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Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man*

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1 The effects of grapefruit juice and naringenin on the activity of the human cytochrome P450 isoform CYP1A2 were evaluated using caffeine as a probe substrate.
2 *In vitro* naringenin was a potent competitive inhibitor of caffeine 3-demethylation by human liver microsomes (K_i = 7–29 μM).
3 *In vivo* grapefruit juice (1.2 l day^{-1} containing 0.5 g l^{-1} naringin, the glycone form of naringenin) decreased the oral clearance of caffeine by 23% (95% CI: 7%–30%) and prolonged its half-life by 31% (95% CI: 20%–44%) (n = 12).
4 We conclude that grapefruit juice and naringenin inhibit CYP1A2 activity in man. However, the small effect on caffeine clearance *in vivo* suggests that in general the ingestion of grapefruit juice should not cause clinically significant inhibition of the metabolism of other drugs that are substrates of CYPIA2.

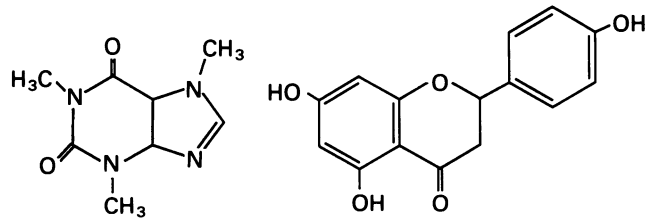
Keywords caffeine grapefruit naringin naringenin flavanones cytochrome P-450 CYP1A2 food-drug interaction saliva

Introduction

Grapefruit juice increases the mean oral bioavailability of nifedipine in man by 34% and of felodipine by 184% (Bailey *et al.*, 1991). These two drugs are completely absorbed following oral administration, but their low bioavailability (approximately 50% for nifedipine and 15% for felodipine) is due to hepatic first-pass metabolism (Reynolds, 1989). Therefore, the effect of grapefruit juice is likely to be mediated by inhibition of the biotransformation of the dihydropyridines, which is primarily carried out by the cytochrome P450 isoform CYP3A4 (Böcker & Guengerich, 1986; for the most recent cytochrome P450 enzyme nomenclature, see Nebert *et al.*, 1991).

Natural grapefruit juice (*Citrus paradisi*) contains several flavonoid glycosides. Naringin (4′, 5, 7-trihydroxyflavanone 7-rhamno-glucoside), the so-called 'bitter principal' of grapefruit, is the most abundant of these compounds and is usually present in concentrations of up to 800 mg l^{-1} of juice (Rousseff *et al.*, 1987; Galensa, personal communication). Following oral administration to man, naringin is thought to be converted to the aglycone naringenin (Figure 1), presumably in the gut (Booth *et al.*, 1958; Buening *et al.*, 1981). Guengerich & Kim (1990) have demonstrated that naringenin inhibits CYP3A4 activity in human liver microsomes. Both *in vivo* and *in vitro* results therefore suggest that the oral bioavailability of drugs with a marked first-pass metabolism, and involving metabolism by CYP3A4, may be increased when coadministered with grapefruit juice. The calcium-channel blockers nitrendipine, isradipine, nimodipine, verapamil and gallopamil belong to this class of drugs.

The specificity of the naringenin effect on CYP3A4, however, is not clearly defined. More than one P450 isoform may be involved since the flavonoid also inhibits benzo(a)pyrene hydroxylation *in vitro*, a metabolic step



Caffeine (1,3,7-trimethylxanthine)

Naringenin (4′,5,7-trihydroxyflavanone)

Figure 1 Chemical structures of caffeine and naringenin.

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*Dedicated to Professor Dr D. Palm on the occasion of his 68th birthday.

mediated by enzymes of the CYP1A subfamily (Buening *et al.*, 1981).

Thus, the objective of the present study was to investigate the effect of grapefruit juice on CYP1A2 in man. Caffeine (Figure 1) metabolism was used as a specific marker substrate for CYP1A2 activity both *in vivo* (Fuhr *et al.*, 1992b) and *in vitro* (Butler *et al.*, 1989) and to provide evidence that grapefruit juice and/or naringenin inhibit caffeine metabolism.

Methods

In vitro

Liver specimens were obtained from patients with adenocarcinoma of the colon undergoing partial hepatectomy for removal of a single liver metastasis. Portions of macroscopically healthy liver taken from the excised lobe were used to prepare microsomes (Fuhr *et al.*, 1990). The tissue specimens originated from two males (R1: 54 years old, non-smoker, 0.52 nmol total cytochrome P-450 mg^{-1} microsomal protein; R6: 75 years old, non-smoker, 0.53 nmol total cytochrome P-450 mg^{-1} microsomal protein).

