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The effect of grapefruit juice on the time-dependent decline of artemether plasma levels in healthy subjects
The effect of grapefruit juice on the time-dependent decline of artemether plasma levels in healthy subjects

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Abstract

Background: Artemether is a new and effective treatment for malaria although relapse is a problem in monotherapy. These relapses could be related to a time-dependent decline in artemether plasma levels described in multiple dose studies and probably caused by autoinduction. The aim of this study was to evaluate the effect of grapefruit juice on the decreasing bioavailability over time of artemether.

Methods: In a randomised, two-phase crossover study, 8 healthy male subjects took 100 mg artemether orally with 350 ml water or 350 ml double strength fresh frozen grapefruit juice once daily for 5 days. On day 1 and day 5 seventeen bloodsamples were collected over a period of 8 hours.

Results: The mean peak artemether plasma concentration (C\text{max}) and the mean area under the concentration-time curve (AUC) after the last dose at day 5 were about one third compared to day 1 without a change in the elimination halflife (t\text{1/2}) after intake with water (P = 0.006 for C\text{max}, P= 0.005 for AUC) as well as grapefruit juice (P<0.001 for C\text{max} and AUC). Grapefruit juice increased C\text{max} (P=0.021) and AUC (P<0.001) twofold on day 1 (P=0.021) and day 5 (P=0.05 for C\text{max} and P=0.004 for AUC). The active metabolite dihydroartemisinin showed a twofold rise in C\text{max} (P=0.006) and AUC (P=0.001) with grapefruit juice, without time-dependent changes of pharmacokinetic parameters.

Conclusions: Grapefruit juice significantly increased the oral bioavailability of artemether but did not prevent the time-dependent reduction in bioavailability. It suggests that CYP3A4 in the gutwall is one of artemether’s metabolizing enzymes but seems not involved in the autoinduction process.

Introduction

Artemether is derived from artemisinin which is a natural product extracted from sweet wormwood (Artemisia annua). Other artemisinin derivatives are arteether, artesunate
and dihydroartemisinin. They are increasingly used in malaria endemic areas for the treatment of malaria having a fast onset of action, little side effects and good effectivity against multi-drugresistant parasites. However, a high rate of recrudescence is reported in monotherapy with these compounds. Factors contributing to this reduced efficacy are the short halflife of 1-3 hours and possibly the recently described remarkable time-dependent pharmacokinetics. For artemisinin it was found that the maximum plasma concentrations ($C_{max}$) and area under the plasma concentration-time curves (AUC) in patients treated for malaria were only 20-30 % after 5 days therapy compared to day 1. An identical phenomenon was found in healthy subjects which suggested that this marked time-dependent decrease in bioavailability was not caused by the disease. In a study in Chinese malaria patients we found that the mean $C_{max}$ of artemether after the 4th dose at 48 hours was only one third compared to the first dose while the metabolite dihydroartemisinin increased over time. It is thought that through autoinduction artemisinin concentrations in plasma decrease over time in multiple dose studies. It is unknown bioavailability during a 5 days oral course and evaluated the effect of concomitant intake with a glass of grapefruit juice on the decline in bioavailability.

