Klinische en farmacologische studies met artemether voor de behandeling van malaria
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Citation for published version (APA):
van Agtmael, M. A. (1999). Klinische en farmacologische studies met artemether voor de behandeling van malaria

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Grapefruit juice increases the bioavailability of artemether
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Abstract Objective: To evaluate the effect of grapefruit juice on the pharmacokinetics of artemether in plasma and saliva after a single oral dose and to detect concentration-dependent electrocardiographic changes (bradycardia and QTc prolongation).

Methods: Six healthy male subjects were given a standard breakfast followed by two tablets of 50-mg artemether administered with water; 1 week later, the tablets were administered with 350 ml double-strength fresh frozen grapefruit juice. For 8 h, 17 blood- and saliva samples were collected, and 17 electrocardiograms were recorded. Drug and metabolite concentrations were measured by means of high-performance liquid chromatography with electrochemical detection. The

Conclusion: Grapefruit juice significantly increases the oral bioavailability of artemether without an effect on the elimination half-life. It suggests a role for intestinal CYP3A4 in the presystemic metabolism of artemether.

Key words Artemisinin derivative • Artemether • Grapefruit juice

Introduction

Artemether, one of the artemisinin drugs, is increasingly used for the treatment of malaria in endemic areas, as it acts very fast, has few side effects and is effective against
pharmacokinetic parameters were determined using a one-compartment model.

Results: Grapefruit juice significantly \( (P = 0.001) \) increased the mean peak concentration \( (C_{\text{max}}) \) of artemether more than twofold from 42 (SD 17) ng/ml to 107 (28) ng/ml. The time to reach \( C_{\text{max}} \) \( (t_{\text{max}}) \) with grapefruit juice was 2.1 (0.6) h compared with 3.6 (17) h with water \( (P = 0.02) \). The area under the concentration–time curve (AUC) almost doubled with grapefruit juice from 177 ng·h/ml to 336 ng·h/ml \( (P = 0.003) \). The elimination half-life remained unchanged \( (1.0 \text{ h vs 1.3 h}) \). No major changes in the kinetics of the metabolite dihydroartemisinin were detected. Low artemether levels and zero dihydroartemisinin levels were found in saliva. No influences of artemether were observed on 17 electrocardiograms during the 8 h after drug intake – in particular there were no signs of bradycardia or QTc prolongation.
mether and is at least twice as active in killing malaria parasites as artemether, in vitro. An increase in bioavailability of artemether could theoretically have a favourable effect on the efficacy. However, grapefruit juice could reduce the efficacy of the drug if it were to inhibit enzymes involved in the formation of dihydroartemisinin. As the metabolism pathways of artemether have not yet been identified, this experiment will provide insight into the role of CYP3A4 in the presystemic metabolism of artemether.

To evaluate a non-invasive mode of sampling artemether, saliva samples were collected for detection of both artemether and its metabolite using the same assay [23].

Finally, we looked at concentration-dependent electrocardiographic changes. Studies in falciparum malaria patients treated with artemether have found asymptomatic prolongation of the QTc interval [24, 25, 26]. It is unknown whether these effects are related to the drug or to the malaria.

**Methods**

The study took place in the Department of Clinical Pharmacology and Pharmacotherapy at the Academic Medical Center in Amsterdam. Six healthy, male, Caucasian subjects were recruited after a physical examination, routine blood- and urine examination, and a 12-lead-electrocardiogram (ECG) had revealed no abnormalities. Written informed consent was obtained before inclusion in the study. Subjects with a history of serious medical disease or any recent use of medication (within 1 month of the study) or a history of smoking, drug- or alcohol abuse were excluded. They were not allowed to drink alcohol, caffeine-containing beverages or grape-

**Data analysis and statistics**

The measured concentrations of artemether and dihydroartemisinin were analysed using a curve-fitting program (Scientist for Windows) [29]. By means of non-linear regression analysis utilising a one-compartment model with a first-order input and output, we calculated, for each individual data set, the pharmacokinetic parameters: lagtime ($t_{lag}$), absorption half-life ($t_{abc1/2}$), time to reach the maximum concentration ($t_{max}$), maximum concentration ($C_{max}$), area under the concentration–time curve (AUC), elimination half-life ($t_{elim1/2}$) and volume of distribution/bioavailability ($V/f$). In this model, artemether was absorbed in two fractions. An earlier study with artemether in healthy subjects had shown that this 'two-fractions' model was superior to a general model, in which the total unfractioned dose was absorbed [30]. The kinetic profile of dihydroartemisinin was described using a modified model, in which the absorption rate constant was replaced by an 'appearance rate constant' of dihydroartemisinin in plasma.

