an additional tool for malaria control, especially in remote geographic locations, and in political and economic circumstances that preclude the availability of modern antimalarial drugs.<sup>5</sup> However, reliable data on the clinical pharmacology, efficacy, and safety of such preparations are extremely scarce, preventing a responsible consideration of their potential benefits and risks in malaria control.

The bark of the tree *Cinchona pubescens* (Peruvian bark) is the source of quinine and has been the mainstay of antimalarial therapy for centuries. Although undoubtedly effective, the use of Cinchona bark today presents an unacceptable risk, especially due to the toxicity of overdoses of quinine.<sup>6</sup>

The herb *Artemisia annua* L. (annual wormwood) has been used in traditional Chinese medicine for the treatment of febrile diseases and malaria for many centuries.<sup>7</sup> The active compound, artemisinin, was isolated by Chinese researchers in the early 1970s.<sup>8</sup> In the last two decades, artemisinin and its semisynthetic derivatives artemether and artesunate have been established as safe and effective antimalarials.<sup>9–11</sup> The toxicity of the artemisinin drugs is much lower than that of quinine, or even that of chloroquine. Significant adverse effects or signs of toxicity have not been reported in human patients treated with therapeutic dosages.<sup>9</sup> Since monotherapy with artemisinin results in relatively high recrudescence rates<sup>12</sup> and there are serious concerns about the possibility of resistance development, the World Health Organization recommends that artemisinin drugs should be used in combination with another effective antimalarial.<sup>13</sup> Artemisinin-based combination therapies are increasingly used in areas of Africa where chloroquine has lost its clinical effectiveness and resistance against sulfadoxine-pyrimethamine is emerging.<sup>4</sup> So far,

there have been no reports on clinical resistance to the artemisinin drugs.<sup>13</sup>

The fast-growing herb *A. annua* can be cultivated with relative ease in poor countries.<sup>14</sup> The artemisinin content of wild *A. annua* L. has been described to vary between 0.02% and 1.1% of the dry weight, depending on plant source and cultivation conditions.<sup>15</sup> Yields of 1.4% of the dry weight can be obtained from a hybrid called *Artemis* that has been developed for commercial artemisinin production.<sup>15</sup> The current pharmacopoeia of the People's Republic of China officially lists the dried herb of *A. annua* as a remedy for fever and malaria.<sup>7</sup> The daily dose is specified as 4.5 to 9 grams of dried herb to be prepared as a tea infusion with boiling water. Artemisinin itself is poorly soluble in water, but may be solubilized in the presence of other plant constituents with amphiphilic properties (e.g., flavonoids or saponins). However, it has not been investigated whether clinically relevant plasma concentrations of artemisinin can be achieved taking such traditional preparations. Therefore, we have carried out an open non-randomized clinical trial in healthy male volunteers to determine artemisinin plasma concentrations after oral intake of an aqueous preparation of *A. annua* L. We also determined the concentration of artemisinin in the dried plant material and in the herbal tea preparation.

### MATERIALS AND METHODS

**Plant material and chemicals.** *Artemisia annua* L. cv. *Artemis*, a hybrid developed by Mediplant (Conthey, Switzerland),<sup>15</sup> was propagated vegetatively and cultivated in the medicinal plant garden of the Pharmaceutical Institute of Tübingen University. The aerial parts of the plant were harvested immediately before flowering and air-dried at ambient temperature. The dried leaves were removed from the stems and sieved (sieve size = 5 mm). This material was used throughout the study. Authentic artemisinin was obtained from Sigma-Aldrich (Taufkirchen, Germany).

**Determination of artemisinin content in plant material.** Extraction of artemisinin from dried plant material was carried out using a method modified from that of Zhao and Zeng.<sup>16</sup> One gram of pulverized dried plant material was extracted with 100 mL of petrol ether at 60–90°C for three hours in a Soxhlet apparatus (Fisher Scientific, Schwerte, Germany).