**Methods**
The study took place in the Department of Clinical Pharmacology & Pharmacotherapy at the Academic Medical Center Amsterdam. Eight healthy male white subjects, all Dutch students, were recruited. Their mean weight and height were 77.6 kg (range 69-93 kg) and 1.90 m (1.82-1.97 m) respectively. A complete physical and laboratory examination (hematology, biochemistry and urinanalysis) and electrocardiogram were done to ensure that all subjects were healthy. Written informed consent was obtained before inclusion. Subjects with a history of serious past medical disease or any recent drug use (within one 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, grapefruit juice or eat fruit in the 24 hours before the study day and on the study day. The protocol has been approved by the Ethics Review Board.
which enzymes are involved in this autoinduction although recently it was found that artemisinin induced omeprazole metabolism in human beings which was associated with increased hydroxylation by CYP 2C19. Autoinduction of drug metabolizing enzymes has also been described for rifampicin, rifabutin and carbamazepine and is generally associated with increased CYP 3A4 activity. Grapefruit juice can increase the oral bioavailability of many drugs by specific inhibition of CYP3A4 in the gut wall, hereby reducing the ‘first-pass’ elimination. Recently we discovered that a glass of grapefruit juice doubled the Cmax and AUC of artemether after a single oral dose in healthy subjects. The rationale for this study was to postulate a role for intestinal CYP 3A4 in the autoinduction of artemether. Autoinduction of intestinal metabolism has been described for oral rifampicin. If one assumes that CYP3A4 is involved in the (auto)induction of artemether, one would expect that grapefruit juice could (partially) block this process. In this study we investigated in healthy subjects the time-dependent decline in artemether of the Academic Medical Center, Amsterdam. After an overnight fast the subjects received 100 mg artemether as two 50 mg tablets (Artenam®, Profarma NV, Belgium) which is a usual clinical dose. The tablets were crushed to reduce variance in dissolution and absorption which was observed in an earlier study. The crushed tablets were administered with either 350 ml plain water or 350 ml double strength grapefruit juice. All subjects received the same type of grapefruit juice from a single batch (Jaffa Choice grapefruitsap 1+3®, Albert Heyn, Zaandam, The Netherlands) which has shown to effectively increase cyclosporine and artemether levels. To randomize for intake with water or grapefruit juice, order randomisation was carried out with closed envelopes. There was a washout period of one month. No food-intake was allowed during 8 hours. Bloodsamples were collected before drugintake 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 hours after artemether intake (total of 17 samples). 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 samples were directly stored in ice and within several hours centrifuged for 10 minutes at 4000 rpm and transferred into plastic aliquots and stored (less than 2 months) at -70°C until analysis. Reversed phase HPLC with electro-chemical detection was used to measure the concentration of artemether and dihydroartemisinin in plasma.\(^\text{17}\) On day 1 and 5 the experiment took place in our laboratory. On day 2, 3 and 4 the subjects took the tablets at home in the morning after an overnight fast with 350 ml of water or grapefruit juice.

**Data analysis and statistics**

Concentrations of artemether and dihydroartemisinin were analysed in a one compartmental model with a lag time (\(t_{lag}\)), a first order absorption (in 2 fractions) and a first order elimination to calculate the pharmacokinetic parameters: \(t_{lag}\), \(t_{abs1/2}\), \(t_{max}\), \(C_{max}\), AUC and elimination halflife (\(t_{1/2}\)) using a non-linear regression program.\(^\text{18}\) A previous single dose study with artemether in healthy

**Results**

Artemether and grapefruit juice were well tolerated by all subjects without any observed adverse effects.

*Artemether.* Figure 1 shows the mean (±s.e.) of the measured artemether concentrations on day 1 and day 5 when administered with water and with grapefruit juice. On day 1 and day 5 more than twofold increase of the concentrations was observed when the tablets were taken with grapefruit juice. Furthermore, the concentrations on day 5 are reduced to about one third of those at day 1. This time dependent reduction was also seen when artemether was taken with grapefruit juice. Table I shows the mean (±s.d.) values of the individually modelled pharmacokinetic parameters for artemether on the 4 study days. The variation in \(t_{max}\) values, explains why in the plot of the mean concentrations (figure 1) the \(C_{max}\) does not reach the \(C_{max}\) values in the tables. Artemether was rapidly absorbed without a significant lagtime and reached a maximum concentration of 68 ng/ml within 2 hours with moderate interindividual variability (s.d. 41 ng/ml). The elimination was fast with a \(t_{1/2}\) less
adults had shown double peaks in the concentration-time profiles probably related to variable dissolution of the tablets or gastric emptying. Therefore we used a similar model in this study where the dose is absorbed in two fractions. Differences between the kinetic parameters on day 1 and day 5 and the pharmacokinetic effects of grapefruit juice were evaluated with a paired two-tailed Student t-test assuming the data to be normally distributed. We expected that the grapefruit effect would give a twofold increase in plasma levels and the autoinduction a twofold decrease in plasma levels. We assumed a moderate variability where the standard deviation $s.d = 0.5 \times \text{mean of a pharmacokinetic variable.}$ Using a standardized difference (difference/$s.d.$) of 2, $n=8$ and $P=0.05$ a power of $>80\%$ would be achieved. Differences were regarded as statistically significant when $P$ was $<0.05$. A correction for multiple comparisons was made using the Bonferroni method ($P/n=0.05/8=0.0063$). The data are expressed as mean ± standard deviation ($s.d.$) in the tables and text and as mean ± standard error of the mean ($s.e.$) in the figures.