Differences between the kinetic parameters from the 'water session' and the 'grapefruit-juice session' were evaluated using a paired Student $t$-test, assuming a normal distribution of the calculated parameters. Conforming with previous grapefruit-juice–drug interaction studies, we estimated that with effective enzyme inhibition, doubling of the AUC of artemether was feasible. Postulating a mean difference for AUC of 100 ng·h/ml with a standard deviation of the difference of 50 ng·h/ml, a power of 80% and a significance level of 0.05, we would need six subjects in a paired study to detect this major difference [31].

**Results**

The mean age of the subjects was 26.8 years (range 23–35 years), mean weight 75.2 kg (65–90 kg), mean height 181.3 m (1.67–1.90 m), mean resting heart rate 65 beats/min (60–80 beats/min) and mean blood pressure 117/69 mmHg (110/55–120/80 mmHg). None of the six subjects experienced side effects during this study. One
fruit juice or to eat fruit either during the 24 h before the study day or on the study days. The protocol was approved by the Ethics Review Board of the Academic Medical Centre, Amsterdam.

After an overnight fast, the subjects were given a standard breakfast (one cheese sandwich), followed 1 h later by a glass of water and 100 mg artemether, administered as two 50-mg tablets (Artenam, Proarma NV, Belgium). Blood samples were taken before drug intake and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 7 and 8 h after artemether administration. Simultaneously, saliva was collected after stimulation by chewing on parafilm for 10 s. During this 8-h period, the subjects were only allowed to drink water. From an indwelling intravenous catheter (Venflon 2, BOC Ohmeda AB, Helsingborg, Sweden), 5-ml samples were drawn into vacuum polystyrene tubes (Venoject II, Terumo Europe, Leuven, Belgium) containing lithium heparinate. The blood samples were centrifuged for 10 min at 2000 g and plasma and saliva were stored in plastic tubes (for less than 2 months) at −70 °C until analysis. Reverse-phase high-performance liquid chromatography (HPLC) with electrochemical detection was used to measure the concentration of artemether and its metabolite, dihydroartemisinin, in plasma and saliva [27]. Seventeen 12-lead ECGs at 25 mm/s (Siemens, Siccard 440, München, Germany) were taken at the same timepoints as blood and saliva were collected. Individual QT intervals were calculated [QT_interval = the rate-corrected QT interval = QT interval /√(R-R interval)].

After a washout period of 1 week, the experiment was repeated (as no order effect was expected, randomisation was not carried out). This time, instead of water, the tablets were administered with a glass of double-strength fresh frozen grapefruit juice (350 ml). All subjects were given the same type of grapefruit juice from a single batch (Jaffa, Albert Heyn, Zaandam, The Netherlands), which has shown to be an effective inhibitor of cyclosporin metabolism [28].

Subject fainted after introduction of the intravenous catheter due to vasovagal syncope.

Figure 1 shows the concentration–time curves of the measured mean (±SE) artemether concentrations. Higher artemether concentrations were seen when the drug was taken with grapefruit juice. It also shows a steeper rise in concentrations during the first 2–3 h. Table 1 shows the mean (±SD) pharmacokinetic
parameters for artemether. Grapefruit juice reduced the absorption half-life significantly from 0.8 h to 0.3 h, contributing to a reduction of the $t_{\text{max}}$ from 3.6 h to 2.1 h. The $C_{\text{max}}$ increased significantly from 42 ng/ml to 107 ng/ml, with a concomitant rise in AUC from 177 ng·h/ml to 336 ng·h/ml. No influence on the lag-time or elimination half-life was seen. Figure 2 shows the concentration–time curves of the measured mean (±SE) dihydroartemisinin concentrations. Like artemether, with grapefruit juice a steeper rise in dihydroartemisinin concentrations was observed, now without a change in $C_{\text{max}}$ or AUC. Table 2 shows the mean (±SD) pharmacokinetic parameters for the metabolite dihydroartemisinin. Grapefruit juice did not influence the pharmacokinetics of the metabolite dihydroartemisinin. Although the $C_{\text{max}}$ was reached almost 1 h earlier with grapefruit juice, this difference was not statistically significant. Figure 3 illustrates the ‘two-fractions’ model used to fit the measured concentrations of artemether (---) and dihydroartemisinin (----) in one of the six individuals taking 100 mg artemether with a glass of grapefruit juice. It shows that, after a lag time, the pharmacokinetic behaviour of the metabolite parallels the parent drug. Furthermore, the plot illustrates the good fit using the ‘two-fractions’ model.

Low levels of artemether were found in saliva (data not shown). Most measurements in saliva were around or under the limit of quantification of the HPLC assay (5 ng/ml) and, therefore, were unreliable for pharmacokinetic analysis. No dihydroartemisinin was detectable in saliva.

