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Quercetin, an *in vitro* inhibitor of CYP3A, does not contribute to the interaction between nifedipine and grapefruit juice

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Quercetin, a flavonoid present in various fruits, is a potent *in vitro* inhibitor of CYP3A. Its role in the reported interaction between grapefruit juice and nifedipine has been determined *in vivo* in humans. Eight healthy volunteers were given in random order 10 mg nifedipine orally, either alone or with 200 ml double strength grapefruit juice, or with 400 mg quercetin. The area under the plasma concentration-time curve (AUC) for nifedipine with grapefruit juice (mean 320 ng ml^{-1} h) was increased significantly ($P < 0.01$) compared with the AUC when nifedipine was given alone (mean 218 ng ml^{-1} h). The time to peak plasma concentration for nifedipine with grapefruit juice (1.5 h) was also increased ($P < 0.05$) compared with control (0.5 h) suggesting delayed absorption. Although quercetin delayed the time to peak nifedipine concentration (1.3 h) it did not alter the AUC of either the parent drug (mean 209 ng ml^{-1} h) or its first-pass metabolite. The results suggest that quercetin does not contribute to the effects of grapefruit juice (which contains <10 mg of quercetin 200 ml^{-1}) on the metabolism of nifedipine. Oral doses of quercetin, similar to those possible from the ingestion of other fruits such as strawberries, do not produce *in vivo* inhibition of CYP3A mediated metabolism of nifedipine.

Keywords quercetin interaction nifedipine grapefruit juice CYP3A

Introduction

The first-pass metabolism and clearance of drugs and other xenobiotics can be influenced by both the genetic constitution of the individual and the environment. It is well recognised that cytochrome P450 isoenzymes can be induced by factors such as cigarette smoking, exposure to pesticides and the consumption of *Brassica* or charcoal broiled meat. However, less attention has been paid to the presence of environmental inhibitors of drug oxidation. There is *in vitro* evidence of inhibition of cytochrome P450 enzymes by flavonoids such as quercetin, kaempferol and naringenin [1, 2, 3, 4]. These flavonoids are widely distributed in edible plants, fruits and vegetables and may be consumed daily in large quantities. Quercetin is present in large amounts in fruits [5] and is a powerful *in vitro* inhibitor of cytochrome P450 oxidations.

The area under the plasma concentration-time curve (AUC) of dihydropyridines such as felodipine and nifedipine are increased when co-administered with grapefruit juice but not orange juice [6] probably through inhibition of first-pass metabolism. Studies on the inhibitory effects of different flavonoids on oxidative metabolism *in vitro* [3] reported similar potencies for quercetin and naringenin; based on the concentrations present in different fruits, the authors suggested that naringenin was probably responsible for the effects observed with grapefruit juice *in vivo* [6]. Subsequent papers on the *in vitro* metabolism of felodipine and nifedipine [4, 7] showed that quercetin and kaempferol were considerably (about 10 times) more potent than naringenin, especially with human liver microsomes: the authors [4] suggested that the previous study [3] may have overestimated the potency of naringenin because of the purity of the material used or the fact that the assay was based on the formation of the pyridine analogue (which is itself metabolised by an isoenzyme of cytochrome P450) [8], rather than the disappearance of the substrate. However, based on the concentrations of naringin (the precursor of naringenin) present in grapefruit juice (3 mM equivalent to 817 mg l^{-1} naringenin) the authors accepted that naringenin could be responsible for the inhibitory effect detected *in vivo*.

Quercetin is present in a variety of fruits, with high concentrations in cherries, apricots, raspberries, blackberries, red and blackcurrants (25–80 mg kg^{-1}) and very

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high concentrations in strawberries (300 mg kg^{-1}) [9]. The aim of the present study was to determine whether quercetin is an *in vivo* inhibitor of CYP3A, and if so if it contributes to the interaction between nifedipine and grapefruit juice.

Methods

Eight healthy volunteers (seven male, one female, two of whom were South Asians) aged 21–23 years and with body weights 56–94 kg participated in the study. The study was approved by the Local Ethics Committee and written informed consent was obtained from all subjects. The study involved administration of nifedipine on 3 study days separated by at least 1 week. The volunteers fasted the night before each study day. At about 09.00 h on the study day they received in random order a) 10 mg nifedipine orally with 200 ml water, b) 10 mg nifedipine orally with 200 ml double strength grapefruit juice and c) 10 mg nifedipine plus quercetin orally with 200 ml water (200 mg quercetin the night before, 100 mg on waking and 100 mg with the dose of nifedipine). The volunteers remained semi-recumbent for 4 h after each of the doses since posture can affect the pharmacokinetics of nifedipine capsules [10]. After 4 h they were given lunch and allowed freedom of movement.

