Possible enhancement of the first-pass metabolism of phenacetin by ingestion of grape juice in Chinese subjects

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Aims This serendipitous study revealed an unexpected effect of Jufeng grape juice on the CYP1A2-mediated metabolism of phenacetin. Investigation of the inhibition of CYP1A2 by grapefruit juice was involved but a translation error led to the grape juice substitution.

Methods Twelve healthy subjects took a single oral dose of phenacetin (900 mg) on two randomized occasions together with 200 ml water or grape juice. Plasma phenacetin and paracetamol concentrations were assessed by h.p.l.c.

Results Ingestion of grape juice was associated with reduced plasma phenacetin concentrations, while paracetamol levels were unaffected. Paracetamol to phenacetin AUC ratios increased from 13.9 ± 3.1 to 24.3 ± 3.8 after ingestion of grape juice.

Conclusions These findings suggest enhanced first-pass metabolism of phenacetin, due to CYP1A2 activation by grape juice or to desaturation of CYP1A2 isoenzymes secondary to a slower rate of phenacetin absorption.

Keywords: cytochrome CYP1A2, drug metabolism, grape juice, paracetamol, phenacetin

Introduction

Constituents of grapefruit juice inhibit CYP3A4, leading to decreased first-pass metabolism of a number of substrates of this important cytochrome P450 [1]. CYP1A2 activity may also be inhibited by grapefruit juice [2], but evidence for this remains inconclusive. In order to investigate this interaction, we designed a study aimed at assessing the effect of grapefruit juice on the disposition of phenacetin, a marker of CYP1A2 activity [3, 4]. Erroneous substitution of grape juice for grapefruit juice led to the serendipitous discovery that ingestion of grape juice markedly reduces plasma phenacetin concentrations, probably due to enhanced first-pass metabolism.

Methods

Twelve healthy Chinese subjects (mean age 36 years, range 29–46 years, and mean body weight 60 kg, range 50–72 kg) were studied. Six subjects (three males, three females) were nonsmokers, and six (three males, three females) smoked more than 10 cigarettes/day. All gave

written informed consent and the protocol was approved by Ethics Committees at both study sites. The clinical part of the work was carried out in the Institute of Clinical Pharmacology, Great Chinese Wall Hospital, Beijing.

After an overnight fast, each subject received a single oral dose of phenacetin (3×300 mg capsules) on two randomized occasions separated by an interval of at least 1 week. On one occasion the phenacetin was taken with 200 ml water and on the other occasion with 200 ml grape juice rather than the intended grapefruit juice. The juice was prepared manually by squeezing fresh Jufeng variety grapes through a cotton gauze, the unbroken seeds being discarded together with the fruit peel. After preparation, the juice was kept frozen at -30°C for up to 3 months, removed from the freezer 12 h before consumption and left to reach room temperature before ingestion. The volunteers were allowed no food or drinks for 4 h after dosing. Blood samples were collected for up to 12 h and plasma stored at -30°C before shipping frozen for assay in Pavia within 3 months.

Plasma concentrations of phenacetin and metabolically derived paracetamol were determined by h.p.l.c [3]. Pharmacokinetic parameters were determined by model-independent analysis as previously described [3, 4]. Phenacetin oral clearance (CL_{O}), an indirect measure

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Figure 1 Plasma concentrations (means $\pm$ s.e. mean) of phenacetin and metabolically derived paracetamol in 12 subjects after a 900 mg phenacetin dose taken with (closed symbols) and without (open symbols) grape juice. For calculations of means, undetectable concentrations were taken as equal to the detection limit ($0.1 \mu\text{g ml}^{-1}$). Phenacetin means were not calculated if more than eight subjects had undetectable levels. Paracetamol was always detectable.

of CYP1A2 activity [3, 4] was calculated as Dose/AUC(0, ∞). Results are given as means $\pm$ s.e. mean. Comparisons were made by Student's *t*-test for paired and unpaired data as applicable; t_{max} values were compared by Wilcoxon's rank test.

Results

Plasma phenacetin concentrations

Ingestion of grape juice was associated with a marked reduction in plasma phenacetin concentrations and a delay in time to peak concentration (Figure 1). Phenacetin C_{max} , AUC and half-life were significantly reduced, while CL_o values were significantly increased (Table 1).