then an hour. On the 5th day, after 4 days of therapy, there is a remarkable reduction in $C_{\text{max}}$ ($P=0.006$) and $AUC$ ($P=0.005$), to one third of the $C_{\text{max}}$ - and AUC -values on day 1 while $t_{1/2}$ remained unchanged. Four of eight subjects had very low concentrations on day 5 (marginally above the detection limit of the assay). In these subjects the measured, and not modelled, $C_{\text{max}}$ and AUC were given. With grapefruit juice a 2 and 2.6 fold rise in $C_{\text{max}}$ was observed on day 1 ($P=0.021$) and day 5 ($P=0.05$) respectively with a concomitant rise in $AUC$ of 2.4 ($P<0.001$) and 3.5 fold ($P=0.004$). The mean of the individual ratios $AUC_{\text{grapefruit}} / AUC_{\text{water}}$ was 3.2 (range 1.4 to 5.0) on day 1 and 2.9 (1.6-19.1) on day 5. Similarly, with grapefruit juice a one third reduction in artemether $C_{\text{max}}$ and AUC from day 1 to day 5 was observed. The mean of the individual ratios $AUC_{\text{day 5}} / AUC_{\text{day 1}}$ was 0.34 (range 0.07-0.58) with water and 0.21 (0.07-0.62) with grapefruit juice. Four hours after drug intake 3/8 subjects on day 1 and 6/8 subjects on day 5 had undetectable artemether levels in their blood. With grapefruit juice 0/8 subjects on day 1 and 3/8 subjects on day 5 had undetectable levels four hours after drug intake.
Figure 1 Mean (± s.e.) of the measured *artemether* concentrations on day 1 and day 5 when 100 mg *artemether* daily was administered with water or with grapefruit juice.
Table I: Mean (±s.d.) values of the individually modelled pharmacokinetic parameters for artemether on the 4 study days.

<table>
<thead>
<tr>
<th></th>
<th>water day 1</th>
<th>water day 5</th>
<th>grapefruit day 1</th>
<th>grapefruit day 5</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>tlag h</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.2 (0.2)</td>
<td>0.3 (0.1)</td>
<td>ns</td>
</tr>
<tr>
<td>tabs1/2 h</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.2)</td>
<td>0.4 (0.2)</td>
<td>0.3 (0.2)</td>
<td>*1</td>
</tr>
<tr>
<td>tmax h</td>
<td>1.7 (0.5)</td>
<td>1.6 (0.8)</td>
<td>1.7 (0.5)</td>
<td>1.9 (0.4)</td>
<td>ns</td>
</tr>
<tr>
<td>Cmax ng/ml</td>
<td>68 (41)</td>
<td>19 (13)</td>
<td>139 (48)</td>
<td>49 (38)</td>
<td>*2</td>
</tr>
<tr>
<td>AUC ng*h/ml</td>
<td>149 (87)</td>
<td>35 (30)</td>
<td>364 (93)</td>
<td>123 (84)</td>
<td>*3</td>
</tr>
<tr>
<td>t1/2 h</td>
<td>0.8 (0.4)</td>
<td>0.5 (0.1)</td>
<td>0.7 (0.2)</td>
<td>0.5 (0.2)</td>
<td>ns</td>
</tr>
</tbody>
</table>

