Pharmacological Reports
2007, 59, suppl.1, 173–176
ISSN 1734-1140

Copyright © 2007
by Institute of Pharmacology
Polish Academy of Sciences

Orange juice has no effect on CYP2D6-dependent drug-metabolism

Przemysław Niewiński[1], Ewa Jaźwińska-Tarnawska[1], Krystyna Orzechowska-Juzwenko[1], Henryk Czarnik-Matusewicz[1], Magdalena Hurkacz[1], Kinga Niewińska[2], Anna Wiela-Hojeńska[1]

[1]Department of Clinical Pharmacology, Wrocław Medical University, Bujwida 44, PL 50-345 Wrocław, Poland

[2]Department of Emergency Medicine, Wrocław Medical University, Bujwida 44a, PL 50-345 Wrocław, Poland

Correspondence: Przemysław Niewiński, e-mail: p.niewinski@gmail.com

Abstract:
Citrus juices can interact with a number of medications. As little as 250 ml of grapefruit or orange juice can change the metabolism of drugs. Most drugs affected by citrus juices are known to be metabolized by CYP3A4. Recently, some studies have shown an *in vitro* inhibition of CYP2D6 metabolism by grapefruit juice. CYP2D6 enzyme is an important factor in the metabolism of 20–25% of clinically important drugs, such as antidepressants, neuroleptics, antiemetics, cardiologic drugs and opioids.
The aim of our study was to investigate the effects of orange juice on human cytochrome CYP2D6 enzyme activity.
The study involved twenty unrelated healthy volunteers. Ten of them received 250 ml (one glass) of orange juice daily for 5 days. Control group consisted of ten persons who received 250 ml (one glass) of tap water. The CYP2D6 phenotype was analyzed before and after 5 days of juice/water ingestion, using sparteine as a model drug. Sparteine and its metabolites were determined in urine by the method of Eichelbaum. Metabolic ratio (MR) was calculated as the ratio of amount of parent drug to the total amount of metabolites.
Mean MR, measured after 5 days of drinking the juice, increased by only 7% in group receiving orange juice, and by 10% in control group receiving tap water.
Our results suggest that orange juice has no significant influence on metabolism and excretion of CYP2D6-dependent drugs. So it is not necessary to advise patients against drinking orange juice at the same time when they take those drugs.

Key words:
orange juice, CYP2D6, drug metabolism

Abbreviations: ADR – adverse drug reaction, AUC – area under curve, C_{max} – maximal concentration

Introduction

It is well known and generally accepted that intake of some foods may affect the pharmacokinetics of drugs. In 1991, the first clinical study of grapefruit juice-drug interaction demonstrated an obvious increase in the AUC and C_{max} of the calcium channel antagonists felodipine and nifedipine [1]. Since then, many studies of grapefruit juice-drug interactions have been conducted [3, 4, 9, 14–17].

Grapefruit juice interacts with a significant number of medications. As little as 250 ml of grapefruit juice can change the metabolism of numerous drugs. Most

drugs affected by grapefruit juice are known to be primarily transported with participation of P-glycoprotein or/and metabolized by a form of cytochrome P450, CYP3A4 [7, 8, 10]. This drug-food interaction occurs because of a common pathway involving enzymes present in both the liver and the intestinal wall. Co-administration of grapefruit juice with P-glycoprotein or CYP3A4-dependent drugs resulted in substantial increases in their bioavailability and higher risk of drug intoxication. Some preliminary studies show an *in vitro* and *in vivo* inhibition of CYP2D6 metabolism by grapefruit juice [19]. CYP2D6 enzyme activity is a crucial factor in the metabolism of 20–25% clinically important drugs, such as tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRI), neuroleptics, antiemetics (ondansetron, tropisetron), cardiologic drugs (propafenon, flecaind) and opioids (codeine, tramadol). Poor, slow metabolism *via* CYP2D6, either genetically determined or caused by enzyme inhibition triggered by drug-food interaction, could cause serious drug toxicity or occurrence of substantial adverse drug reactions (ADRs) [2, 5, 6, 10, 11].

