COMMENTARIES

Grapefruit Juice, a Glass Full of Drug Interactions?

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Numerous reports have documented drug interactions with grapefruit juice (GFJ) that occur via inhibition of CYP3A enzymes. As reported by Glaeser *et al.*[1] in the March 2007 issue of this journal, there is increasing recognition that GFJ may also affect the activity of influx (*e.g.*, OATPs) and efflux (*e.g.*, P-glycoprotein) transporters. This commentary focuses on these interactions between GFJ and drug transporters.

The grapefruit juice (GFJ)-drug interaction was accidentally discovered when GFJ was used to mask the taste of ethanol in a felodipine–ethanol interaction study.[2] As a result, many *in vitro* and *in vivo* studies, summarized in recent reviews,[3,4] have identified furanocoumarins (*e.g.*, bergamottin and 6,7-dihydroxybergamottin) as mechanism-based inhibitors of CYP3A enzymes. These studies have also found that even a single glass of GFJ causes a significant decrease in intestinal metabolism of CYP3A substrates. On repeated consumption of GFJ, hepatic CYP3A activity is reduced as well.[5] Consequently, numerous GFJ-drug interactions have been reported with orally administered drugs that are significantly metabolized by CYP3A enzymes (see Saito *et al.*[4] for an excellent review). Because CYP3A is involved in the disposition of a large number of approved drugs, the potential for clinically significant GFJ-drug interactions is high, especially with drugs that have significant first-pass metabolism. For this reason, product labeling of some CYP3A substrates with narrow therapeutic indices have included warnings regarding GFJ consumption. In a draft guidance from the FDA, "Guidance for Industry: Drug Interaction Studies—Study Design, Data Analysis, and Implications for Dosing and Labeling" (http://www.fda.gov/cder/guidance/6695dft.htm), GFJ is listed as a moderate CYP3A inhibitor that may result in a two- to five-fold increase in the area under the plasma concentration-time curve (AUC) of CYP3A substrates. A category of drugs that may be susceptible to GFJ interactions was described as orally administered drugs that are substrates of CYP3A and have low bioavailability because of extensive presystemic extraction contributed by enteric CYP3A. The guidance also acknowledges that, because of wide variability in composition of GFJ, the magnitude of interactions can be quite variable.

The effect of GFJ on CYP3A activity is well documented, but its effect on transporters is not. There is increased recognition that both efflux—*e.g.*, P-glycoprotein (P-gp)—and influx—*e.g.*, OATPs—transporters play an important role in the disposition of drugs. For example, P-gp, an efflux transporter, is considered one of the most important transporters involved in drug disposition. This is because, like CYP3A enzymes, P-gp has broad substrate selectivity (which significantly overlaps with that of CYP3A enzymes) and is expressed in tissues important to drug disposition, such as the intestine, liver, and kidneys. OATPs are expressed throughout the body. Those that are most relevant to the absorption and hepatic extraction process are located in the apical membrane of the intestine and the sinusoidal membrane of the liver, respectively (see **Figure 1**).[6] Thus, they act in an opposing fashion to modulate systemic drug exposure (the former increasing and the latter decreasing drug exposure), which complicates the interpretation as well as prediction of OATP drug interactions. The OATP family has a broad spectrum of substrates ranging from endogenous compounds (bile acids, thyroid hormones, and conjugated steroids) to xenobiotics including fexofenadine (FEX), digoxin, pravastatin, methotrexate,[6] repaglinide,[7] and levothyroxine.[8]

There is increasing evidence that GFJ can affect the activity of both efflux and influx transporters. GFJ and some of its components have been shown to inhibit transport of P-gp and OATP (1A2 and 2B1) substrates *in vitro* and in the rat.[3,9] Clinical studies with a variety of P-gp substrates have been carried out to assess the potential of GFJ to cause P-gp mediated drug interactions. Surprisingly, in humans GFJ decreased the AUC of the poorly metabolized P-gp probe drugs talinolol (by 44%) and celiprolol (by 87%), whereas no significant change was seen with digoxin, although the apparent absorption rate was decreased.[3] A decrease in AUC or absorption rate is inconsistent with inhibition of P-gp, but would be consistent with inhibition of an influx (uptake) transport