The substrate caffeine (0, 0.125, 0.25, 0.5, 1 and 2 mM) was incubated with either naringin or naringenin (0, 30, 100, and 300 μM), a NADPH generating system and human liver microsomes as described previously (Fuhr *et al.*, 1990). The flavanones were dissolved in DMSO, at concentrations adjusted to give a final DMSO concentration in the incubation medium of 1% (v/v). The microsomes of both donors were used separately and incubations were carried out in duplicate. Caffeine 3-demethylation, measured as 1,7-dimethylxanthine (17X) formation, was used as an index of the activity of human CYP1A2 (Fuhr *et al.*, 1990).

Enzyme constants were obtained from Hanes and Dixon plots. A least squares linear regression without value weighting was used in both plots. The medians of the coordinates of the points of intersection found for each pair of regression lines in the Dixon plots were used to calculate K_i and V_{max} values from inhibition experiments (Fuhr *et al.*, 1990).

In vivo

The effects of grapefruit juice on caffeine pharmacokinetics were measured using a randomised, crossover study design. The Ethics Committee of the University Hospital Frankfurt gave its approval to the study which was conducted according to the revised declaration of Helsinki (Hong Kong, 1989). The subjects were 12 healthy volunteers who gave their written informed consent. Both male ($n = 4$) and female ($n = 8$) subjects, aged between 21 and 56 years and including smokers ($n = 6$) and non-smokers ($n = 6$), participated in the study. Women on oral contraceptives were included if the drug used and the dosage were the same for both study periods. The weights of the subjects ranged between 39 and 98 kg. Individual characteristics of the volunteers are listed in Table 1. All had normal pre-study blood chemistry and haematology values.

Following an overnight period of methylxanthine abstinence the subjects received 167 mg of caffeine in the morning administered as 5.0 g of instant coffee (Nescafé Classic) in both study periods. In addition, the subjects drank 300 ml of either grapefruit juice or water 0.5 h before and every 6 h following caffeine intake until the end of the sampling period. In order to exclude any contamination that might be present in commercially processed grapefruit juice, only the freshly pressed juice from 'Outspan' fruit of South African origin was administered. The naringin content determined by h.p.l.c. (Greiner & Wallrauch, 1984) in two fruits of the batch used was 530 and 421 $mg\ l^{-1}$. Both plasma (predose and 2, 5, 9, and 24 h following caffeine intake) and saliva samples (predose, and 1, 2, 3, 5, 7, 9, 12, 15, 18, 24, 30, and 36 h postdose) were collected. Food and beverages containing methylxanthines and citrus fruits other than the test substances were not permitted during the sampling period. The washout time between study periods was 1 week.

Caffeine concentrations in plasma and saliva were measured by h.p.l.c. Protein precipitation with trichloroacetic acid was carried out on plasma specimens before analysis (Fuhr *et al.*, 1990). An extraction procedure, previously described for the determination of caffeine and its metabolites in cell incubation media, was used to process saliva samples (Fuhr *et al.*, 1992b). The

Table 1 Demographic details of the subjects studied

Subject	*Sex (m/f)*	*Age (years)*	*Height (cm)*	*Weight (kg)*	*Oral contraceptives*	*Smoking habits (cigarettes/day)*
1	f	22	169	55	yes*	0
2	f	29	160	58	yes*	20
3	m	30	190	98	—	0
4	m	56	174	80	—	4
5	m	29	185	81	—	0
6	f	25	171	60	no	0
7	f	26	158	39	yes*	35
8	f	35	163	57	no	0
9	f	52	171	78	no	22
10	f	21	175	74	no	15
11	m	23	172	76	—	10
12	f	56	162	60	no	0

*the preparations and the dosage are identical for both limbs of the study.

h.p.l.c. procedure has been reported (Fuhr *et al.*, 1990). The analysis gave peak responses to spiked concentrations which were linear in the range 500 nM to 100 μM for plasma and 100 nM to 50 μM for saliva with coefficients of variation of less than 10% for repeated measurements at the lower limit of determination, and of less than 5% at concentrations which were four fold greater than the limits of determination. Grapefruit juice, naringin and naringenin did not interfere with the h.p.l.c. methods.