The solvent was evaporated, and the residue was dissolved in 10.0 mL of ethanol and analyzed by high-performance liquid chromatography (HPLC) as described by Zhao.<sup>17</sup> Extraction with ethyl acetate instead of petrol ether gave identical results.

**Preparation of tea and extraction of artemisinin from tea.** For each preparation method, 1.0 liters of boiling distilled water was added to either five or nine grams of dried herb. In method A, the mixture was left to cool to room temperature before the plant material was removed by filtration. In method B, the mixture was boiled for 30 minutes, the tea was cooled to room temperature, and subsequently the plant material was removed by filtration. In method C, after the addition of the boiling water, the mixture was briefly stirred and the container was covered for 10 minutes. Subsequently, the plant material was removed by filtration and squeezed gently to release residual water. The tea was allowed to cool to room temperature. For detection of artemisinin, 200 mL of each herbal tea were extracted twice with 200 mL of petrol ether at 60–90°C. The organic phase was dried with sodium sulfate, the solvent was evaporated, and the residue was dissolved in 10.0 mL of ethanol.

**Subjects.** Healthy male volunteers were recruited through advertisements from students and staff of Tübingen University. Inclusion criteria were an age between 18 and 35 years and physical examination results, electrocardiogram, and routine laboratory parameters without clinically significant abnormalities. Exclusion criteria were any medication taken within seven days prior to the study and smoking. The study protocol was reviewed and approved by the Ethics Committee of Tübingen University Hospital and each subject gave written informed consent. Adverse events were monitored throughout the study period.

**Study design.** An open, single-center study was carried out involving 14 Caucasian subjects. After an overnight fast and a standardized light breakfast, the subjects received orally one liter of freshly prepared *A. annua* tea (9 grams of herb/liter, artemisinin content = 94.5 mg/liter) divided into five doses of 200 mL. Each dose was taken over a three-minute period so that the intake of the tea was completed within 15 minutes. All subjects received similar snacks and meals at predetermined intervals after dosing.

**Preparation of plasma samples.** Blood samples (18 mL) were taken before and 0.5, 1, 2, 3, 4, and 8 hours after the beginning of medication with *A. annua* tea using EDTA monovettes (Sarstedt, Nümbrecht, Germany). The content of the monovettes was mixed gently and samples were centrifuged immediately at  $2,800 \times g$  at 4°C for five minutes. The plasma was separated from the cells and stored at –20°C.

**Extraction of artemisinin from plasma.** Extraction was carried out using a method modified from that of Titulaer and others.<sup>18</sup> One milliliter of plasma was extracted twice with 800  $\mu$ L of heptane. Separation of the phases was facilitated by centrifugation. Organic phases were combined and the solvent was evaporated. The residue was dissolved in 100  $\mu$ L of ethanol.

**Detection of artemisinin by HPLC.** The method was based on that of Edlund and others.<sup>19</sup> Eighty microliters of the ethanolic solution obtained from extraction of plasma, tea, or dried herb material were separated on a  $250 \times 4.6$  mm Multiphase RP-18  $5\mu$ m column (Chromatography Service, Langenwehe, Germany). A linear gradient of 45–100% acetonitrile in water (20 minutes, 1 mL/minute) was used. For on-line post-column derivatization, 0.3 M aqueous KOH was added to the eluate at a rate of 0.2 mL/minute. The resulting mixture was passed through a 5-meter steel capillary (0.5 mm internal diameter) immersed in a water bath of 70°C. Ultraviolet absorption was monitored at 289 nm.

trile in water (20 minutes, 1 mL/minute) was used. For on-line post-column derivatization, 0.3 M aqueous KOH was added to the eluate at a rate of 0.2 mL/minute. The resulting mixture was passed through a 5-meter steel capillary (0.5 mm internal diameter) immersed in a water bath of 70°C. Ultraviolet absorption was monitored at 289 nm.

