ARTEMISININ PHARMACOKINETICS IS TIME-DEPENDENT DURING REPEATED ORAL ADMINISTRATION IN HEALTHY MALE ADULTS

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ABSTRACT:
The pharmacokinetics of the antimalarial artemisinin exhibited an unusual time dependency during a 7-day oral daily regimen of 500 mg in 10 healthy, male Vietnamese adults. Artemisinin areas under the plasma concentration-time curve (AUC) decreased to 34% (median) by day 4 with a further decrease by day 7 to only 24% of values obtained after the first day of administration. In seven subjects restudied after a 2-week washout period, artemisinin AUCs had almost normalized, demonstrating the reversibility of the time-dependent drug disposition. The results suggest artemisinin exhibits an auto-inductive effect on drug metabolism of an unusual magnitude. This may partly explain why some patients on standard doses, due to subparasiticidal drug levels toward the end of a standard regimen, do not completely clear parasites. Further, the possibility of drug-drug metabolic interactions during combination regimens is implicated.

Artemisinin (fig. 1) is isolated from Artemisia annua L., a plant with a long history of medical use against malaria in China (Klayman, 1985). Numerous trials have demonstrated its clinical efficacy, characterized by rapid fever and parasite clearance times (Qinghaosu Antimalarial Coordinating Research Group, 1979; Hien and White, 1993; Sy et al., 1993; Hassan Alin et al., 1996a). Importantly, the artemisinin class of endoperoxides, including derivatives artether and artesunate, are effective against Plasmodium falciparum strains resistant to other antimalarials (Hassan Alin et al., 1992). The artemisinin compounds have become first-line drugs in several regions in Southeast Asia (Li et al., 1994; Hien, 1994). In Vietnam, a 5-day regimen of 500 mg/day, which may be divided into two daily doses, is in current use.

The major outstanding problems with the artemisinin class of antimalarials are, respectively, a high recrudescence rate and an unknown risk for toxicity after long-term use. Improper use of these antimalarials may reduce cost-effectiveness due to recurring malaria that, by extension, may provoke development of parasite resistance.

We have previously observed a marked change in artemisinin pharmacokinetics resulting in drug levels at the end of treatment being 20–30% of the initial; this is an unusual change not only in consideration of its magnitude but also by its rapid development within 5–6 days (Ashton et al., 1996; Hassan Alin et al., 1996a). In the present study, we have sought to verify whether a similar time dependency occurs also in healthy subjects. The rapidity of onset of change in drug kinetics as well as its reversibility were furthermore considered.

Materials and Methods

Ten healthy male Vietnamese adults, 26 ± 6 years of age (mean ± SD) and weighing 55 ± 4 kg, were administered 500 mg of artemisinin orally for 7 days. Study drug was presented as hard gelatin capsules containing 250 mg of artemisinin (Institute of Materia Medica, Hanoi, batch 021191, 99.8% pure by 400 MHz [1H]NMR). On study days 1, 4, and 7, artemisinin was given as a single 2 × 250-mg morning dose to ensure detectable drug plasma levels. On intermediate study days (2–3, 5–6), the daily 500-mg dose was divided into a morning and evening administration each of 250 mg. Seven subjects were restudied after a 2-week washout period by receiving another single 2 × 250-mg oral dose on day 21.

Subjects were fasted overnight before administration and refrained from food or beverages for 2 hr after drug administration on all study days. Tea, coffee, or cola soft drinks were not allowed during days of sampling for...
TABLE 1

Mean ± SD artemisinin pharmacokinetic parameters on days 1, 4, and 7 of a 7-day oral regimen of 500 mg of artemisinin daily to 10 healthy, male Vietnamese adult subjects, of which 7 were restudied on day 21 after a 2-week washout period.

Values in parentheses represent the 95% confidence intervals around the (geometric) averages based on log-normal distributions.