*1 p=0.006 day 1 water vs grapefruit
*2 p=0.006 day 1 vs day 5 (water), p<0.001 day 1 vs day 5 (grapefruit), p= 0.021 day 1 water vs grapefruit, p=0.05 day 5 water vs grapefruit
*3 p=0.005 day 1 vs day 5 (water), p<0.001 day 1 vs day 5 (grapefruit), p<0.001 day 1 water vs grapefruit, p=0.004 day 5 water vs grapefruit
Figure 2 Mean (± s.e.) of the measured dihydroartemisinin concentrations on day 1 and day 5 when 100 mg artemether daily was administered with water or with grapefruit juice.
Table II Mean (±s.d.) values of the individually modelled pharmacokinetic parameters for dihydroartemisinin on the 4 study days.

<table>
<thead>
<tr>
<th></th>
<th>water</th>
<th></th>
<th>grapefruit</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 1</td>
<td>day 5</td>
<td>day 1</td>
<td>day 5</td>
</tr>
<tr>
<td>t_{lag} h</td>
<td>0.2 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.3 (0.1)</td>
<td>0.3 (0.1)</td>
</tr>
<tr>
<td>t_{app 1/2} h</td>
<td>0.2 (0.2)</td>
<td>0.4 (0.3)</td>
<td>0.5 (0.1)</td>
<td>0.4 (0.2)</td>
</tr>
<tr>
<td>t_{max} h</td>
<td>1.7 (0.5)</td>
<td>1.6 (0.8)</td>
<td>1.7 (0.5)</td>
<td>1.9 (0.4)</td>
</tr>
<tr>
<td>C_{max} ng/ml</td>
<td>100 (43)</td>
<td>78 (28)</td>
<td>146 (61)</td>
<td>179 (63)</td>
</tr>
<tr>
<td>AUC ng*h/ml</td>
<td>269 (117)</td>
<td>231 (65)</td>
<td>438 (149)</td>
<td>486 (118)</td>
</tr>
<tr>
<td>t_{1/2} h</td>
<td>1.0 (0.3)</td>
<td>0.8 (0.3)</td>
<td>0.8 (0.3)</td>
<td>0.8 (0.3)</td>
</tr>
</tbody>
</table>

*1 p=0.03 day 1 water vs grapefruit, p=0.025 day 5 water vs grapefruit
*2 p=0.009 day 1 water vs grapefruit
*3 p=0.006 day 5 water vs grapefruit
*4 p=0.001 day 1 water vs grapefruit, p<0.001 day 5 water vs grapefruit
Dihydroartemisinin. Dihydroartemisinin paralleled the pharmacokinetics of artemether but reached a higher $C_{\text{max}}$ and AUC. However, no changes were observed on the 5th day compared to the first day. (Figure 2) Grapefruit juice increased $C_{\text{max}}$ 1.5 fold on day 1 ($p=0.062$) and 2.3 fold on day 5 ($p=0.006$). The AUC showed a 1.6 fold increase on day 1 and a 2.1 fold increase on day 5. Again no changes were found in the $t_{1/2}$ (Table II). The mean of the individual [artemether] / [dihydroartemisinin] ratios was 1.2 on day 1 with water or a mean ratio(s.d) of 0.51(0.40), almost 1:1 on day 1 with grapefruit juice (0.93 (0.53)), 1:7 on day 5 with water (0.15(0.20) and 1:4 on day 5 with grapefruit juice (0.25(0.16)). Overall this ratio seemed not concentration-dependent in the 0-300 ng/ml range with a 100 mg dose. Six hours after drug intake 2/8 subjects on day 1 and 4/8 subjects on day 5 had undetectable dihydroartemisinin levels in their blood. Six hours after drug intake with grapefruit juice 1/8 subjects on day 1 and 1/8 subjects on day 5 had undetectable levels.