**Pharmacokinetic data analysis.** Plasma artemisinin concentration-time profiles were analyzed for each subject using non-compartmental methods. Peak concentration in plasma ( $C_{\max}$ ) and time to  $C_{\max}$  ( $t_{\max}$ ) were derived directly from original data. The elimination rate constant,  $k$ , was calculated by linear regression analysis using the last four or five data points of the log plasma concentration-time curve. The elimination half-life ( $t_{1/2}$ ) was calculated by the equation  $t_{1/2} = \ln 2/k$ . The area under the plasma concentration-time curve (AUC) to the last quantifiable concentration ( $AUC_{0-t}$ ) was calculated using the trapezoidal with the computer program GraphPad Prism version 2 (GraphPad Software, Inc., San Diego, CA). The total area under the plasma concentration-time curve ( $AUC_{0-\infty}$ ) was obtained by adding  $AUC_{0-t}$  and  $AUC_{t-\infty}$ .  $AUC_{t-\infty}$  was extrapolated by dividing the last quantifiable blood concentration by  $k$ . The extrapolated area  $AUC_{t-\infty}$  was less than 10% of  $AUC_{0-\infty}$ .

## RESULTS

**Artemisinin content in cultivated *Artemisia annua* L.** The artemisinin content of the dried plant material of *A. annua* L. cv. *Artemis* was determined as 1.39%, using extraction with petrol ether and pre-column derivatization as described by Zhao and Zeng.<sup>16</sup> Identical results were obtained using post-column derivatization (see Materials and Methods).

**Artemisinin concentration in tea preparations of *A. annua* L.** Three different methods of tea preparations were used to determine the most efficient extraction method. The results are shown in Table 1. Up to 86% of the total artemisinin could be extracted from the dried plant material with boiling water. Extended boiling (30 minutes) reduced the yield considerably, presumably due to the known chemical lability of artemisinin. The most efficient method of tea preparation (method C) yielded 94.5 mg of artemisinin in one liter of tea prepared from nine grams of leaves. This tea preparation was used in the subsequent pharmacokinetic study. This amount corresponds to only 19% of the usual daily dose of artemisinin in adults.<sup>11</sup> However, bioavailability of artemisinin from the current available preparations is poor,<sup>9</sup> and obviously the

TABLE 1  
Artemisinin concentration in tea preparations of *Artemisia annua* L.\*

Preparation method	Amount of <i>Artemisia annua</i> L. dried leaves	Artemisinin concentration in tea preparation	Efficiency of extraction (percentage of artemisinin extracted from leaves into tea preparation)
A	5.0 g	57.5 mg/L	83%
	9.0 g	88.2 mg/L	71%
B	5.0 g	36.5 mg/L	53%
	9.0 g	37.8 mg/L	30%
C	5.0 g	60.0 mg/L	86%
	9.0 g	94.5 mg/L	76%

\* Method A = dried plant material plus one liter of boiling water, allow tea to cool, and filter. Method B = dried plant material plus one liter of boiling water; boil for 30 minutes, allow tea to cool, and filter. Method C = dried plant material plus one liter of boiling water, cover and allow tea to stand for 10 minutes, filter, squeeze plant material gently to remove residual water, and allow tea to cool. For further details, see Materials and Methods.

possibility existed that bioavailability may be higher from the tea preparation.

**Development of an HPLC method for the detection of artemisinin in plasma.** The best established and most widely used method for the determination of artemisinin in pharmacokinetic studies is separation by HPLC followed by post-column derivatization with aqueous KOH and detection by UV absorption.<sup>20–22</sup> We therefore adopted this method using heptane extraction of the plasma. The analytical procedure was validated in our laboratory with human plasma samples to which different quantities of authentic artemisinin were added. The detection limit was approximately 5 ng/mL plasma, which is similar to results from earlier studies.<sup>21,23</sup> Linearity was confirmed between 10 and 500 ng/mL ( $r^2 = 0.985$ ). At 30 ng/mL, the coefficient of variation was 9.9% (100 ng/mL = 5.3%, 300 ng/mL = 4.1%).