The calculation of pharmacokinetic parameters of caffeine, obtained from the data after oral application, were based on the following assumptions:

- rapid absorption and 100% bioavailability of oral caffeine (Benet & Williams, 1990; Newton *et al.*, 1981).
- a constant ratio between caffeine concentrations in saliva and plasma. This was evaluated by comparing the kinetic parameters determined using measured plasma concentrations and those based on plasma concentrations calculated from saliva concentrations (see discussion).
- first-order elimination of caffeine during the observation period (Campbell *et al.*, 1987; Parsons & Neims, 1978).

Calculated plasma caffeine concentrations were obtained by dividing measured saliva concentrations by 0.790, the factor describing the ratio between caffeine concentrations in saliva and plasma (see results). An open one-compartment-model was used to calculate pharmacokinetic parameters, including oral clearance values, from measured and calculated plasma concentrations.

Since for practical reasons, methylxanthine abstinence was started only 12 h before caffeine intake, caffeine was present in all pre-dose samples, reaching 13.5 ± 13.7% of the post-dose maxima and making a correction of caffeine concentrations necessary. This was achieved by using the magnitude of the pre-dose caffeine concentrations and the elimination rate constants for caffeine determined in the individual subjects.

The Wilcoxon matched pairs sign rank test was used to assess statistical differences in caffeine kinetic parameters.

Non-parametric 95% confidence intervals (CI) were calculated to assess the statistical significance of changes in the kinetic parameters related to grapefruit juice intake (Steinijans & Diletti, 1983). The Mann-Whitney U test was used to compare baseline kinetics between smokers and non-smokers. Saliva:plasma concentration ratios and kinetic parameter ratios were estimated using linear correlation. The possibility that saliva:plasma concentration ratios were a function of sampling time after dose was examined using the Spearman rank correlation coefficient. This coefficient was also used to evaluate a possible correlation between naringin dose and relative changes in kinetic parameters. Statistical significance was assumed when the corresponding P values were lower than $\alpha = 0.05$.

Table 2 Enzyme constants describing caffeine 3-demethylation by human liver microsomes

	Microsome sample R1	R6
K_m Caffeine (μM)	268	177
K_i Naringenin (μM)	7	29
V_{max} in absence of naringenin (pmol 17 X min^{-1} mg^{-1} protein)	48	45
V_{max} in presence of naringenin (pmol 17 X min^{-1} mg^{-1} protein)	45	47

17 X = 1,7-dimethylxanthine; for incubation conditions see methods.

Results

In vitro

Hanes plots showed a linear relationship between substrate concentration and the substrate concentration: 1,7-dimethylxanthine formation rate quotient. A linear relationship is indicative of a single enzyme (i.e. CYP1A2) mediating the reaction (Fuhr *et al.*, 1992b). Naringenin was a potent competitive inhibitor of CYP1A2 mediated caffeine 3-demethylation in both microsome samples (Figure 2). The derived enzyme constants are shown in Table 2. Inhibition constants K_i were one order of magnitude lower than K_m values. Naringin, however, did not show a direct influence on caffeine metabolism in the concentration range tested.

In vivo

The saliva:plasma concentration ratio was 0.790 ± 0.078 (overall mean ± s.d.). Its value depended neither on the post-dose sampling time nor on the study period. There was a close correlation between kinetic parameters calculated from measured plasma caffeine concentrations and plasma caffeine concentrations derived from saliva concentrations ($r > 0.96$ for oral clearance, elimination half-life and AUC values). These observations indicate that saliva samples can be used to obtain reliable estimates

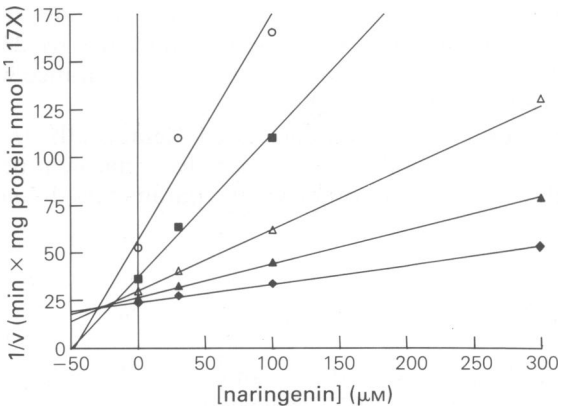

Figure 2 Dixon plot illustrating the competitive inhibitory effect of naringenin on caffeine 3-demethylation by hepatic microsomes from donor R6. 17X = 1,7-dimethylxanthine. Caffeine concentrations ○ 125 μM, ■ 250 μM, △ 500 μM, ▲ 1000 μM, ◆ 2000 μM.