<table>
<thead>
<tr>
<th>Study Day</th>
<th>AUC (hr ng/ml)</th>
<th>Cmax (ng/ml)</th>
<th>t1/2 (hr)</th>
<th>CL/F (liter/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1373 ± 912</td>
<td>311 ± 232</td>
<td>3.0 ± 1.2a</td>
<td>502 ± 283</td>
</tr>
<tr>
<td></td>
<td>(754, 1779)</td>
<td>(167, 397)</td>
<td>(2.0, 3.8)</td>
<td>(281, 664)</td>
</tr>
<tr>
<td>4</td>
<td>402 ± 251</td>
<td>148 ± 93</td>
<td>3.8 ± 2.0b</td>
<td>3033 ± 4636</td>
</tr>
<tr>
<td></td>
<td>(147, 605)</td>
<td>(76, 199)</td>
<td>(2.3, 5.2)</td>
<td>(826, 3398)</td>
</tr>
<tr>
<td>7</td>
<td>346 ± 398</td>
<td>110 ± 104</td>
<td>4.8 ± 5.0b</td>
<td>8048 ± 12895</td>
</tr>
<tr>
<td></td>
<td>(61, 485)</td>
<td>(36, 151)</td>
<td>(1.2, 9.8)</td>
<td>(1031, 8190)</td>
</tr>
<tr>
<td>21</td>
<td>862 ± 503</td>
<td>195 ± 126</td>
<td>2.7 ± 0.9b</td>
<td>553 ± 186</td>
</tr>
<tr>
<td></td>
<td>(137, 2180)</td>
<td>(61, 361)</td>
<td>(1.3, 3.6)</td>
<td>(364, 767)</td>
</tr>
</tbody>
</table>

a N = 8–9.  
N = 5.  
' N = 6–7.

pharmacokinetic investigation. Compliance with all drug intake was under investigators’ supervision. All subjects were smokers, but only two subjects smoked more than 10 cigarettes per day.

Venous blood was collected through an antecubital catheter (Venflon, 1.0 × 30 mm) into heparinized Vacutainer vials just before and then at 1, 2, 3, 4, 5, 6, 7, 8, and 10 hr after drug administration on study days 1, 4, 7, and 21. Sampling handling and quantitation by HPLC was as previously described (Ashton et al., 1996). All samples from one subject were analyzed on the same day and with the interspersement of six quality control samples. The limit of quantitation was 10 ng/ml (intra-assay CV < 15%, N = 10).

Subjects were interviewed about possible adverse events on days 1, 4, 7, and 14. Blood hematology (erythrocyte particle concentration, volume fraction, hemoglobin, reticulocyte fraction, leukocyte concentration, and differentiated white blood cells) was studied on days 1, 7, 14, and 21.

Artemisinin pharmacokinetic parameters were estimated by conventional noncompartmental methods identical to our earlier studies (Ashton et al., 1996; Hassan Alin et al., 1996a). In the present study, oral clearance values (CL/F) were approximated by dividing the oral dose by the area under the plasma concentration-time curve from the time of drug administration until the last concentration point in time above or equal the limit of quantitation (AUC). Values are reported as arithmetic averages ± standard deviation (95% confidence interval). For the pharmacokinetic parameters, the 95% confidence interval round the geometric average was calculated by log transformation of the data and by taking the anti-logarithm of the calculated interval.

The study was performed in January-February of 1995 at the Institute of Malariology, Parasitology and Entomology, Hanoi. The subjects gave their written, informed consent to participate in the study, which was performed according to the ethics standards of the Helsinki Declaration and in lieu of a domestic ethics committee, with the protocol reviewed by the Ministry of Health, Hanoi, and separately approved by the ethics committee of the Medical Faculty, Uppsala University, and by the Medical Products Agency, Uppsala, Sweden.

Results

Artemisinin plasma concentrations decreased significantly during the administration regimen, with AUC values on day 4 only 31% (95% confidence interval: 19, 43%) of those on day 1 (table 1). A further decrease was observed between days 4 and 7, resulting in AUCs on day 7 being 25% (11, 39%) compared with the first day of drug administration. A similar change was seen for Cmax values. Of the two subjects differing by not exhibiting the same frank time-dependent pharmacokinetics (fig. 2), one had low plasma concentrations resulting in poor estimation of pharmacokinetic parameters. In seven subjects restudied after a washout period of 2 weeks, AUCs were 68% (30, 103%) compared with day 1 values, indicating a normalization of drug disposition. Despite the marked changes in AUCs between study days, there was little change in drug half-lives. The median oral clearance (CL/F) increased from 491 liters/h on day 1 to 1152 and 2184 liters/h on days 4 and 7, respectively, returning to 538 liters/h on day 21. Interindividual pharmacokinetic variability was generally high.