Recently, reports have been published regarding interactions between citrus juices other than grapefruit juice and drugs. In the first clinical study of citrus juice and drug interaction, orange juice, in contrast to grapefruit juice, did not affect at all the pharmacokinetics of felodipine. Subsequently, orange juice was even sometimes used as a negative control in studies of grapefruit juice-drug interactions. However, notable clinical results that might change this long-held stance have been reported. In one study, orange juice reduced the AUC and C_{max} of fexofenadine [4]. Orange juice also substantially reduced the C_{max}, AUC and urinary excretion of celiprolol [9]. Those findings, suggesting induction of metabolism, reveal that not only grapefruit juice, but also orange juice could influence drug metabolism. Therefore, there is a possibility that orange juice intake could cause changes in CYP2D6 metabolism.

The aim of our study was to investigate the effects of orange juice on human cytochrome CYP2D6 enzyme activity. Our goal was to assess the possible importance of orange juice as: a) a potential risk factor of adverse drug reactions (ADRs) occurrence or increasing toxicity of drugs, or b) quite opposite – a factor decreasing efficacy of drugs, which are metabolized by cytochrome CYP2D6.

Materials and Methods

Subjects

Study involved twenty-two individuals, healthy, non-pregnant unrelated volunteers with normal kidney and liver function. All were of native Polish origin, living in the southwestern (Lower Silesia) region of Poland. The characteristics of study participants are summarized in Table 1.

Tab. 1. Characteristics of persons in whom oxidation phenotype was determined

Group	Number of persons			Age (years)	
	Females	Males	Total	Mean	± SD
Orange juice	5	5	10	24.7	4.4
Controls (tap water)	6	4	10	23.2	3.8
All subjects	11	9	20	23.9	4.2

Informed consent was obtained in every case. The protocol for the study was approved by the Bioethics Committee of the Wrocław Medical University.

The CYP2D6 genotype was analyzed before juice/water ingestion.

Genotyping

DNA was obtained from leukocytes isolated from peripheral blood. Genotyping for the defective CYP2D6*3 (single base-pair deletion in exon 5), CYP2D6*4 (G1934 to a point mutation) and CYP2D6*5 (deletion of the whole CYP2D6 gene) alleles was performed by polymerase chain reaction amplification and restriction fragment length polymorphism (PCR-RFLP) modified techniques based on method described by Smith et al. [18]. Alleles carrying neither CYP2D6*3, CYP2D6*4 nor CYP2D6*5 mutations were classified, using this method, as CYP2D6*1 (wild-type) alleles. Homozygotes carrying two wild-type CYP2D6*1 alleles and heterozygotes carrying one wild-type and one mutant (CYP2D6*3, CYP2D6*4 or CYP2D6*5) allele were classified as extensive metabolizers (EM). Homozygotes carrying two mutant alleles were classified as poor metabolizers of sparteine (PM).

Phenotyping

The CYP2D6 phenotype was analyzed before and after 5 days of juice/water ingestion.

The subjects received 100 mg of sparteine sulfate. All the urine excreted during 6 h was collected and stored at –20°C until analyzed. Sparteine (SP) and its metabolites 2- and 5-dehydrosparteine (DHS) were determined in urine by the gas chromatographic method of Eichelbaum [5] with minor modification (such as 6 h instead of 12 h urine collection).

The metabolic ratio (MR) was calculated as:

$$\frac{\%\text{ of dose excreted as sparteine}}{\%\text{ of dose excreted as 2- and 5- dehydrosparteine}}$$

Individuals with a sparteine metabolic ratio lower than 2.5 were classified as extensive metabolizers ($EM_{sparteine}$), and with MR higher than 2.5 as intermediate metabolizers of sparteine ($IM_{sparteine}$).

Sparteine (7,14-methano-2H-dipyrido[1,2-a:1',2'-e][1,5]diazocine) was obtained from Polfa Kutno (Kutno, Poland). Internal standard was a generous gift from Prof. M. Eichelbaum (Dr. Margarete Fischer-Bosch Institute, Stuttgart, Germany).

Data analysis

The statistical analysis of the results was performed by using nonparametric Fisher Exact Test, Wilcoxon Matched-Pairs Signed-Ranks Test and Mann-Whitney U Test. Hardy-Weinberg law was applied to calculate expected genotype frequency. For the analysis of the susceptibility of drug metabolism to inhibition the odds ratio (OR) was calculated.