Discussion

artemether did not significantly inhibit mefloquine, halofantrine or primaquine metabolism which suggests that liver CYP3A4 is not important in artemether’s metabolism.\textsuperscript{22-24} However in a previous single dose study we found that grapefruit juice, being a specific gut CYP3A4 inhibitor, doubled the AUC of artemether.\textsuperscript{14} In this multiple dose study we have shown that grapefruit juice significantly increases plasma levels of artemether on the first and last day of the regimen without an influence on the time-dependency of artemether kinetics. It suggests that CYP3A4 is involved in intestinal metabolism of artemether but that its autoinduction is not caused by increased intestinal CYP3A4 activity. However, as grapefruit juice does not have an effect on liverenzyme activity, it can not be excluded that liver CYP3A4 is involved. Probably autoinduction and the ‘grapefruit effect’ have different underlying mechanisms. The decline in artemether levels coincided with a decrease in drug/metabolite ratio from 1:2 on day 1 to 1:7 on day 5, consistent with induction of metabolism. However, the absolute
This is the first study describing a time-dependent decline in plasmalevels of artemether in healthy subjects. This decline in plasma levels can be considered as a major pharmacokinetic phenomenon as it reached statistical significance in this small study (n=8) comparing $C_{\text{max}}$ and AUC on day 1 and day 5, even after Bonferroni correction for multiple comparisons (using $P < 0.0063$). Recently we demonstrated in patients that artemether was subject to autoinduction, like its parent compound artemisinin. This induction was apparent after 2 days which is remarkably fast because generally for enzyme-induction more time is required for de novo synthesis of enzymes. Although generally (auto)induction of drug metabolizing enzymes will predominantly take place in the liver, autoinduction of enzymes in the gut wall has also been described for rifampicin. It is not clear whether the autoinduction by artemisinin drugs takes place in the liver or in the intestine or both and which enzymes are involved. In vitro it was found that CYP3A4 is the primary enzyme involved in the metabolism of arteether into dihydroartemisinin. Interaction studies have been done in human liver microsomes where dihydroartemisinin concentrations were not higher on day 5 compared to day 1. Why does the induction not lead to increased metabolite concentrations on day 5? Four theoretical explanations can be given for the absence of time-dependency in the metabolite pharmacokinetics. First, the autoinduced enzyme is not responsible for the formation of dihydroartemisinin but for an other unidentified metabolite of artemether. Second, the formation and elimination of dihydroartemisinin are both induced which would netto not influence the dihydroartemisinin concentrations. Third, induction of first pass metabolism will lower artemether plasma levels and decrease metabolite concentrations if the metabolite is not a product of the first pass metabolism. Fourth, an important part of dihydroartemisinin is formed by non-enzymatic hydrolysis in the acid environment of the stomach as described for arteether. The lack of an intravenous formulation of artemether makes it impossible to calculate the absolute bioavailability. It is thought that the oral bioavailability of artemether is low. In a rat perfusion model artemisinin exhibited high
jejunal permeability and was no substrate or inducer of P-glycoprotein. Our observed interaction with grapefruit juice suggests that artemether is subject to a significant intestinal first pass metabolism. Previous studies have shown higher concentrations of dihydroartemisinin after oral than intramuscular artemether administration which supports that there is metabolism of artemether in the digestive tract.

In conclusion, an oral treatment course with artemether is probably more effective when the tablets are taken with grapefruit juice. We demonstrated in this study a 2-fold rise in the amount of drug and active metabolite in plasma, during the full treatment course. Although grapefruit juice could not antagonise the autoinduction of artemether, theoretically by increasing and prolonging effective plasma levels it could reduce the recrudescence rate in monotherapies with artemether. However, caution is necessary concerning neurotoxicity, described in animals after high dose artemether administration. The efficacy and side effects of concomitant grapefruit juice administration should be evaluated in a clinical study in an

endemic malaria area where artemether monotherapy is common use. For low health-budget countries, adding grapefruit juice to a drug regimen would be more cost-effective then doubling the dose. Finally, drug-drug interactions (in malaria combination-therapy, in women using oral anticonceptives etc.) need to be studied in vitro and in vivo to analyze the strong inductive capacities of artemisinin compounds and to identify which enzymes are involved in this induction process.

References