Since cigarette smoking induces CYP1A2-mediated phenacetin metabolism [5], nonsmokers and smokers were compared. Grape juice reduced phenacetin AUC and increased CL_o values to a similar extent both in non smokers (AUC: from 7.6 ± 2.4 to $4.3 \pm 1.6 \mu\text{g ml}^{-1} \text{h}$; CL_o : from 3.8 ± 1.5 to $6.9 \pm 2.1 \text{ l h}^{-1} \text{kg}^{-1}$) and in smokers (AUC: from 2.8 ± 0.9 to $1.2 \pm 0.2 \mu\text{g ml}^{-1} \text{h}$; CL_o : from 8.3 ± 2.2 to $13.1 \pm 1.9 \text{ l h}^{-1} \text{kg}^{-1}$), though the difference was statistically significant ($P < 0.05$) only in the former.

Metabolically derived paracetamol

Plasma paracetamol levels with or without grape juice were superimposable apart from a modest delay in time to peak (Figure 1 and Table 1). Paracetamol to phenacetin molar AUC ratios increased two-fold after ingestion of the juice (from 13.9 ± 3.1 to 24.3 ± 3.8 , $P < 0.01$).

Discussion

The complete absorption and extensive CYP1A2-mediated presystemic metabolism of phenacetin makes its oral clearance a good index of CYP1A2 activity [3, 4]. Furthermore, potentially short-lived effects on first-pass metabolism are reflected in changes in the oral clearance of phenacetin.

To date, most studies on the metabolic modulators in fruit juice on drug metabolism have concerned grapefruit,

Table 1 Pharmacokinetic parameters (means $\pm$ s.e. mean and 95% CI for the differences between the two groups) derived from plasma concentrations of phenacetin and metabolically derived paracetamol after a single 900 mg phenacetin dose taken with or without (control) grape juice in 12 healthy subjects.

	Phenacetin				Paracetamol	
	Control	Grape juice	95% CI	Control	Grape juice	95% CI
C_{max} ($\mu\text{g ml}^{-1}$)	2.4 ± 0.6	$1.1 \pm 0.4^{**}$	(-2.1; -0.5)	6.9 ± 0.3	7.1 ± 0.6	(-0.8; 1.3)
t_{max} (h)†	1.5 (1; 3)	2 (2; 3)**		2 (2; 4)	3 (2; 5)	
$t_{1/2}$ (h)	0.95 ± 0.07	$0.79 \pm 0.05^*$	(-0.31; -0.01)	2.64 ± 0.18	2.50 ± 0.14	(-0.55; 0.27)
AUC ($\mu\text{g ml}^{-1} \text{h}$)	5.2 ± 1.4	$2.8 \pm 0.9^{**}$	(-3.9; -1.01)	37.6 ± 2.8	38.8 ± 3.0	(-2.4; 4.7)
CL_o ($\text{l h}^{-1} \text{kg}^{-1}$)	6.1 ± 1.4	$10.0 \pm 1.6^*$	(0.3; 7.5)			

†Median and range.

* $P < 0.05$

** $P < 0.01$

which contains inhibitors of CYP3A4- and, possibly, CYP1A2-mediated drug oxidation [1]. Now serendipitous substitution of grapefruit juice with grape juice resulted in significantly increased phenacetin oral clearance. Both reduced absorption or increased first-pass metabolism could decrease plasma phenacetin concentrations. However, although phenacetin absorption rates were probably decreased by grape juice (as indicated by a modest delay in $t_{\max}$ for both parent drug and metabolite), a decrease in the extent of absorption is unlikely because the paracetamol AUC was unchanged. Increased paracetamol to phenacetin AUC ratios could also be caused by impaired phenacetin absorption associated with decreased elimination of the metabolite, but the paracetamol half-lives were unaffected by juice administration. Therefore, enhanced CYP1A2-mediated metabolism, consistent with the decreased phenacetin half-life, is a more likely explanation.

The decrease in phenacetin concentrations within 0.5 h of juice intake is incompatible with enzyme induction and suggests enzyme activation [6]. Some synthetic and naturally occurring flavonoidic substances such as 5,6-benzoflavone, 7,8-benzoflavone, 4'-bromoflavone, flavone, nobiletin and tangeretin have been reported to activate the CYP1A-mediated hydroxylation of benzo-(a)pyrene and aflatoxin B1 oxidation [7, 8]. Although metabolic activation affecting drug disposition in humans has not been reported, flavonoids in grape juice (or formed during its storage) have increased hepatic or intestinal CYP1A2 activity. Alternatively, desaturation of CYP1A2 enzymes secondary to slower absorption rates may have enhanced phenacetin first-pass metabolism. This explanation depends on the first-pass extraction of phenacetin being concentration-dependent, and there is no other evidence for this. Moreover, even those subjects showing no delay in time to peak phenacetin concentrations or paracetamol formation in association with grape juice showed a marked juice-related fall in plasma phenacetin concentrations.

Since CYP1A2 isoenzymes are involved in the metabolism of a large number of drugs and toxins [9], even a transient enhancement of activity by fruit juice may have clinical consequences.

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