No abnormalities or changes in comparison with the pre-dose ECG were observed in the 17 ECGs carried out during the 8-h period after drug intake. Special attention was paid to bradycardia and prolongation of the QTc interval. No relationship between artemether concentrations and electrocardiographic effects could be demonstrated; therefore, no pharmacokinetic-dynamic analysis was done. Figure 4 shows the course of the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Water</th>
<th>Grapefruit</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{\text{lag}}$ (h)</td>
<td>1.1 (0.5)</td>
<td>0.7 (0.3)</td>
<td>n.s.</td>
</tr>
<tr>
<td>$t_{\text{app/2}}$ (h)</td>
<td>0.7 (0.2)</td>
<td>0.6 (0.2)</td>
<td>n.s.</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>3.7 (0.7)</td>
<td>2.8 (0.5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>67 (34)</td>
<td>85 (26)</td>
<td>n.s.</td>
</tr>
<tr>
<td>$t_{\text{lim/2}}$ (h)</td>
<td>0.9 (0.3)</td>
<td>0.8 (0.2)</td>
<td>n.s.</td>
</tr>
<tr>
<td>AUC (ng·h/ml)</td>
<td>239 (105)</td>
<td>276 (83)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
Table 1 Mean (± SD) pharmacokinetic parameters of artemether in six healthy subjects who took 100 mg artemether orally with a glass water or a glass grapefruit juice. $t_{lag}$: lag time; $t_{abs1/2}$: absorption half-life; $t_{max}$: time to reach $C_{max}$; $C_{max}$: maximum concentration; $t_{elim1/2}$: elimination half-life; AUC: area under the concentration–time curve; $P$: level of significance using Student’s t-test for paired samples.

<table>
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<th>Grapefruit</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{lag}$ (h)</td>
<td>0.8 (0.5)</td>
<td>0.7 (0.3)</td>
<td>n.s.</td>
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<tr>
<td>$t_{abs1/2}$ (h)</td>
<td>0.8 (0.1)</td>
<td>0.3 (0.2)</td>
<td>&lt;0.001</td>
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<tr>
<td>$t_{max}$ (h)</td>
<td>3.6 (0.9)</td>
<td>2.1 (0.6)</td>
<td>0.02</td>
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<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>42 (17)</td>
<td>107 (28)</td>
<td>0.001</td>
</tr>
<tr>
<td>$t_{elim1/2}$ (h)</td>
<td>1.0 (0.3)</td>
<td>1.3 (0.2)</td>
<td>n.s.</td>
</tr>
<tr>
<td>AUC (ng·h/ml)</td>
<td>177 (49)</td>
<td>336 (53)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Fig. 2 Mean (± SE) measured dihydroartemisinin concentrations over time in the plasma of six healthy subjects who took 100 mg artemether orally either with a glass water or a glass grapefruit juice.

Mean (± SE) QTc interval (in ms) during those 8 h. It illustrates the great overlap between the ‘water’ and ‘grapefruit’ sessions and no tendency for QTc prolongation around the peak concentration (2–3.5 h). During the absorption phase of artemether, between 1 h and 3 h, a discrete shortening of the QTc interval can be observed in both groups. Hereafter, the QTc slowly increases. These changes, however, are minor and within 5–10% of the QTc before drug intake. Of 204 ECGs, in two the QTc was prolonged 10–15% and in one 15–20%. These three ECGs were from three different volunteers in the grapefruit group, two at 1.75 h and one at 7 h after drug intake. They experienced no arrhythmia or clinical symptoms. No relationship was found with a
Fig. 3 The ‘two-fractions’ model used to fit the measured concentrations of artemether (— —) and dihydroartemisinin (— — ) in one individual taking 100 mg artemether with a glass of grapefruit juice.

Discussion

The results show that a glass of grapefruit juice increases the bioavailability of artemether after a single oral dose. More than a doubling of the C_{max} and almost a doubling of the AUC was observed. This is the first study to demonstrate a grapefruit–drug interaction for artemether, with an effect similar to that found for previously described drugs [22, 32]. The results suggest that CYP3A4 plays an important role in the presystemic metabolism of artemether. In other words, artemether were too low, even after food and grapefruit juice, to find significant artemether levels in saliva well above the limit of quantification of the assay (5 ng/ml). A saliva/plasma ratio close to the unbound drug fraction in plasma has been described for artemether in rabbits [36]. In humans, an unbound fraction of 13% for artemisinin was found and 21% for arteether [23, 37]. No data have been published on protein binding for artemether in humans. Due to the low dose used in this study, we were unable to draw conclusions concerning protein binding and saliva concentrations of artemether.