**Artemisinin plasma levels after oral intake of tea prepared from *A. annua*.** Fourteen Caucasian volunteers were recruited into the study (mean age = 28 years, range = 28–31 years; mean body weight = 75 kg, range = 62–91 kg). The subjects received orally one liter of freshly prepared *A. annua* tea (artemisinin content = 94.5 mg); intake of the complete dose required on average of 15.4 minutes (range = 10–24 minutes). Blood samples were taken before and 0.5, 1, 2, 3, 4, and 8 hours after the beginning of the medication, and plasma artemisinin concentrations were determined. Individual results for each subject are shown in Table 2, and the mean plasma concentration-time curve for all 14 subjects is shown in Figure 1. Artemisinin was absorbed very fast, and maximum plasma concentrations were observed 30 minutes after the beginning of medication ( $C_{\max} = 240$  ng/mL, SD = 75 ng/mL). In comparison, eight previous studies with oral intake of 500 mg of pure artemisinin in the form of hard gelatin capsules<sup>20,24–30</sup> resulted in an average  $C_{\max}$  of 531 ng/mL of artemisinin (range = 311–776 ng/mL);  $C_{\max}$  was reached in those studies in an average of 2.3 hours after intake. Another study that used an oral dose of 100 mg of artemisinin in the form of capsules showed an average peak concentration of 162 ng/mL reached 2.4 hours after intake.<sup>20</sup> The tea medica-

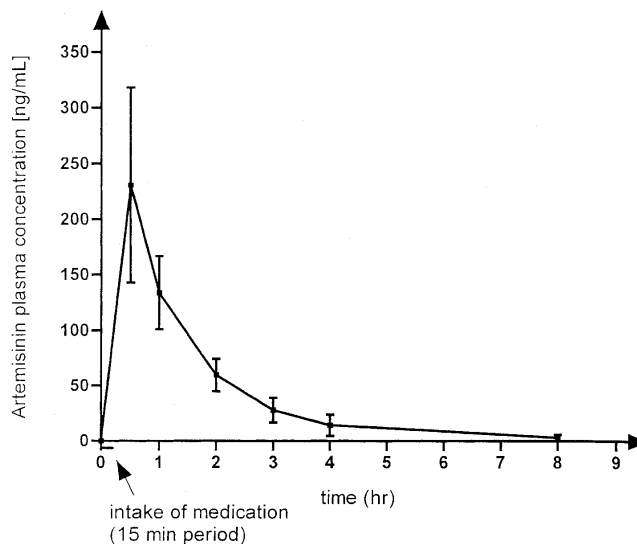


FIGURE 1. Artemisinin plasma concentration time curve after oral intake of one liter of *Artemisia annua* tea containing 94.5 mg of artemisinin. Data represent the mean  $\pm$  SD from 14 healthy volunteers.

tion in the present study (94.5 mg of artemisinin) resulted in an AUC of artemisinin in the plasma of 336 ng/mL  $\times$  hr (SD = 71 ng/mL  $\times$  hr), compared with an average AUC of 2,072 ng/mL  $\times$  hr (range = 1,373–2,611 ng/mL  $\times$  hr) reported in the studies using 500 mg of artemisinin in the form of capsules. Therefore, the bioavailability of artemisinin from the tea preparation is very similar to that from artemisinin in capsules. The elimination half-life of artemisinin in our study was 0.87 hr (SD = 0.23 hr), similar to previous results.

The study medication was well tolerated by the subjects. One subject developed signs and symptoms of an allergic reaction, including cough and a skin rash, shortly after intake of the herbal tea. These symptoms resolved quickly without requiring treatment. No other adverse events were observed.