Table 3 Pharmacokinetic parameters for caffeine in plasma[a] during coadministration of either water (W) or grapefruit juice (G)

Subject	AUC (μmol l^{-1} h) with W	AUC (μmol l^{-1} h) with G	CL_0[b] (ml min^{-1}) with W	CL_0[b] (ml min^{-1}) with G	$t_{½,z}$ (h) with W	$t_{½,z}$ (h) with G	V^b (l kg^{-1}) with W	V^b (l kg^{-1}) with G
1	332	525	42	27	7.9	13.0	0.53	0.56
2	109	125	130	105	2.6	3.4	0.50	0.53
3	122	121	117	120	5.7	6.3	0.56	0.66
4	105	134	133	104	3.9	4.9	0.55	0.55
5	148	206	98	67	5.8	7.1	0.61	0.51
6	171	153	74	95	4.8	5.2	0.51	0.72
7	108	156	107	78	1.8	2.7	0.43	0.47
8	252	388	55	37	6.4	10.2	0.53	0.57
9	97	90	151	155	2.9	3.4	0.49	0.59
10	105	138	144	105	4.7	6.0	0.78	0.74
11	51	83	248	177	2.7	3.6	0.76	0.72
12	162	224	89	67	4.4	6.3	0.56	0.61
Mean	147	195	115	94	4.5	6.0	0.57	0.60
s.d.	77	132	54	44	1.8	3.0	0.10	0.09
Intraindividual relative changes due to grapefruit juice coadministration:								
point estimator	+28%*		−23%*		+31%*		+7%	
95% CI	+11%/+46%		−30%/−7%		+20%/+44%		−3%/+16%	

a) plasma caffeine concentrations were calculated using saliva concentrations.
b) estimate relative 100% bioavailability.
* significant differences between periods.

of caffeine kinetics in plasma. Using saliva has the additional advantage that estimates of kinetic parameters can be based on more data points. For these reasons, the kinetic parameters shown are those based on transformed saliva concentrations. Kinetic parameters derived from measured plasma caffeine concentrations, which gave essentially the same results (data not presented), served principally to validate the use of saliva:plasma concentration ratios.

Smokers exhibited significantly higher oral caffeine clearance values (152 ± 49 s.d. ml min^{-1}) than non-smokers (79 ± 28 s.d. ml min^{-1}) in periods without grapefruit juice coadministration.

Concomitant administration of grapefruit juice *in vivo* (see Table 3) increased caffeine AUC values by 28% (95% CI: 11%–46%) and prolonged caffeine half-life by 31% (95% CI: 20%–44%). The oral caffeine clearance, which reflects systemic clearance of caffeine (see method section), was decreased significantly by 23% (95% CI: 7%–30%). A reduction of oral clearance was observed in 9 of the 12 subjects.

Changes in half-lives correlated significantly (r = 0.743) with the body weight normalised naringin dose (mg day^{-1} kg^{-1}), but other kinetic parameters did not.

Discussion

This study was designed to examine possible effects of grapefruit juice on CYP1A2 activity in man using caffeine as a model substrate for this isoform. Concomitant administration of grapefruit juice resulted in a small but significant decrease in oral caffeine clearance, with naringenin identified *in vitro* as being a possible major cause of this effect.

The interpretation of results obtained in a homogeneous group of subjects, e.g. young, nonsmoking, male volunteers, is not always applicable to subjects not meeting these standards. Therefore inclusion criteria used here were permissive and admitted both sexes and did not exclude smokers. Differences in baseline caffeine kinetics in subgroups were taken into account by using intraindividual comparisons.

The use of saliva samples to estimate plasma concentrations of caffeine is supported by data in the literature (Campbell *et al.*, 1987; Newton *et al.*, 1981; Parsons & Neims, 1978; Zylber-Katz *et al.*, 1984) where similar ratios for saliva and plasma concentrations (mean values 0.74–0.92) have been reported.

The elimination of caffeine is subject to considerable interindividual variability with elimination half-lives in healthy volunteers ranging from 2 to 12 h (Campbell *et al.*, 1987). A similar variability in caffeine kinetics was found in the present study. This diversity is, in part, caused by exogeneous compounds which inhibit or induce CYP1A2 activity.

