Clinical pharmacology of the tyrosine kinase inhibitors imatinib and sunitinib

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Absorption of cytochrome P450 3A4 inhibiting furanocoumarins from grapefruit juice after oral administration

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Abstract

Purpose: Furanocoumarins in grapefruit juice are potent cytochrome P450 3A4 (CYP3A4) inhibitors, however it appears that this only effects intestinal CYP3A4. The reason for an absent effect on hepatic CYP3A4 is yet unknown and we hypothesize that this might be the result of limited absorption of these substrates after oral administration.

Therefore, the absorption of bergamottin (BG) and 6',7'-dihydroxybergamottin (DHB) was studied in healthy volunteers after drinking grapefruit juice. Additionally, the concentration of BG and DHB in different brands and lots of grapefruit juice was analyzed.

Patients and Methods: Six Caucasian healthy volunteers consumed 3 times 400 mL (equal to a BG and DHB dose of 3 x 2.45 mg and 3 x 3.22 mg respectively) grapefruit juice at t= 0, 3 and 6 hour. The serum concentrations of BG and DHB were determined at t= 1, 7 and 8 hour using a validated HPLC-UV method.

Results: BG and DHB levels were undetectable in all volunteers both after single and after multiple dosing, indicating that these inhibitors can only act via the intestinal and not via the hepatic CYP3A4. The variability of BG and DHB concentrations between the different brands and lots of grapefruit juice is substantial. However, the variability within one lot is small.

Conclusion: Since the furanocoumarins BG and DHB are not absorbed after a single or multiple consumptions of grapefruit juice, they are intestinal CYP3A4 inhibitors rather than hepatic CYP3A4 inhibitors. The large variability in concentration BG and DHB between different brands and lots of grapefruit juice necessitates quantification of these ingredients for selecting grapefruit juice for interaction studies.

Introduction

In 1991 the first report was published describing the potential interaction between grapefruit juice and felodipine[1]. Grapefruit juice resulted in an increased felodipine plasma concentration, which led to a decrease in blood pressure. In the following years the prominent role of the metabolic enzyme cytochrome P450 3A4 (CYP3A4) underlying this drug interaction became clear. The effect of grapefruit juice on CYP3A4 appears to be the result of a irreversible inactivation of this enzyme[2, 3]. The pronounced effect of grapefruit juice on the oral availability of multiple drugs that are substrates of CYP3A4, has since the first serendipitous observation, been described thoroughly in many studies[4-10]. Additionally, an inhibitory effect of grapefruit juice on the ATP binding pocket B1 transporter (P-glycoprotein) and on the organic anion transporting polypeptide 1A2 (OATP1A2) and OATP2B1 was postulated[8, 11-15].

In contrast to orally administered drugs, grapefruit juice appears to have only a little effect on intravenously administered drugs[16-19]. Grapefruit juice acts by inhibiting intestinal CYP3A4 activity during uptake of the drug from the intestinal lumen to the systemic circulation and it is thought that hepatic CYP3A4 is largely unaffected, but this has never been studied in detail. Possible explanations for this divergent effect of grapefruit juice on intestinal and hepatic CYP3A4 could be poor absorption of the CYP3A4 inhibiting ingredients or dilution of these substances to concentrations below their effective enzyme inhibitory concentrations[20].

Grapefruit juice is a complex and rich mixture of several hundred ingredients. Much effort has been invested to identify the chemical substance responsible for the inhibiting effect on CYP3A4. The flavonoids; naringin, naringenin, quercetin, kaempferol, and the furanocoumarins; bergamottin, 6',7'-dihydroxybergamottin and its dimers bergapten, bergaptol, 6',7'-epoxybergamottin have been suggested to contribute to the grapefruit juice – drug interactions[2, 12, 20, 21]. The administration of the purified forms of these different compounds to human volunteers, pointed into the direction of the furanocoumarins as being the group of substances most responsible for the CYP3A4 inhibiting effect[10, 22, 23]. The most abundant furanocoumarins present in grapefruit juice are bergamottin (BG) and 6',7'-dihydroxybergamottin (DHB). A complicating factor is that among grapefruit juices brands the concentrations of these furanocoumarins exhibit substantial variability, potentially resulting in a more or less pronounced effect on CYP3A4[24]. To find an explanation for the pronounced effect of BG and DHB on the intestinal but absent effect on the hepatic CYP3A4 enzyme, we investigated whether BG and DHB are absorbed after drinking grapefruit juice with a predetermined dose of BG and DHB. Additionally, BG and DHB were quantified in different brands and batches of grapefruit juice.