## DISCUSSION

The present study investigated tea preparations of the Chinese traditional medicinal plant *A. annua* L. Somewhat unexpectedly, most of the artemisinin contained in dried *A. annua* leaves could be extracted with boiling water. Although the solubilization of lipophilic agents by other plant constituents is a well known phenomenon, this is a very striking example for such an effect. The tea preparation, based on the dosage recommendations of the current pharmacopoeia of the People's Republic of China, contained a total amount of 94.5 mg of artemisinin in one liter of tea. This is low in comparison to the 500 mg of artemisinin that are commonly used as the daily dose of artemisinin for adults in the form of tablets or capsules.<sup>11</sup> Our data show that artemisinin was absorbed faster from the tea preparation than from capsules. We found peak concentrations of 240 ng/mL after intake of herbal tea, i.e., approximately 40% of the peak concentrations reported after intake of 500 mg of artemisinin in the form of capsules.<sup>20,24–30</sup> The AUC found in the present study was only 16% of the average AUC reported after intake of 500 mg of artemisinin in the form of capsules, reflecting the

TABLE 2

Pharmacokinetic data obtained from 14 volunteers after intake of an aqueous preparation from *Artemisia annua* L. containing 94.5 mg of artemisinin\*

Subject	AUC (ng/mL $\times$ hr)	$C_{\max}$ (ng/mL)	$t_{\max}$ (hr)	$t_{1/2}$ (hr)
1	359	297	0.5	0.6
2	274	210	0.5	0.8
3	378	217	0.5	1.4
4	404	169	1.0	1.0
5	332	182	1.0	1.0
6	303	186	0.5	1.1
7	245	133	1.0	0.9
8	258	206	0.5	0.8
9	483	383	0.5	0.8
10	303	349	0.5	0.5
11	408	339	0.5	0.7
12	262	225	0.5	0.8
13	396	262	0.5	1.0
14	293	203	0.5	0.9
Mean(SD)	336 (71)	240 (75)	0.6 (0.2)	0.9 (0.2)

\* The artemisinin concentration was determined in venous plasma samples. AUC = area under the plasma concentration-time curve;  $C_{\max}$  = maximum artemisinin concentration;  $t_{\max}$  = time to  $C_{\max}$ .

lower dose contained in the tea (19% of the dose of a capsule). Thus, the bioavailability of artemisinin from the herbal tea preparation is similar to the bioavailability of artemisinin from capsules.

Few data are available on the minimum dose (or minimum plasma concentration) of artemisinin required for clinical efficacy in the treatment of malaria. The minimum concentration required for growth inhibition of *Plasmodium falciparum* *in vitro* has been estimated as 9 ng/mL.<sup>31</sup> The artemisinin plasma concentrations achieved in the present study clearly exceeded this value for at least four hours in the current dosage regimen. Artemisinin and dihydroartemisinin are known to be strongly accumulated in infected erythrocytes,<sup>32</sup> and the exact relationship between the concentrations in plasma and in erythrocytes are unknown. The plasma concentrations that we found in the present study appear sufficient to explain a possible growth inhibition of *P. falciparum* and thus a possible clinical effect of the traditional *A. annua* preparations used in ancient and modern China. However, the plasma concentrations are considerably lower than those that are achieved by modern artemisinin preparations. Therefore, our study shows that the tea preparation is not equivalent to modern artemisinin drugs. This is confirmed by a recent clinical trial with a similar tea preparation of *A. annua* in malaria patients, which showed a rapid reduction of parasitemia and clinical improvements during therapy, but unacceptably high recrudescence rates after termination of the treatment.<sup>13</sup> Monotherapy with tea preparations from *A. annua* can therefore not be recommended as a treatment option for malaria also due to concerns about possible resistance development against artemisinin.

Received June 12, 2003. Accepted for publication September 18, 2003.

Acknowledgments: We thank Dr. Richard Fux, Svitlana Igel, Bettina Lutze, and Dr. Markus Müller for help in the clinical trial.