The leukocyte particle concentration increased on average by 2.0 give milli units (0.7, 3.4 × 10^9 mm^-3) between days 1 and 7, returning to predrug levels on days 14 and 21, but all individual values were inside the normal range. There were no other remarkable hematological changes or other adverse events in evidence.

Discussion

We have previously demonstrated that artemisinin exhibits time-dependent pharmacokinetics in malaria patients (Ashton et al., 1996; Hassan Alin et al., 1996a) and now report similar findings in healthy subjects. The high values for oral artemisinin clearance indicate either poor absorption or high first-pass extraction. The explanation for the time dependency would then lie with either of these two processes. Although artemisinin exhibits good in vitro permeability in the Caco-2 line of intestinal epithelial cells (Augustijn et al., 1996), one possible explanation is an induction of intestinal exsorption such as that mediated by P-glycoprotein. A decrease in plasma protein binding is an unlikely explanation because binding is moderate at about 88% (Sidhu and Ashton, 1997). A more probable cause is a capacity for...
auto-induction of drug-metabolizing enzymes. The pathways of artemisinin elimination are poorly understood. Four metabolites, deoxy-
artemisinin, dihydrodeoxyartemisinin, deoxydihydroartemisinin, and the so-called “crystal-7,” all lacking the endoperoxide bridge necessary for antiparasitic effect, were identified in urine after oral administration of artemisinin to humans (Zhu et al., 1983; Lee and Hufferd, 1990). The available data suggest hepatic metabolism as the main route of elimination. In comparison with other tissues, artemisinin disposition of artemisinin to humans (Zhu et al., 1990). The available data suggest hepatic metabolism as the main metabolism in rat liver microsomes, indicating the cytochrome P450 system may be involved in its metabolism (Lesko-vac and Theoharides, 1991).

The lack of simultaneous change in drug elimination half-life is consistent with an extensive liver extraction, such that systemic clearance, and therefore also drug half-life, is blood flow dependent, whereas AUCs after oral administration are governed by intrinsic hepatic clearance (Wilkinson and Shand, 1975). The high interindividual pharmacokinetic variability found in the present study is in contrast to an earlier report in Vietnamese subjects (Duc et al., 1994) and is more in agreement with our earlier findings in patients with uncomplicated malaria in both Vietnam and Tanzania (Ashton et al., 1996; Hassan Alin et al., 1996a, 1996b). This high variability may, in itself, suggest that artemisinin is eliminated primarily by one specific isozyme. It was not readily understood why one or possibly two subjects in the present study differed as far as development of time dependency is concerned, and extended population data would be desirable for confirmation.

The time-dependent pharmacokinetics may contribute toward explaining why radical cure is not achieved in 15–40% of the patients (Hien and White, 1993; Sy et al., 1993). Lower antimalarial drug levels toward the end of a treatment period may in some individuals become insufficient for radical parasite clearance. This may, however, be counteracted by an increased formation of unknown active metabolites. Future studies will be required to show whether the inductive effect is a class phenomenon of the endoperoxide antimalarials or whether it is unique to the parent compound artemisinin. Because of the high recrudescence rates, the combination of artemisinin and its derivatives with drugs with longer half-lives has been increasingly advocated for achievement of radical cure. The putative induction by artemisinin may lead to increased metabolism of drugs used either in such combinations or, such as anticonvulsants, used concomitantly during treatment. Decreased plasma levels of mefloquine after concomitant intake of the derivatives artesunate and artemether have been reported (Karbwang et al., 1994; Na-Bangchang et al., 1995).

In conclusion, artemisinin pharmacokinetics in healthy subjects exhibit the same marked time dependency as earlier observed in malaria patients, putatively caused by an autoinduction of drug metabolism in man.

References