Material and methods

BG and DHB in grapefruit juice

Materials

Five different brands and different lots of a single brand of commercially available grapefruit juices were obtained from local grocery stores in The Netherlands. BG and DHB were purchased from Sigma-Aldrich (St. Louis, MO, USA). The internal standard fenprocoumon was kindly supplied by F. Hoffmann-La Roche (Basel, Switzerland).

Analysis of bergamottin and 6', 7'-dihydroxybergamottin in grapefruit juice

The concentrations BG and DHB were determined using a validated high pressure liquid chromatography – ultraviolet detection (HPLC-UV) method. The used assay is based on a previously published method with minor modifications[24]. Briefly, the juice was homogenized by shaking. Grapefruit juice (0.5mL) was mixed with 10 µL internal standard (100 µg/mL, in methanol) and 2 mL ethyl acetate. Calibration standards contained 0.2 – 4 µg/mL BG and 0.1 – 2 µg/mL DHB were prepared at the start of each analytical run. The standard stock solution contained BG and DHB (100 and 50 µg/mL in DMSO:methanol(1:3)). The extraction was performed by shaking for 30 minutes and separation by centrifugation; 4,000 rpm for 3 minutes. The organic phase was collected and evaporated (40ºC; N_2). The residue was reconstituted with 100 µL of DMSO/acetonitril solution (1:3 v/v). A volume of 30 µL of each sample was applied to a HPLC separation system (Unexas 2104, Knauer, Berlin, Germany). The compounds of interest were separated on a Hypersil ODS RP analytical column (4.6 x 100 mm, i.d 3 µm) and eluted over 22 minutes with a flow rate of 1 mL/min and the following gradient [time scale (minutes - minutes)/ percentage of solvent A (water 2500/phosphoric acid 1.25)/ percentage of solvent B (acetonitril)]: 0-7/70/30, 7-17 70/30 → 0/100, 17-18/0/100, 18-19 0/100 → 70/30, 19-22/70/30. The effluent was monitored with a diode array detector (Dionex, UVD340U, Germering, Germany). The UV absorption profile was monitored between 210 – 350 nm. DHB, fenprocoumon and BG eluted at 10.9, 12.8 and 16.5 minutes, respectively. Linearity was confirmed over the range of 0.2 – 24 µg/mL for BG and 0.1 – 12 µg/mL for DHB. The within day and between day precision and accuracy were within 15%.

Study in healthy volunteers

Study design

The study was designed to evaluate the absorption of BG and DHB from the gastrointestinal tract after consuming volumes of grapefruit juice concordant to the volumes described to cause significant drug interactions. Six Caucasian healthy volunteers (4 females, 2 males; age 26 – 40) consumed 3 times 400 mL of a preselected grapefruit juice batch at 0, 3 and 6 hour. To determine serum concentrations of BG and DHB, blood samples were collected at 1, 7 and 8 hour. Blood samples were centrifuged at 4,000 rpm for 5 min and serum was divided into two tubes and stored at –20˚C until the day of analysis. The study was approved by the institutional ethics committee (Leiden University Medical Center, Leiden, The Netherlands).

Analysis of bergamottin and 6', 7'-dihydroxybergamottin in serum

The analytical method used to determine BG and DHB in serum is identical to the method used to determine BG and DHB in juice. Sample preparation was moderately adjusted; 0.5mL serum, 0.5mL phosphate buffer pH 3.0, 0.5M, 10 µL internal standard (10 µL/mL in methanol) and 4mL ethyl acetate were mixed and processed similar to the method described for grapefruit juice. Calibration standards contained 0.02 – 0.4 µg/mL BG and 0.01 – 0.2 µg/mL DHB were prepared at the start of each analytical run. The standard stock solution contained BG and DHB (10 and 5 µg/mL in DMSO:methanol(1:3)). Linearity was confirmed over the range of 0.04 – 1.60 µg/mL for BG and 0.02 – 0.80 µg/mL for DHB. The within day and between day precision and accuracy were within 15%. Stability was studied over a period of 30 days at four conditions; room temperature, refrigerated, frozen and after 3 freeze-thaw cycles and accuracy and precision remained within 15%. The LLQ levels for BG and DHB were 0.04 µg/mL and 0.02 µg/mL, respectively. The LLQ easily met the criteria of accuracy and precision of < 20% and the BG and DHB response at the LLQ was at least 5 times the blank response[25].