In many species polycyclic aromatic hydrocarbons are potent inducers of CYP1A2. Tobacco smoke is the most common cause of exposure of humans to these compounds (Parsons & Neims, 1978). This was reflected by the observation that the mean oral caffeine clearance in non-smokers was half that in smokers as a result of CYP1A2 induction. On the other hand, numerous reports describe inhibitory effects of a variety of drugs on caffeine metabolism, including quinolone antibiotic agents, cimetidine, and verapamil (Broughton & Rogers, 1981; Fuhr *et al.*, 1992a,c).

An additional factor which may contribute to the variability of caffeine pharmacokinetics within a subject and between individuals is the presence of CYP1A2 inhibitors in many foods. The competitive inhibition of

caffeine 3-demethylation by naringenin indicates that the effects of grapefruit juice were, at least in part, caused by this flavanone. Similar flavonoids, or their corresponding glycosides occur widely in many plant species. Some of them have already been shown to inhibit cytochromes P450 *in vitro* (Buening *et al.*, 1981). Therefore, other interactions between fruits or vegetables and drugs may be expected. In this context, it is possible that the marked variability in the kinetics of drugs which are substrates of CYP1A2 (caffeine, theophylline) and CYP3A4 (verapamil, dihydropyridines) is attributable to such phenomena. Moreover, effects of flavanoid inhibitors may not be limited to these two isoforms. Our results underline the necessity of specifying diet in bioavailability and other comparative drug metabolism studies.

The extent of CYP1A2 inhibition by grapefruit juice, observed *in vivo*, is not expected to have therapeutically relevant consequences in most cases. However, naringin concentration in commercially available grapefruit juice may reach almost twice that of the juice given in the present study (Rousseff *et al.*, 1987). Therefore, extensive ingestion of grapefruit juice may occasionally cause a marked effect, not only on the pharmacokinetics and pharmacodynamics of caffeine, but also on those of other drugs which are metabolised completely or in part by this cytochrome P450 isoform (e.g. theophylline).

Both CYP1A2 and CYP3A4 are involved in the activation of carcinogens and mutagens (Guengerich, 1990; Guengerich *et al.*, 1990). Naringenin decreases the metabolism of premutagens to their active forms in human liver microsomes (Buening *et al.*, 1981; Guengerich & Kim, 1990). As a result, cancer chemoprevention by grapefruit juice through inhibition of cytochromes P450 has been discussed (Guengerich & Kim, 1990). An additional antimutagenic mechanism may be present since mutagenicity induced by a direct acting carcinogen has been decreased by naringin in the Ames test (Francis *et al.*, 1989). In contrast, mutagenic effects of hydrolysates of citrus fruit juices, including grapefruit juice, have been reported (Mazaki *et al.*, 1982). Whether or not these properties of grapefruit and/or its flavanones may influence cancer incidence *in vivo* remains to be evaluated.

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References

Bailey, D. G., Spence, J. D., Munoz, C. & Arnold, J. M. O. (1991). Interaction of citrus fruit juices with felodipine and nifedipine. *Lancet*, **337**, 268–269.

Benet, Z. L. & Williams, R. L. (1990). Design and optimization of dosage regimens; pharmacokinetic data. In Goodman and Gilman's, *The pharmacological basis of therapeutics*, 8th edition, eds Gilman, A. G., Rall, T. W., Nies, A. S. & Taylor, P., pp. 1650–1735. New York: Pergamon Press.

Böcker, R. & Guengerich, F. P. (1986). Oxidation of 4-aryl- and 4-alkyl-substituted 2,6-dimethyl-3,5-bis (alkoxycarbonyl)-1,4-dihydropyridines by human liver microsomes and immunochemical evidence for the involvement of a form of cytochrome P-450. *J. med. Chem.*, **29**, 1596–1603.

Booth, A. N., Jones, F. T. & De Eds, F. (1958). Metabolic and glucosuria studies on naringin and phlorizidin. *J. biol. Chem.*, **233**, 280–282.

Broughton, L. J. & Rogers, H. J. (1981). Decreased systemic clearance of caffeine due to cimetidine. *Br. J. clin. Pharmac.*, **12**, 155–159.

Buening, M. K., Chang, R. L., Huang, M.-T., Fortner, J. G., Wood, A. W. & Conney, A. H. (1981). Activation and inhibition of benzo(a)pyrene and aflatoxin B1 metabolism in human liver microsomes by naturally occuring flavonoids. *Cancer Res.*, **41**, 67–72.

Butler, M. A., Iwasaki, M., Guengerich, F. P. & Kadlubar, F. K. (1989). Human cytochrome $P450_{PA}$ (P-450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demthylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc. Natl. Acad. Sci. USA*, **86**, 7696–7700.