Financial support: This study was supported by the University of Tübingen and University Hospital Tübingen.

Authors' addresses: Karin R ath, Katja Taxis, Shu-Ming Li, and Lutz Heide, Pharmazeutische Biologie, Pharmazeutisches Institut, Universit t T bingen, Auf der Morgenstelle 8, 72076 T bingen, Germany, Telephone: 49-7071-297 2460, Fax: 49-7071-29 5250, E-mail: heide@uni-tuebingen.de. Gitta Walz and Christoph H. Gleiter, Abteilung f r Klinische Pharmakologie, Universit tsklinikum T bingen, Otfried-M ller-Strasse 45, 72076 T bingen, Germany, Telephone: 49-7071-297 8277, Fax: 49-7071-29 4942.

## REFERENCES

- Riley EM, 2000. The London School of Hygiene and Tropical Medicine: a new century of malaria research. *Mem Inst Oswaldo Cruz* 95 (suppl 1): 25–32.
- Yamey G, 2000. African heads of state promise action against malaria. *BMJ* 320: 1228.
- Yamey G, 2001. Global campaign to eradicate malaria. *BMJ* 322: 1191–1192.
- World Health Organization and UNICEF, 2003. *The Africa Malaria Report 2003*. <http://mosquito.who.int>.
- Bodeker G, Willcox M, 2000. New research initiative on plant-based antimalarials. *Lancet* 355: 761.
- H nsel R, Keller K, Rimpler H, Schneider G, 1992. *Hagers Handbuch der Pharmazeutischen Praxis*. Berlin: Springer Verlag.
- Pharmacopoeia of the People's Republic of China*. Volume 1. English Edition, 2000. Peking: Chemical Industry Press.
- Klayman DL, 1985. Qinghaosu (artemisinin): an antimalarial drug from China. *Science* 228: 1049–1055.
- de Vries PJ, Dien TK, 1996. Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. *Drugs* 52: 818–836.
- Guerin PJ, Olliaro P, Nosten F, Druilhe P, Laxminaraya R, Binka F, Kilama WL, Ford N, White NJ, 2002. Malaria: current status of control, diagnosis, treatment, and a proposed agenda for research and development. *Lancet Infect Dis* 2: 564–573.
- McIntosh HM, Olliaro P, 2001. Artemisinin derivatives for treating severe malaria (Cochrane Review). *The Cochrane Library* 2. Oxford: Update Software.
- Meshnick SR, Taylor TE, Kamchonwongpaisan S, 1996. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiol Rev* 60: 301–315.
- World Health Organization, 2001. *The Use of Antimalarial Drugs*. Geneva: World Health Organization. WHO/CDS/RBM/2001.33.
- Mueller MS, Karhagomba IB, Hirt HM, Wemakor E, 2000. The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects. *J Ethnopharmacol* 73: 487–493.
- Delabays N, Simonnet X, Gaudin M, 2001. The genetics of artemisinin content in *Artemisia annua* L. and the breeding of high yielding cultivars. *Curr Med Chem* 8: 1795–1801.
- Zhao SS, Zeng MY, 1985. Studies on the analysis of qinghaosu by high-pressure liquid chromatograph and spectrometry (HPLC). *Planta Med* 51: 233–237.
- Zhao SS, 1987. High-performance liquid chromatographic determination of artemisinin (qinghaosu) in human plasma and saliva. *Analyst* 112: 661–664.
- Titulaer HA, Zuidema J, Kager PA, Wetsteyn JC, Lugt CB, Merkus FW, 1990. The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to volunteers. *J Pharm Pharmacol* 42: 810–813.
- Edlund PO, Westerlund D, Carlqvist J, Wu BL, Jin YH, 1984. Determination of artesunate and dihydroartemisinin in plasma by liquid chromatography with post-column derivatization and UV-detection. *Acta Pharm Suec* 21: 223–234.
- Gordi T, Hai TN, Hoai NM, Thyberg M, Ashton M, 2000. Use of saliva and capillary blood samples as substitutes for venous blood sampling in pharmacokinetic investigations of artemisinin. *Eur J Clin Pharmacol* 56: 561–566.
- Gordi T, Huong DX, Hai TN, Nieu NT, Ashton M, 2002. Artemisinin pharmacokinetics and efficacy in uncomplicated malaria patients treated with two different dosage regimens. *Antimicrob Agents Chemother* 46: 1026–1031.
- Svensson US, Alin H, Karlsson MO, Bergqvist Y, Ashton M, 2002. Population pharmacokinetic and pharmacodynamic modelling of artemisinin and mefloquine enantiomers in patients with falciparum malaria. *Eur J Clin Pharmacol* 58: 339–351.
- Sidhu JS, Ashton M, Huong NV, Hai TN, Karlsson MO, Sy ND, Jonsson EN, Cong LD, 1998. Artemisinin population pharmacokinetics in children and adults with uncomplicated falciparum malaria. *Br J Clin Pharmacol* 45: 347–354.
- Alin HM, Ashton M, Kihamia CM, Mtey GJ, Bjorkman A, 1996. Multiple dose pharmacokinetics of oral artemisinin and comparison of its efficacy with that of oral artesunate in falciparum malaria patients. *Trans R Soc Trop Med Hyg* 90: 61–65.
- Alin MH, Ashton M, Kihamia CM, Mtey GJ, Bjorkman A, 1996. Clinical efficacy and pharmacokinetics of artemisinin monotherapy and in combination with mefloquine in patients with falciparum malaria. *Br J Clin Pharmacol* 41: 587–592.
- Ashton M, Gordi T, Trinh NH, Nguyen VH, Nguyen DS, Nguyen TN, Dinh XH, Johansson M, Le DC, 1998. Artemisinin pharmacokinetics in healthy adults after 250, 500 and 1000 mg single oral doses. *Biopharm Drug Dispos* 19: 245–250.
- Ashton M, Hai TN, Sy ND, Huong DX, Van Huong N, Nieu NT, Cong LD, 1998. Artemisinin pharmacokinetics is time-dependent during repeated oral administration in healthy male adults. *Drug Metab Dispos* 26: 25–27.
- Ashton M, Sy ND, Gordi T, Hai TN, Thach DC, Huong NV, Farah MH, Johansson M, Cong LD, 1996. Evidence for time-