Results

Bergamottin and 6',7'-dihydroxybergamottin concentration in grapefruit juice

The BG and DHB concentrations as determined in different brands and lots of grapefruit juice are summarized in Table 1 and showed considerable variability. Moreover, the variation in BG and DHB concentrations within one lot (analyzed in three packets) was relatively small (< 10%). For the performance of the study in healthy volunteers Brand B2 was selected.

Amount of grapefruit juice consumed by healthy volunteers

We aimed to investigate the absorption of a BG dose in the range of at least 1.7-2 mg BG from the intestines, which is a dose capable of inducing a significant effect on felodipine exposure[10]. To administer a sufficient amount of BG in our experiment 400mL (= 2.45 mg BG) of grapefruit juice was administered.

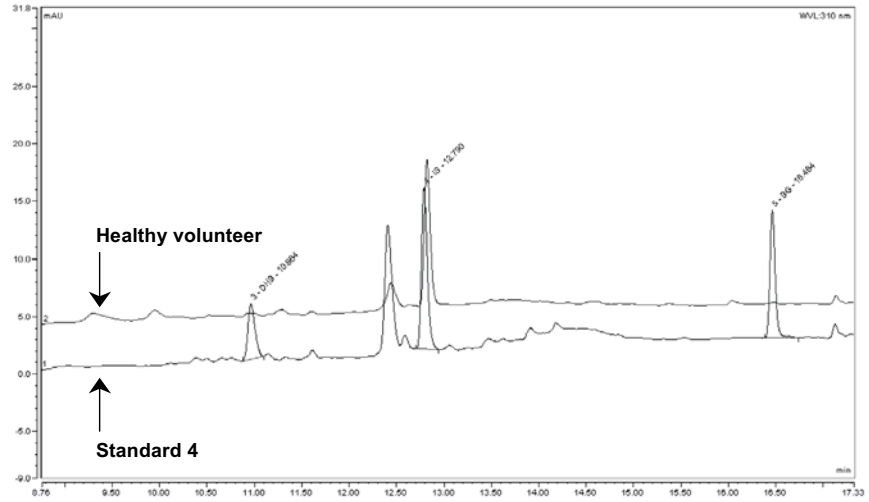
Serum bergamottin and 6', 7'-dihydroxybergamottin concentration

The serum concentrations BG and DHB after a single dose and after multiple doses of 400mL grapefruit juice were not detectable (< 0.04 µg/mL BG and <0.02 µg/mL DHB =LLQ).

Table 1 BG and DHB concentration in different batches grapefruit juice

Product	Bergamottin (mg/L)	6',7'-dihydroxybergamottin (mg/L)
Brand A lot 1	11.9	2.0
Brand A lot 2	8.0	2.1
Brand A lot 3	8.4	2.4
Brand B lot 1	10.0	7.5
Brand B lot 2	6.1	8.1
Brand C	2.6	1.0
Brand D	5.5	1.1
Brand E	4.3	0.4

Figure 1 Chromatograms DHB, IS and BG in Standard and Healthy volunteer



Discussion

In this study the absorption of the two major furanocoumarins in grapefruit juice, capable of inhibiting CYP3A4, was investigated in order to clarify their effect on intestinal and hepatic CYP3A4 activity. Indeed, the effect of grapefruit juice on CYP3A4 is thought to be the result of an irreversible inactivation of the intestinal and not hepatic CYP3A4 enzyme but the reason for this divergent effect is yet unclear[20]. Our study shows for the first time that the furanocoumarins BG and DHB are not absorbed after single or multiple consumptions of grapefruit juice which could explain their inhibiting effects on CYP3A4 located in the gastrointestinal tract but the absent effect on the same enzymes located in the liver. In addition, a large variability in furanocoumarin concentrations was observed in different brands and in different lots of one brand of grapefruit juice.

Besides inhibition of intestinal CYP3A4, grapefruit juice also inhibits the transporters ABCB1, OATP1A2 and OATP2B1 and this may additionally contribute to the effect that grapefruit juice has on the exposure of co-administered drugs[8, 11-15, 26]. Our results also implicate that interaction of BG and DHB with these transporters located outside the intestinal wall, such as in the kidney, will be limited[16, 17].