Results and Discussion

Genotyping results revealed among 22 healthy volunteers the presence of 20 (91%) extensive CYP2D6 metabolizers (EM) and 2 (9%) poor metabolizers (PM). Frequency of CYP2D6 homo- and heterozygotes, carrying CYP2D6*1 CYP2D6*3, CYP2D6*4 and CYP2D6*5 alleles were in general concordance with expected values calculated using Hardy-Weinberg law. Poor metabolizers (PM), with non-functional CYP2D6 enzyme, unable to metabolize significant amount of drugs, were excluded from further study.

Extensive CYP2D6 metabolizers (EM) were randomly divided into 2 groups, 10 subjects in each group. The subjects received 100 mg of sparteine. All the urine excreted during 6 h was collected and the metabolic ratio (MR) was calculated. To prove that both groups are similar according to CYP2D6 metabolic capacity, distribution of MR in both groups was compared, using Mann-Whitney U Test, and frequency of sparteine extensive metabolizers ($EM_{sparteine}$) and intermediate metabolizers ($IM_{sparteine}$) was compared using Fisher Exact Test. Both tests showed no statistically significant difference ($p > 0.1$ and $p > 0.05$, respectively).

Mean metabolic ratio (MR), measured again after 5 days of drinking the juice or tap water, increased by 7% in group receiving orange juice, and by 10% in control group receiving water (Fig. 1), showing only minimal inhibition of CYP2D6 metabolism. Changes in frequency distribution of MR, content of unchanged sparteine and sparteine metabolites excreted with urine, compared before and after 5 days of juice or/water ingestion (evaluated by the use Wilcoxon

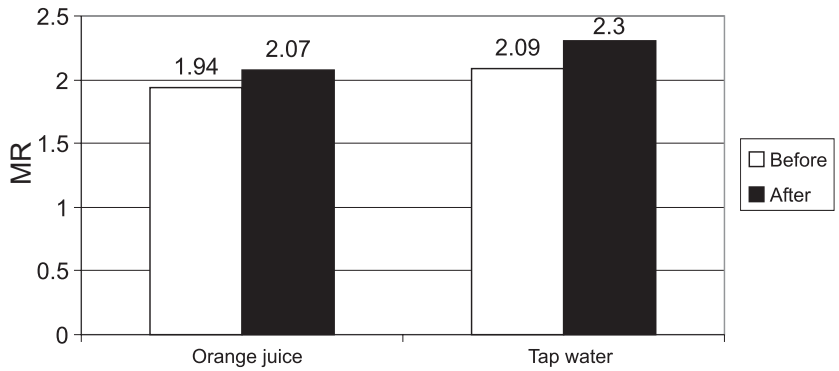

Fig. 1. Mean metabolic ratio (MR), measured before and after 5 days of drinking juice or water

Matched-Pairs Signed-Ranks Test) all were not statistically significant in both groups ($p > 0.05$). Proportion of extensive metabolizers ($EM_{sparteine}$), and intermediate metabolizers of sparteine ($IM_{sparteine}$) in both groups, (evaluated by the use of Fisher Exact Test) did not changed significantly ($p > 0.05$). Also relative risk of drug metabolism inhibition, calculated using the odds ratio (OR = 0.84) was statistically insignificant.

Frequency of CYP2D6 poor metabolizers (9%) among our subjects is close to frequency in the Polish population (8%) [13].

Metabolic ratio increase by 7% is an equivalent of similar decrease in detoxification potential of hepatic enzymes, involved in metabolism of CYP2D6-dependent drugs. Those numbers are significantly lower than 34% increase after 5 days of drinking grapefruit juice, revealed in our previous study [12]. Our results show neither relevant inhibition nor induction of CYP2D6 metabolism (contrary to inhibition by grapefruit juice previously analyzed by us). Also the decrease in unchanged drug excretion induced by orange juice was not significant. Ingestion of orange juice did not result in an increased risk of CYP2D6-dependent drug inefficacy or toxicity, contrary to the documented significant influence on CYP3A4 metabolism [4, 6].