Blood samples were taken prior to the dose and at 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0 2.5, 3.0 3.5 and 4 h and then hourly up to 8 h and at 12 and 24 h (post-dose). All samples were taken into lithium heparin tubes, centrifuged immediately and stored at −20° C until extraction and analysis. Sample processing was performed under sodium lighting to prevent photo-degradation. Nifedipine and its first-pass metabolite were measured by a reverse phase h.p.l.c. method based on that of Waller *et al.* [11]. Pharmacokinetic parameters were calculated by standard non-compartmental methods. The terminal half-life was derived by least squares regression analysis applied to the post-peak, log-linear part of the plasma drug concentration-time curve. The AUC was determined by the linear trapezoidal method and extrapolated to infinity by dividing the last measurable concentration by the terminal slope. The maximum concentration (C_{max}) and the time of C_{max} (t_{max}) are the observed values. Data were evaluated statistically by Wilcoxon's signed-rank test with a value of $P < 0.05$ taken as significant.

Analysis of grapefruit juice for quercetin

Grapefruit juice (0.2 ml) was mixed with methanol (9.8 ml) and centrifuged. An aliquot (20 μl) was injected onto a reverse phase C18 h.p.l.c. column (Waters Associates) and eluted with methanol/water (50/50 by volume). Quercetin was detected by its absorption at 375 nm and had a retention time of 2.6 min under these conditions. A series of standards was prepared by spiking increasing concentrations of quercetin (0–0.1 mg in 10 μl methanol) into the 0.2 ml grapefruit juice. A graph of peak height against concentration added was linear from 0.01–0.1 mg 0.2 ml^{-1}.

A very small peak of quercetin was detected in the unspiked grapefruit juice, which using the slope and intercept indicated that about 1.4 μg was present per 0.2 ml, i.e. about 1 mg 200 ml^{-1}. Because the standard curve was not linear below 0.01 mg 0.2 ml^{-1} the peak height in the absence of added quercetin was also compared with that in the standard containing 0.01 mg 0.2 ml^{-1}. The peak height of the 0.01 mg 0.2 ml^{-1} standard was over twice that in the unspiked grapefruit juice; therefore allowing for non-linearity the maximum concentration in grapefruit juice would be 0.01 mg 0.2 ml^{-1}, i.e. 10 mg 200 ml^{-1}.

Results

The pharmacokinetic parameters for nifedipine and its first-pass metabolite, under each of the dosing conditions, i.e. control, with quercetin and with grapefruit juice, are shown in Table 1. The times to reach peak

Table 1 The influence of grapefruit juice and quercetin on the pharmacokinetics of nifedipine given as a single oral dose in capsule form

	Control	*Quercetin*	*Grapefruit juice*
Nifedipine			
AUC (ng ml^{-1} h)	218 (146–290)	209 (131–287)	320 (202–437)**
C_{max} (ng ml^{-1})	136 (75–197)	99 (58–139)	141 (61–221)
t_{max} (h)	0.5 (0.3–0.8)	1.3 (0.2–2.5)*	1.5 (0.9–2.0)*
Half-life (h)	2.6 (2.1–3.1)	2.7 (2.2–3.3)	3.7 (1.8–5.6)
MRT (h)	3.1 (2.3–3.9)	3.9 (2.9–4.9)	4.7 (3.2–6.2)
Nitropyridine metabolite			
AUC (ng ml^{-1} h)	96 (74–118)	102 (85–118)	129 (99–159)
C_{max} (ng ml^{-1})	60 (42–78)	52 (24–79)	46 (32–59)
t_{max} (h)	0.6 (0.3–0.8)	1.2 (0.1–2.2)	1.4 (0.9–2.0)*
Half-life (h)	2.2 (1.8–2.6)	3.7 (2.5–5.0)*	3.5 (2.2–4.9)*
MRT (h)	2.8 (2.4–3.3)	4.8 (3.6–5.9)**	5.1 (3.7–6.5)**
Ratio $\frac{\text{AUC nifedipine}}{\text{AUC metabolite}}$	2.5 (1.4–3.5)	2.1 (1.3–2.9)	2.5 (1.8–3.3)

The results are the mean for eight volunteers with 95% confidence intervals in parentheses.
*$P < 0.05$; **$P < 0.01$ compared with control by Wilcoxon's signed rank test.