Less variability of C_{max} and AUC was observed in the ‘grapefruit results’ than the ‘water results’. The coefficient of variation (= (SD/mean) * 100%) for C_{max} was 26% compared with 40% for the ‘water results’. For AUC, the variation reduced from 28% to 16%. It is known that there is a 20- to 50-fold difference in CYP 3A activity in the normal population [38]. This is probably one of the reasons for the high variation found in pharmacokinetic studies. If grapefruit juice could convert a normal population to approximately the same level of CYP 3A activity, this would reduce this variability, as we found in this study [14].

Although other studies in falciparum malaria patients treated with artemether have shown electrocardiographic changes during treatment, especially bradycardia and QT\(_c\) prolongation, we did not find any important changes in our study [26]. The minor changes found can be considered as natural variation of the QT\(_c\) interval [39]. This suggests that the changes found on ECG tracings in malaria patients are either secondary to malaria or related to a treatment with artemether containing a dose higher than 100 mg.
seems liable to a significant first-pass metabolism that can be reduced by grapefruit juice.

The faster absorption with grapefruit juice can be explained either by the larger fluid intake (350 ml) than with the water session (160 ml), thus facilitating the absorption, or by an effect of grapefruit juice itself (accelerated gastric emptying or faster dissolution of the tablet).

Why is the increase in artemether not followed by an increase in the metabolite dihydroartemisinin? There are several options to explain this. The dihydroartemisinin concentration is the sum of that formed during absorption of artemether (in the gut) and that formed from absorbed artemether (in the liver). If the majority of dihydroartemisinin is formed during absorption and now blocked by grapefruit juice, the increase in dihydroartemisinin concentrations can be minor. Studies have shown a higher dihydroartemisinin/artemether ratio after oral than intramuscular administration of artemether, which suggests that indeed some dihydroartemisinin is formed in the gastrointestinal tract [33]. Besides, formation of dihydroartemisinin possibly takes place also in the acid environment of the stomach; this has been described for oral arteether [34, 35].

We measured low levels of artemether and no detectable dihydroartemisinin in saliva. The plasma levels

Comparison with other studies

Food prolonged the \( t_{\text{max}} \) significantly (3.6 h vs 0.8 h) and increased \( C_{\text{max}} \) and AUC of artemether and dihydroartemisinin almost twofold compared with the results of a previously published study we did in seven healthy white subjects who received 100 mg artemether orally with water on an empty stomach [30]. This effect of food was also found in a cross-over study in 16 male Chinese volunteers [40].

In a recent study, we administered 100 mg artemether orally to eight healthy Vietnamese volunteers in the south of Vietnam either with water or (with a washout period of 2 weeks) with 350 ml freshly squeezed juice from local grapefruits, and the results were similar to this study, although less pronounced (different grapefruit juice, not double concentrated). Combined with juice, the mean AUC of artemether increased from 206 ng·h/ml to 309 ng·h/ml \( (P = 0.058) \). The mean \( C_{\text{max}} \) increased from 79 ng/ml to 103 ng/ml \( (P = 0.14) \). No changes were found for dihydroartemisinin. The same local grapefruit juice was evaluated in eight Vietnamese patients with uncomplicated falciparum malaria who were treated with 200 mg artemether on \( t = 0 \) h
Fig. 4 Mean (± SE) QTc interval (in ms) measured on 17 electrocardiograms over an 8-h period from six healthy subjects who took 100 mg artemether orally with a glass water or a glass grapefruit juice.
(with water), 8 h (with water) and 16 h (with grapefruit juice), followed by 750 mg mefloquine on t = 24 h. No differences were observed between the mean artemether $C_{\text{max}}$ (357 ng/ml vs 352 ng/ml) and AUC (1239 ng·h/ml vs 1292 ng·h/ml) after the first and the third gift (van Agtmael, unpublished data). First, these results show that locally squeezed Vietnamese grapefruit juice also increases the bioavailability of artemether in healthy Vietnamese subjects, although less pronounced than double-strength fresh frozen grapefruit juice. Second, in Vietnamese patients, a two fold higher dose of 200 mg resulted in a four fold higher $C_{\text{max}}$ and six fold higher AUC than in healthy Vietnamese subjects who were given 100 mg. This suggests that either malaria increases drug levels or artemether is subject to a dose-dependent saturable first-pass metabolism [41]. Saturation kinetics would explain why grapefruit juice had no effect on artemether when a higher dose was given.

We have shown that the bioavailability of a single oral dose of 100 mg artemether in healthy subjects can be doubled when taken with a glass of grapefruit juice. Considering the marked decline in bioavailability of artemether over time during a multiple-dose regimen, this simple method could theoretically increase the efficacy (and lower the cost) of the malaria treatment. This should be evaluated in a randomised clinical study.