The results suggest that orange juice has no significant influence on metabolism and excretion of CYP2D6-dependent drugs, such as tricyclic antidepressants, SSRI, neuroleptics, antiemetics, cardiologic drugs and opioids. Therefore, it is not necessary to advise patients against drinking orange juice, contrary to ingesting grapefruit juice, at the same time when they take those drugs.

References:

1. Bailey DG, Spence JD, Munoz C, Arnold JM: Interaction of citrus juices with felodipine and nifedipine. Lancet, 1991, 337, 268–269.
2. Baliharova V, Velik J, Fimanova K, Lamka J, Szotakova B, Savlik M, Skalova L: Inhibitory effect of albendazole and its metabolites on cytochromes P450 activities in rat and mouflon *in vitro*. Pharmacol Rep, 2005, 57, 97–106.
3. Bressler R: Grapefruit juice and drug interactions. Exploring mechanisms of this interaction and potential toxicity for certain drugs. Geriatrics, 2006, 61, 12–18.
4. Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson PA, Freeman DJ, Kim RB: Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. Clin Pharmacol Ther, 2002, 71, 11–20.
5. Eichelbaum M, Gross AS: The genetic polymorphism of debrisoquine/sparteine metabolism: clinical aspects. Pharmacol Ther, 1990, 46, 377–394.
6. Haduch A, Bromek E, Kot M, Jemnitz K, Veres Z, Vereczkey L, Daniel WA: Effect of mirtazepine on the CYP2D activity in the primary culture of rat hepatocytes. Pharmacol Rep, 2006, 58, 979–984.
7. Haduch A, Wojcikowski J, Daniel WA: Direct effects of neuroleptics on the activity of CYP2A in the liver of rats. Pharmacol Rep, 2005, 57, 867–871.
8. Ho PC, Saville DJ, Wanwimolruk S: Inhibition of human CYP3A4 activity by grapefruit flavonoids, furanocoumarins and related compounds. J Pharm Pharm Sci, 2001, 4, 217–227.
9. Lilja JJ, Juntti-Patinen L, Neuvonen PJ: Orange juice substantially reduces the bioavailability of the beta-adrenergic-blocking agent celiprolol. Clin Pharmacol Ther, 2004, 75, 184–190.
10. Meisel Ch, Gerloff T, Kirchheiner J, Mrozikiewicz PM, Niewiński P, Brockmöller J, Roots I: Implications of pharmacogenetics for individualizing drug treatment and for study design. J Mol Med, 2003, 81, 154–167.
11. Nebert DW: Polymorphisms in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? Am J Hum Genet, 1997, 60, 265.
12. Niewiński P, Jaźwińska-Tarnawska E, Czarnik-Matusewicz H, Wiela-Hojeńska A, Niewińska K: Grapefruit juice as a potential risk factor of intoxication by CYP2D6-metabolized drugs. Clin Toxicol, 2006, 44, 497.
13. Niewinski P, Orzechowska-Juzwenko K, Hurkacz M, Rzemislawska Z, Jaźwinska-Tarnawska E, Milejski P, Forkasiewicz Z: CYP2D6 extensive, intermediate and poor phenotypes and genotypes in a Polish population. Eur J Clin Pharmacol, 2002, 58, 533–536.
14. Row E, Brown SA, Stachulski AV, Lennard MS: Development of novel furanocoumarin dimers as potent and selective inhibitors of CYP3A4. Drug Metab Dispos, 2006, 34, 324–330.
15. Saito M, Hirata-Koizumi M, Matsumoto M, Urano T, Hasegawa R: Undesirable effects of citrus juice on the pharmacokinetics of drugs. Drug Safety, 2006, 28, 677–694.
16. Schmidt LE, Dalhoff K: Food-drug interactions. Drugs, 2002, 62, 1481–1502.
17. Singh BN: Effects of food on clinical pharmacokinetics. Clin Pharmacokinet, 1999, 37, 213–255.
18. Smith C, Gough A, Leigh P, Summers B, Harding A: Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. Lancet, 1992, 339, 1375–1377.
19. Tassaneeyakul W, Guo LQ, Fukuda K, Ohta T, Yamazoe Y: Inhibition selectivity of grapefruit juice components on human cytochromes P450. Arch Biochem Biophys, 2000, 378, 356–363.

Received:
March 28, 2007; in revised form: July 10, 2007.