REVIEW ARTICLE

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Clinical Implications and Applications of Grapefruit Juice Intake during Oral Therapy of Drugs Known to be Metabolized by Cytochrome P450 3A4 Isozyme*

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The significance of the use of grapefruit juice as it relates to drug-drug interaction potential, via cytochrome P450 (CYP) 3A4 isozyme, has been a topic of considerable interest for over a decade now. Drugs belonging to diversified chemical classes have exhibited a significant pharmacokinetic and/or pharmacodynamic interaction when concomitantly administered with grapefruit juice. Owing to the inhibition of the intestinal CYP 3A4 enzyme, the exposure levels of the ingested drug substrate have been increased by several folds following a single intake of grapefruit juice. While many investigations have been performed to identify active ingredient(s) in grapefruit juice responsible for this profound interaction resulting in an increased bioavailability, some recent reports suggest that grapefruit juice may impede the bioavailability of a very few drugs due to alteration of the micro-environment of absorption. Therefore, from a clinical viewpoint, caution needs to be exercised when co-administering CYP 3A4 substrates with grapefruit juice. Additionally, molecules that are substrates to P-glycoprotein efflux transporter pumps may also get affected by the inhibitory interaction of grapefruit juice. In some situations, the inhibitory interaction exerted by grapefruit juice may be capitalized to allow dose reduction of CYP3A4 substrate(s). This review covers many aspects of drug-drug interaction potential upon the ingestion of grapefruit juice.

Over a decade ago, Bailey *et al.* (1991)[1] reported a significant pharmacokinetic and pharmacodynamic interaction of felodipine (a calcium antagonist) when the drug was given with concomitant intake of grapefruit juice; this investigation was carried out in a cohort of subjects who had a borderline hypertension[1]. As a result of the pharmacokinetic interaction, parameters such as peak plasma concentration (C_{max}) and area under the plasma concentration-curve (AUC) increased approximately by 2.2 fold and 2.5 fold of the control treatment (water alone)[1]. In a similar fashion, the pharmacodynamic interaction resulted in a significant 2-fold decrease in diastolic blood pressure and a 2-fold increase in heart rate measures compared to the control treatment. Interestingly, the concomitant administration of felodipine with orange juice to the same subjects did not alter the pharmacokinetics or pharmacodynamics of felodipine. Contemporary work during the same time frame showed that other dihydropyridine substrates such as nifedipine[2], nitrendipine[3] and nisoldipine[4] when administered with grapefruit juice exhibited increased rate and extent of absorption when compared to the respective control treatments. In conjunction with the increase in exposure, the hemodynamic responses of the various dihydropyridine substrates appeared to be enhanced to varying degrees by grapefruit juice. Since appropriate patient population was not enrolled in the above pharmacokinetic investigations, it was rather difficult to assess the clinical consequences of the interaction. However, since ischaemic complications leading to myocardial infarction have been reported in se-

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vere hypertensive patients treated with dihydropyridine congeners[5] such an interaction between dihydropyridines and grapefruit juice is clinically important to understand.

In several studies performed over the next several years, in addition to dihydropyridines, other class of compounds has been shown to have a significant interaction when administered with grapefruit juice. Terfenadine, an antihistamine, when coadministered with grape fruit juice displayed augmented plasma levels both the parent compound and its active metabolite, and produced a significant prolongation of the QT_c interval when compared to the control treatment[6,7]. In the presence of grapefruit juice, the rate and extent of absorption of midazolam (psychotropic agent) was significantly increased from the control treatment; pharmacodynamically, the combination treatment significantly altered the psychometric performance[8]. Cyclosporine (immunosuppresant) also showed elevated plasma levels resulting in an increased bioavailability following intake of grapefruit juice[9]. In a cohort of transplant patients, Proppe *et al.* (1995)[10] observed a stable creatine clearance in all but one patient, in spite of significant alteration in the pharmacokinetics of cyclosporine following intake of grapefruit juice. In a group of ovariectimized women patients, it has been shown that the intake of grapefruit juice significantly inhibited the metabolism 17β-estradiol (hormone supplement) and as a result significantly increased the bioavailable amounts 17β-estradiol and its metabolite, estrone[11]. However, it appeared that this pharmacokinetic interaction between grapefruit juice and 17 β -estradiol may not be clinically important[11].