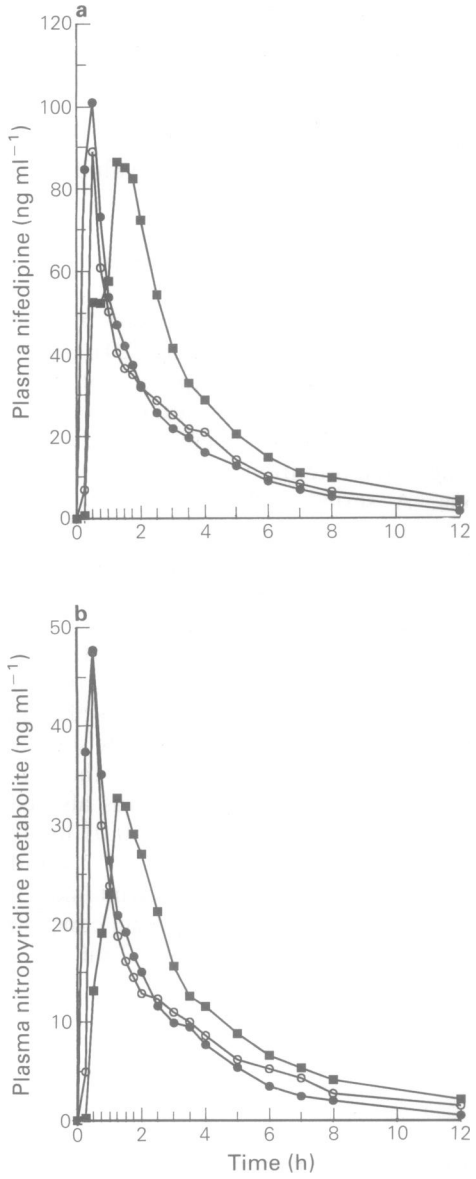

Figure 1 Plasma concentrations of nifedipine (a) and its first-pass metabolite (b), when given alone (●), with quercetin (○) or with grapefruit juice (■). The data are the means for eight subjects; statistically significant differences ($P < 0.05$) were detected between control and grapefruit juice for both nifedipine (between 1.25 h and 12 h) and its first-pass metabolite (between 1.25 h and 2.5 h and between 5 h and 12 h).

plasma concentration (t_{max}) of both nifedipine and its metabolite were significantly increased with grapefruit juice ($P < 0.05$) compared with control (Figure 1). Administration of quercetin also delayed the absorption of nifedipine since the t_{max} was 1.3 h compared with 0.5 h under control conditions. The AUC of nifedipine with grapefruit juice (mean 320 ng ml^{-1} h) was significantly increased ($P < 0.01$) compared with the AUC under control conditions (mean 218 ng ml^{-1} h) but there was no significant change after administration of quercetin. Neither grapefruit juice nor quercetin reduced the AUC of the first-pass nitropyridine metabolite or altered the ratio of nifedipine to its metabolite (Table 1). Both grapefruit juice and quercetin significantly increased the terminal half-life and mean residence time of the first-pass metabolite, but not of nifedipine itself. The increase in half-life may have arisen in part from the delayed time to peak and resulting extended duration of measurable concentrations of the metabolite. Calculation of the kinetic parameters using 0–8 h data only, reduced the calculated half-lives of the metabolite [mean values 2.1 h (1.7–2.6), 3.0 h (2.2–3.8) and 3.1 h (2.0–4.3) after control, quercetin and grapefruit juice respectively] but did not remove the statistical significance of the differences. Similarly, the mean residence times remained significantly different between treatments, using 0–8 h data, since this would reflect both the longer terminal half-life and the slower absorption when nifedipine was given with quercetin or grapefruit juice.

Discussion

The present study has demonstrated a significant increase in the AUC for nifedipine when given with grapefruit juice compared with control. This finding is consistent with previous reports of increased bioavailability of nifedipine and felopidine with grapefruit juice [6]. Nifedipine is metabolised by CYP3A [12], and undergoes extensive first-pass metabolism [11] to the pyridine analogue. It is therefore likely that the augmented AUC of nifedipine is due to a component in grapefruit juice inhibiting its first-pass metabolism. The increased time to reach peak plasma concentration (t_{max}) for nifedipine with grapefruit juice and quercetin indicated delayed gastric emptying and prolonged absorption. The AUC of the nitropyridine metabolite was not statistically significantly reduced with grapefruit juice but was increased by about 35%; a significant increase (22%) was reported by Bailey *et al.* [6]. The increase in the AUC of the metabolite may indicate that grapefruit juice also inhibits the further metabolism of the pyridine metabolite (which is probably *via* a P450 isoenzyme but not CYP3A [8]). The increase in the terminal half-life of the metabolite and the fact that the ratios of the AUC of nifedipine to that of its metabolite remained unaltered support this possibility. The inhibitory effect of grapefruit juice *in vivo* is not specific to CYP3A since the oral clearance of caffeine (a substrate for CYP1A2) was decreased when given with grapefruit juice [3].

