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

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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.1 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.2–4 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.5 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.6

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.7 The active compound, artemisinin, was isolated by Chinese researchers in the early 1970s.8 In the last two decades, artemisinin and its semisynthetic derivates artether and artemunate have been established as safe and effective antimalarials.9–11 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.9 Since monotherapy with artemisinin results in relatively high recrudescence rates12 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.13 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.4 So far, there have been no reports on clinical resistance to the artemisinin drugs.13

The fast-growing herb A. annua can be cultivated with relative ease in poor countries.14 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.15 Yields of 1.4% of the dry weight can be obtained from a hybrid called Artemisia that has been developed for commercial artemisinin production.15 The current pharmacopeia of the People’s Republic of China officially lists the dried herb of A. annua as a remedy for fever and malaria.7 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),15 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.16 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.\(^{17}\) 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 tablet of T. annua Univer he, 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.

**Pharmacokinetic data analysis.** Plasma artemisinin concentration-time profiles were analyzed for each subject using non-compartmental methods. Peak concentration in plasma (C\text{max}) and time to C\text{max} (t\text{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\text{1/2}) was calculated by the equation t\text{1/2} = ln2/k. The area under the plasma concentration-time curve (AUC) to the last quantifiable concentration (AUC\text{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\text{0–∞}) was obtained by adding AUC\text{0–t} and AUC\text{t–∞}. AUC\text{t–∞} was extrapolated by dividing the last quantifiable blood concentration by k. The extrapolated area AUC\text{t–∞} was less than 10% of AUC\text{0–∞}.

### 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.\(^{16}\) 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.\(^{11}\) However, bioavailability of artemisinin from the current available preparations is poor,\(^{9}\) and obviously the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Artemisinin concentration in tea preparations of <em>Artemisia annua L.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preparation method</strong></td>
<td><strong>Amount of <em>Artemisia annua</em> L. dried leaves</strong></td>
</tr>
<tr>
<td>A</td>
<td>5.0 g</td>
</tr>
<tr>
<td>B</td>
<td>9.0 g</td>
</tr>
<tr>
<td>C</td>
<td>5.0 g</td>
</tr>
<tr>
<td>C</td>
<td>5.0 g</td>
</tr>
<tr>
<td>C</td>
<td>9.0 g</td>
</tr>
<tr>
<td>C</td>
<td>9.0 g</td>
</tr>
</tbody>
</table>

\(^{*}\) 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 30 minutes, filter, squeeze plant material gently to remove residual water, and allow tea to cool. For further details, see Materials and Methods.
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. 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, which is similar to results from earlier studies. 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 ($C_{\text{max}}$ = 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 resulted in an average $C_{\text{max}}$ of 353 ng/mL of artemisinin (range = 311–776 ng/mL); $C_{\text{max}}$ 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. The tea medication in the present study (94.5 mg of artemisinin) resulted in an AUC of artemisin in the plasma of 336 ng/mL × hr (SD = 71 ng/mL × x hr), compared with an average AUC of 2,072 ng/mL × hr (range = 1,373–2,611 ng/mL × 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 artemisin 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.

### TABLE 2
Pharmacokinetic data obtained from 14 volunteers after intake of an aqueous preparation from *Artemisia annua* L., containing 94.5 mg of artemisinin*

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

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

### 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 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. 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 artemisin in the form of capsules. The AUC found in the present study was only 16% of the average AUC reported after intake of 500 mg of artemisin in the form of capsules, reflecting the
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. 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, 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.

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.

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