Campbell, M. E., Spielberg, S. P. & Kalow, W. (1987). A urinary metabolite ratio that reflects systemic caffeine clearance. *Clin. Pharmac. Ther.*, **42**, 157–165.

Francis, A. R., Shetty, T. K. & Bhattacharya, R. K. (1989). Modulating effect of plant flavonoids on the mutagenicity of N-methyl-N′-nitro-N-nitrosoguanidine. *Carcinogen*, **10**, 1953–1955.

Fuhr, U., Anders, E.-M., Mahr, G., Sörgel, F. & Staib, A. H. (1992a). Inhibitory potency of quinolone antibacterial agents on cytochrome P450IA2 activity *in vivo* and *in vitro*. *Am. J. Antimicrob. Agents Chemother.*, **36**, 942–948.

Fuhr, U., Doehmer, J., Battula, N., Wölfel, C., Kudla, C., Keita, Y. & Staib, A. H. (1992b). Biotransformation of caffeine and theophylline in mammalian cell lines genetically engineered for expression of single cytochrome P450 isoforms. *Biochem. Pharmac.*, **43**, 225–235.

Fuhr, U., Wolff, T., Harder, S., Schymanski, P. & Staib, A. H. (1990). Quinolone inhibition of cytochrome P-450-dependent caffeine metabolism in human liver microsomes. *Drug Metabol. Dispos.*, **18**, 1005–1010.

Fuhr, U., Woodcock, B. G. & Siewert, M. (1992c). Verapamil and drug metabolism by the cytochrome P450 isoform CYP1A2. *Eur. J. clin. Pharmac.*, **42**, 463–464.

Greiner, G. & Wallrauch, S. (1984). Naringin als Nachweis für den Zusatz von Grapefruitsaft zu Orangen- und Tangerinensaft. *Flüssiges Obst*, **12**, 626–628.

Griffiths, R. R. & Woodson, P. P. (1988). Caffeine physical dependence: a review of human and laboratory animal studies. *Psychopharmac.*, **94**, 437–451.

Guengerich, F. P. (1990). Characterisation of roles of human cytochrome P-450 enzymes in carcinogen metabolism. *Asia Pacific J. Pharmac.*, **5**, 327–345.

Guengerich, F. P. & Kim, D. H. (1990). In vitro inhibition of dihydropyridine oxidation and aflatoxin B1 activation in human liver microsomes by naringenin and other flavonoids. *Carcinogen*, **11**, 2275–2279.

Guengerich, F. P., Shimada, T., Iwasaki, M. & Martin, M. V. (1990). Activation of mutagens by human cytochrome P-450 enzymes. *Mut. Environ.*, **B**, 87–96.

Mazaki, M., Ishii, T. & Uyeta, M. (1982). Mutagenicity of hydrolysates of citrus fruit juices. *Mutat. Res.*, **101**, 283–291.

Nebert, D. W., Nelson, D. R., Coon, M. J., Estabrook, R. W., Feyereisen, R., Fujii-Kuriyama, Y., Gonzalez,

F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Loper, J. C., Sato, R., Waterman, M. R. & Waxman, D. J. (1991). Corrigendum. The P450 Superfamily: Update on new sequences, gene mapping, and recommended nomenclature. *DNA Cell Biol.*, **10**, 397–398.

Newton, R., Broughton, L. J., Lind, M. J., Morrison, P. J., Rogers, H. J. & Bradbrook, I. D. (1981). Plasma and salivary pharmacokinetics of caffeine in man. *Eur. J. clin. Pharmac.*, **21**, 45–52.

Parsons, W. D., Neims, A. H. (1978). Effect of smoking on caffeine clearance. *Clin. Pharmac. Ther.*, **24**, 40–45.

Reynolds, J. E. F., ed. (1989). *Martindale: The Extra Pharmacopoeia*, 29th edition, pp. 1499–1513. London: The Pharmaceutical Press.

Rousseff, R. L., Martin, S. F. & Youtsey, C. O. (1987). Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in citrus. *J. Agric. Food Chem.*, **35**, 1027–1030.

Steinijans, V. W. & Diletti, E. (1983). Statistical analysis of bioavailability studies: Parametric and nonparametric confidence intervals. *Eur. J. clin. Pharmac.*, **24**, 127–136.

Zylber-Katz, E., Granit, L. & Levy, M. (1984). Relationship between caffeine concentrations in plasma and saliva. *Clin. Pharmac. Ther.*, **36**, 133–137.

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