More recently, a single intake of grapefruit juice has been shown to increase the exposure of diltiazem, a calcium channel antagonist[12]. Another recent example cites the increase in the AUC value of halofantrine, an antimalarial agent, by approximately 3.2 fold when ingested with grapefruit juice[13]. Also, the authors reported a better correlation of the QTc interval prolongation with increased halofantrine concentrations, suggesting a significant pharmacodynamic interaction with grapefruit juice[13]. In another study, a 2-fold increase in the exposure of midazolam was observed following grapefruit juice administration in patients with liver cirrhosis[14]. In this article, authors concluded that liver cirrhotic patients are more dependent on the intestinal metabolism of midazolam than the normal subjects who have the liver function intact[14]. Nagy *et al.* (2002)[15] have reported an approximately 3.2 fold increase in the exposure of albendazole, when grapefruit juice was ingested with the drug. In another study, the bioavailability of dextromethorphan was significantly increased by the presence of grapefruit juice, such that it almost took 3 days for the bioavailability to reach half its original value following a washout[16]. The bioavailability of sildenafil was enhanced, while the absorption was delayed when grapefruit juice was administered with seldanifil[17]. Both C_{max} and AUC values of cisapride were both tremendously increased by concomitant grapefruit juice ingestion, however, neither T_{max} nor elimination half-life values were altered[18]. Castro *et al.* (2002)[19] reported a significant increase in the human exposure of praziquantel following pretreatment with grapefruit juice. In the case of simvastatin, an acute effect of interaction with grapefruit juice was observed in the exposure values; however, the delay in the ingestion of grapefruit juice in relation to the time of the administered dose appeared to blunt the increase in exposure of simvastatin[20]. A report of a dramatic alteration of the metabolism of amiodarone such that the formation of a key metabolite (N-desethylamiodarone) was blocked has been published as a consequence of ingestion of grapefruit juice with amiodarone[21]. In the case of saquinavir, a HIV protease inhibitor, concomitant intake of grapefruit juice increased the bioavailability of saquinavir by approximately 1.7 fold[22].

There are only a few reports of grapefruit juice drug interactions where the investigators have evaluated the pharmacokinetics of the individual enantiomers of a racemic CYP3A4 substrate when coadministered with grapefruit juice[23,24]. Although grapefruit juice was found to increase the concentrations of individual enatiomers of cisapride, the increase was found to be similar between the two enantiomers[23]. Similarly, the bioavailability of verapamil enantiomers increased following the concomitant intake of grapefruit juice, however, the increment in bioavailability appeared to be similar between the two enantiomers of verapamil[24]. Overall, it appears that based on limited published data, ingestion of grapefruit juice has not been implicated in stereoselective differences in the increased bioavailability of racemic substrates.

The mechanism underlying the interaction between grapefruit juice and therapeutically important drugs has received considerable attention. Because felodipine, nifedipine, nisoldipine, nitrendipine, terfenadine, midazolam, cyclosporine, triazolam, 17β-estradiol and several others are all ideal substrates to undergo metabolism by cytochorome P4503A isozymes, it has been postulated that inhibition of this enzyme system resulted in an increased bioavailability of the various drugs. In addition, some specific crossover pharmacokinetic investigations of oral and

intravenous treatments of either cyclosporine[8], midazolam[9], or nifedipine[25] with concomitant intake of grapefruit juice, have implicated that effects of grapefruit juice are mainly due to inhibition of CYP3A isozymes present in the gastrointestinal tract rather than those found in the liver. Because the intake of grapefruit juice also augmented the bioavailability of metabolites of felodipine[1], terfenadine[7], cyclosporine[8], midazolam[9], or 17β-estradiol[11], it is postulated that grapefruit juice perhaps may also be involved in the inhibition of other cytochrome P450 isozymes. To underscore this point, it has been demonstrated that the intake of grapefruit juice resulted in a significant increase in AUC and prolongation of half-live value of caffeine, a known substrate for cytochrome P4501A2 isozyme[26]. Recently, Spahn-Langguth and Langguth (2001)[27] have shown that grapefruit juice can inhibit the p-glycoprotein-related efflux transport of talinolol both *in vitro* and *in vivo* situations. Previously, it was confirmed that in vitro efflux of vinblastine, mediated by p-glycoprotein transporter, was inhibited by grapefruit juice[28]. On the contrary, Parker *et al.* (2003)[29] have used digoxin as a probe substrate and have concluded that inhibition of intestinal p-glycoprotein may not play a role in explaining drug interactions with grapefruit juice.

Interestingly, researchers have modeled the pharmacokinetics of felodipine-grapefruit juice interaction using an irreversible enzyme inhibition model[30]. On the basis of this exercise, they have concluded that the observed pharmacokinetic interaction would amplify as a function of the amount and frequency of grapefruit juice ingestion[30]. More perspectives on the clinical interaction of grapefruit juice with coadministered substrates can be obtained from a recently published review article[31].

While reports of grapefruit juice causing an increased C_{max} and exposure are on the rise, there are a few reports which suggest that pretreatment with grapefruit juice has the potential to decrease plasma concentrations of a few oral agents. Lilja *et al.* (2003)[32] have reported a decrease in the concentrations of celiprolol following grapefruit juice pretreatment. The authors have attributed a physical interaction of grapefruit juice such that it interferes with the absorption phase of celiprolol. The data is well supported by the fact that itraconazole's treatment increases the plasma concentrations of celiprolol due to itraconazole's known inhibitory role on CYP3A4 and p-glycoprotein. In another example, oral etoposide bioavailability was unexpectedly reduced by about 25% when the patients were pretreated with grapefruit juice[33]. It was reported that indinavir's absorption was delayed with slight but non-significant reduction in bioavailability due to increased gastric pH following pretreatment with grapefruit juice[34]. Such recent reports suggest that grapefruit juice may have the potential to change the micro environment (local pH; cause physical interference and incompatibility) of absorption for a few agents.