The AUC for nifedipine and the nitropyridine metabolite showed no significant change when given with quercetin compared with control. The amount of quercetin administered (400 mg in divided doses) was at least 40 times the maximum amount present in the grapefruit juice and was equivalent to the amount ingested if an individual consumed about 600 g of strawberry on the evening preceding and on the morning of the study day. The lack of inhibition by quercetin *in vivo* contrasts with the *in vitro* inhibitory effect demonstrated with both rat and human liver microsomal P450 activity [2, 3, 4, 7]. The absence of an inhibitory effect of quercetin *in vivo* may be due to inadequate concentrations reaching the P450 enzymes because of poor absorption and/or its own metabolism by the liver or gut wall. *In vivo* inhibition of nifedipine metabolism is unlikely to arise from the dietary consumption of fruits containing quercetin.

From this study we conclude that the inhibition of

nifedipine metabolism by grapefruit juice is not due to quercetin but some other component, possibly naringenin. Alternatively, vasoactive substances in grapefruit juice such as urodiolenone may have increased liver blood flow thereby reducing first-pass extraction [14]. In addition nootkatone the immediate precursor of urodiolenone may compete with nifedipine for CYP3A4 metabolism thereby enhancing the AUC of nifedipine [14].

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References

1 Buening MK, Chang RL, Huang M, *et al.* Activation and inhibition of benzo(a)pyrene and aflatoxin B_1 metabolism in human liver microsomes by naturally occurring flavonoids. *Cancer Res* 1981; **41**: 67–72.

2 Sousa RL, Marletta MA. Inhibition of cytochrome P-450 activity in rat liver microsomes by the naturally occurring flavonoid, quercetin. *Arch Biochem Biophys* 1985; **240**: 345–357.

3 Guengerich FP, Kim DH. *In vitro* inhibition of dihydropyridine oxidation and aflatoxin B_1 activation in human liver microsomes by naringenin and other flavonoids. *Carcinogenesis* 1990; **11**: 2275–2279.

4 Miniscalco A, Lundahl J, Regardh CG, Edgar B, Ericksson UG. Inhibition of dihydropyridine metabolism in rat and human liver microsomes by flavonoids found in grapefruit juice. *J Pharmac exp Ther* 1992; **261**: 1195–1199.

5 Kühnau J. The flavonoids; a class of semi essential food components. Their role in human nutrition. *Wld Rev Nutr Diet* 1976; **24**: 117–191.

6 Bailey DG, Spence JD, Munoz C, Arnold JMO. Interaction of citrus juices with felodipine and nifedipine. *Lancet* 1991; **337**: 268–269.

7 Lundahl J, Miniscalco A, Edgar B, Regardh CG, Eriksson UG. Effects of flavonoids on the oxidative metabolism of felodipine and nifedipine. *Abstracts of the Third North American ISSX Meeting*, San Diego, 1990; p24.

8 Guengerich, FP. Oxidative cleavage of carboxylic esters by cytochrome P450. *J biol Chem* 1987; **262**: 8459–8462.

9 Wildanger W, Herrmann K. Die phenolischen Inhaltsstoffe des Obstes. II Die Flavonole des Obstes. *Z Lebensmittel Unters* 1973; **151**: 103–108.

10 Renwick AG, Ahsan CH, Challenor VF, *et al.* The influence of posture on the pharmacokinetics of orally administered nifedipine. *Br J clin Pharmac* 1992; **34**: 332–336.

11 Waller DG, Renwick AG, Gruchy BS, George CF. The first-pass metabolism of nifedipine in man. *Br J clin Pharmac* 1984; **18**: 951–954.

12 Nebert DW, Nelson DR, Adesnik M, *et al.* The P450 Superfamily: Updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA* 1989; **8**: 1–13.

13 Fuhr U, Klittich K, Staib AH. Inhibitory effect of grapefruit juice and the active component, naringenin, on CYP1A2 dependent metabolism of caffeine in man. *Br J clin Pharmac* 1993; **35**: 431–436.

14 Chayen R, Rosenthal T. Interaction of citrus juices with felodipine and nifedipine. *Lancet* 1991; **337**: 854.

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