We investigated the absorption of BG and DHB after a single dose but also after multiple dosing since one may argue that BG and DHB is only absorbed after saturation of CYP3A4 e.g. after the first dose. Indeed, one study has reported an effect of grapefruit juice on the elimination half life of midazolam and the production of $^{14}CO_2$ after intravenous erythromycin administration and therefore an effect on hepatic CYP3A4 activity after consuming double strength grapefruit juice 240 mL tid for three days[27]. In contrast, an effect of grapefruit juice on hepatic CYP3A4 activity studied with other compounds (lovastatin and simvastatin) was not observed with similar amounts of double strength grapefruit juice[28, 29]. In all three studies the concentration of BG and DHB in the juices was not quantified. Contrastingly, in the our study for the first time the quantity of BG and DHB administered was measured and related to the amount of BG and DHB absorbed. Double quantities of grapefruit juice (400mL) were used to simulate the double strength used in the described studies, which is approximately double the amount normally used in interaction studies[10, 16, 27, 30]. Additionally, 1.7mg and 2mg bergamottin causes a significant drug interaction with felodipine. The 400mL used in this study equalizes 2.45mg BG and 3.22mg DHB. Repeated doses were administered with short time intervals (3hours) to saturate the intestinal CYP3A4 and prevent the formation of new CYP3A4 (CYP3A4 $t_{½} \cong 7 – 23$hours)[4, 5]. The sampling times, 1 hour after de first dose and 1 and 2 hours after the third dose of grapefruit juice, were selected based on the time to maximal BG concentration after consumption of BG capsules; ~1 hour[10]. However, also after multiple dosing non-detectable serum levels of BG and DHB were found. The lower limit of quantification of the validated HPLC-UV method was 0.02 µg/mL for BG and 0.04 µg/mL for DHB, which makes the method suitable for detecting clinically relevant BG and DHB concentrations. Indeed, BG and DHB were able to inhibit CYP3A4 mediated testosterone hydroxylation by 50% and 87.5% at 0.17 – 0.04 µg/mL and 0.15 – 0.04 µg/mL in *in vitro* experiments, respectively[20]. Serum BG and DHB levels below the lower limit of quantitation of the assay are therefore very unlikely to result in any clinical effect on hepatic CYP3A4.

These results indicate that these grapefruit juice compounds are not or only in a very limited amount absorbed after oral consumption and therefore only result in a local effect on the transporters and enzymes in the intestinal wall.

Our study confirms earlier findings regarding high variability of furanocoumarins concentrations in different brands and lots of grapefruit juices[24]. The concentrations BG and DHB measured in grapefruit juices are in the same range as earlier reported. The variability was postulated to be the result of the kind of grapefruit used (white, pink or red) and the storage conditions of the juices[24].

The variable concentrations in the different juices have important implications both clinically and experimentally. Drug-interactions with grapefruit juice could be strongly influenced by the juice that is used since higher concentrations of BG and DHB would logically result in a more pronounced inhibition of intestinal CYP3A4. Therefore, in pharmacological interaction studies a standardized quantity of BG and DHB should be administered in order to interpret the results and make the comparison with other studies possible. An international standardized quantity of 2mg BG could be used since this amount has demonstrated to result in a significant drug interaction in humans[10]. DHB has always demonstrated to exhibit a greater potency as BG *in vitro*, however the magnitude of the difference varied from ~2 - >20-fold[20]. An international standardized quantity of 1mg DHB could therefore be safely suggested for interaction studies.

In the current study we have focused on the most abundant furanocoumarins, BG and DHB and therefore we can not totally exclude an effect of other active compounds in grapefruit juice on hepatic CYP3A4 activity. However, interaction studies with the purified form of the different compounds of grapefruit juice make the furanocoumarins the group that most likely results in an inhibitory effect of CYP3A4[10, 22, 23]. Theoretically, by the design of our study we can not exclude the absorption of BG and DHB across the intestinal wall followed by an extremely high extraction ratio for these components, which could result in undetectable levels of BG and DHB in serum due to a complete first pass effect. However this theoretical large effect of BG and DHB on hepatic enzymes has not been confirmed in interaction studies so far.

In conclusion, BG and DHB are not absorbed in clinically relevant amounts after oral administration of grapefruit juice. This explains why grapefruit juice has an effect on orally administered CYP3A4 substrates whereas it has no effect on CYP3A4 substrates when administered intravenously. The observation that the contents of BG and DHB are highly variable among different brands and lots of grapefruit juices has important consequences for both the interpretation of clinical grapefruit juice – drug interactions and the design of interaction studies.

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