- dependent artemisinin kinetics in adults with uncomplicated malaria. *Pharm Pharmacol Lett* 6: 127–130
29. Dien TK, de Vries PJ, Khanh NX, Koopmans R, Binh LN, Duc DD, Kager PA, van Boxtel CJ, 1997. Effect of food intake on pharmacokinetics of oral artemisinin in healthy Vietnamese subjects. *Antimicrob Agents Chemother* 41: 1069–1072.
  30. Duc DD, de Vries PJ, Nguyen XK, Le Nguyen B, Kager PA, van Boxtel CJ, 1994. The pharmacokinetics of a single dose of artemisinin in healthy Vietnamese subjects. *Am J Trop Med Hyg* 51: 785–790.
  31. Alin MH, Bjorkman A, 1994. Concentration and time dependency of artemisinin efficacy against *Plasmodium falciparum* *in vitro*. *Am J Trop Med Hyg* 50: 771–776.
  32. Gu HM, Warhurst DC, Peters W, 1984. Uptake of [3H] dihydroartemisinin by erythrocytes infected with *Plasmodium falciparum* *in vitro*. *Trans R Soc Trop Med Hyg* 78: 265–270.
  33. Mueller MS, Runyambo N, Wagner I, Borrmann S, Dietz K, Heide L, 2004. Randomised controlled trial of a traditional preparation of *Artemisia annua* L. (Annual Wormwood) in the treatment of malaria. *Trans R Soc Trop Med Hyg*, in press