The search for the active ingredient(s) in the grapefruit juice responsible for CYP3A enzyme inhibition has also been a topic of considerable interest. Several flavonoids present in grapefruit juice such as naringin, quercetin, and kaempferol have been demonstrated to have appreciable potency for the inhibition of *in vitro* metabolism of one or more CYP3A substrates such as felodipine, nifedipine, midazolam and quinidine[2, 35-37]. However, neither naringin nor quercetin, when tested *in vivo* failed to show any inhibition of felodipine (or nifedipine, respectively. In a recent publication, Bailey and co-workers (2000)[38] have examined the effects of unprocessed whole grapefruit, grapefruit segments, grapefruit juice and the likely active ingredients from each system employed to further understand the felodipine-grapefruit juice interaction. On the basis of this work, bergamottin was found to be most important in predicting drug interaction following grapefruit juice ingestion; while, both 6',7'-dihydroxybergamottin and naringin, which are present in high proportions in the segments, could be important if the segmental regions of grapefruit is consumed[38]. All the active ingredients of grapefruit juice produced marked *in vitro* inhibition of CYP3A4 activity3[8]. Sahi *et al.* (2002)[39] have specifically studied the effects of bergamottin administration on the *in vivo* cytochrome P450 levels using beagle dogs. In this study, beagle dogs were treated with bergamottin (1 mg/kg) orally for about 10 d prior to harvesting of liver and intestine (jejunal region). Microsomal preparations made from liver and jejunum were analyzed for the activities of CYP3A12, CYP2B11, CYP1A1/2, and tolbutamide hydroxylase. In hepatic microsomes, bergamottin pretreatment resulted in reduced CYP3A12 (approximately 50%) and CYP1A1/2 (approximately 75%) activities; while CYP2B11 activity was moderately induced and tolbutamide hydroxylase activity was unchanged[39]. In jejunum microsomal preparations, CYP3A12 activity was almost doubled while other CYP /tolbutamide hydroxylase activities were not detected. Studies done by Bailey and co-workers have further confirmed that ibergamottin has no role to play in the inhibition of CYP3A4[40]. More recently, Wangensteen and co-workers (2003)[41] have identified epoxybergamottin obtained from the peel of grapefruit to be a very potent inhibitor of CYP3A4. The authors suggested that epoxybergamottin may get hydrolyzed to 6',7'-

dihydroxybergamottin during the manufacturing of grapefruit juice – the hydrolyzed product has been shown to be an important CYP3A4 inhibitor[41].

Although the active ingredient(s) *per se* in grapefruit juice is/are still being elucidated and mechanism(s) being understood, the pharmacokinetic and pharmacodynamic interaction of the grapefruit juice with several cytochrome P450 substrates given orally are staggering. There are many more CYP3A substrates belonging to important therapeutic classes that have the potential to interact with grapefruit juice is often consumed with breakfast, during which time drugs are also very frequently administered, the observed interaction has potential clinical consequences in the targeted patent population. It could be argued that when patients are hospitalized for therapy (in-patient), it is very unlikely that grapefruit juice will be consumed because of strict control on dietary intake. However, in an out-patient situation or in the population where medications are prescribed intermittently or in semi-chronic drug interventions, restriction on dietary intake cannot be easily imposed. This is also true in a self -medication situation when over the counter (OTC) drugs are consumed. Therefore, it is important for biomedical community including clinicians, physicians, pharmacists, and nurses to be aware that during oral therapy, if the drug of interest is a substrate of cytochrome P450 isozymes (i.e. CYP3A) ingestion of grapefruit juice, seemingly innocuous, has the potential to produce clinical consequences in the targeted population.

On the other hand, it should also be noted that the interaction of grapefruit juice with CYP3A substrates has the potential to provide opportunities for optimization of a cost-effective drug therapy. For example, owing to the increased cost and chronic nature of cyclosporine therapy, many researchers have co-administered cyclosporine with other CYP3A inhibitors, in an attempt to reduce the administered dose of cyclosporine. This co-therapy is especially relevant in patients where therapeutic levels of cyclosporine are not achievable due to extensive pre-systemic metabolism of the drug. Although drugs such as erythromycin, ketoconazone, verapamil, or diltiazem can produce significant increase of cyclosporine levels when administered concomitantly with cyclosporine, such co-therapies are also associated with side effects and adverse events owing to the pharmacological activity of the individual agent(s). However, grapefruit juice offers an advantage in increasing levels of cyclosporine without the risk of any adverse systemic effect and also it is cheaper compared to the therapy with other CYP3A inhibitors. Additionally, the intake of grapefruit juice may also potentially benefit the oral therapy of other important CYP3A metabolized drugs by reduction in the dose of the drug which may translate in lower occurrence of the adverse events. It is of paramount importance; however, in order to confirm and claim the benefits of grapefruit juice-drug interaction, there is a need to carryout rational clinical studies looking into pharmacokinetic, pharmacodynamic, and safety aspects in the targeted population.

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