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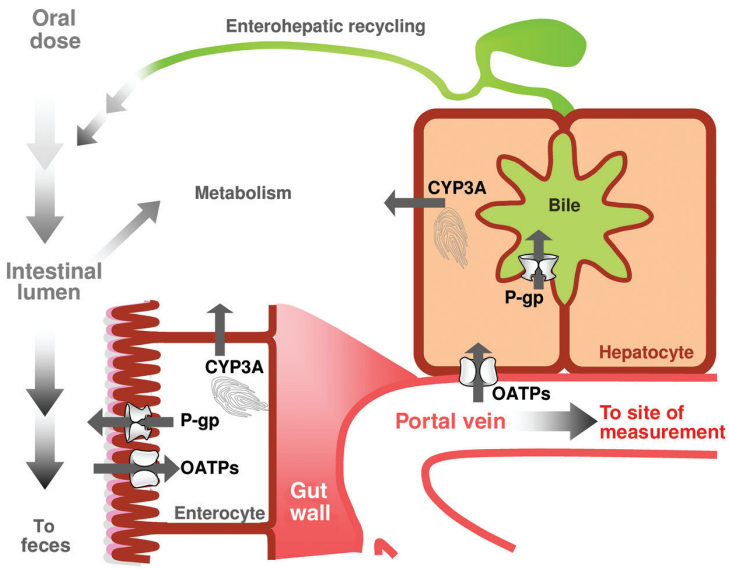

Figure 1 OATPs and P-gp in the intestinal enterocytes act in an opposing fashion to respectively increase and decrease absorption of drugs. In the hepatocytes, these transporters work in tandem to eliminate drugs from the body.

process (*e.g.*, OATPs). *In vitro* data indicate that digoxin, talinolol, and celiprolol are substrates of influx transporters. Similar to the effect on CYP3A, multiple doses of GFJ may affect systemic activity of transporters as shown by lengthening of celiprolol plasma half-life from 4.8 to 8.9 hours.[10] Even though *in vitro* experiments show that GFJ inhibits P-gp activity, conclusive evidence of the effect of GFJ on P-gp activity *in vivo* is complicated by the contribution of other transporters (both efflux and influx) involved in the disposition of these probe drugs. In fact, the above data suggest that GFJ is not a significant inhibitor of *in vivo* P-gp activity, but seems to be an inhibitor of an influx transporter(s). Indeed, as outlined below, there is evidence to suggest that GFJ can significantly inhibit one of these influx transporters *in vivo*, namely OATP1A2.

The AUC of FEX, another commonly used P-gp and OATP substrate, was reduced (to 37%) when administered with GFJ,[11] an observation explained by GFJ inhibition of absorption of FEX mediated by OATPs that are expressed in the intestine. Since this initial observation, FEX interactions with fruit juice have been addressed in several recent studies focusing on the volume, strength, and type of juice administered.[3,4] These studies have shown that multiple fruit juices, including apple and orange juice, alter FEX AUC to different degrees. Recent *in vitro* and animal studies have begun to determine components and extracts of fruit juices responsible for inhibition of P-gp and OATPs.[9,12] However, the OATPs expressed in the human intestine or the particular member(s) of this family that transports FEX have not been comprehensively determined. In the March 2007 issue of this journal, Glaeser *et al.*[1] addressed these issues as well as the influence of time between administration of GFJ and FEX on the GFJ-FEX interaction.

Glaeser *et al.*[1] found that transcripts of many transporters as well as CYP enzymes are expressed in the intestine, including many of the OATPs, OCTNs, MRPs, BCRP, and P-gp. Most notable is the expression of OATP1A2, which was formerly believed to be expressed mostly in the brain; OATP1B1 and 1B3, formerly believed to be liver specific[6]; and the lack of OCT1 expression, which has been detected in the jejunum.[13] Using immunohistochemical staining, OATP1A2 was shown to co-localize with P-gp at the apical membrane of the intestinal villi. Administration of GFJ (~90 minutes before intestinal biopsies) did not have a significant effect on the intestinal expression of OATP1A2 or P-gp, but did, as expected, show a trend to lower CYP3A expression. *In vitro* studies with HeLa cells transfected with a variety of human (OATP1A2, 1B1, 1B3, and 2B1) and rat (Oatp1a1, 1a4, 1a5, and 1b2) OATPs indicate that within the OATP family, transport of FEX is mediated only by human OATP1A2, whereas multiple rat isoforms are capable of transporting FEX. Moreover, GFJ can inhibit the transport of FEX by cells expressing recombinant OATP1A2 (ref. 11). In addition, Glaeser *et al.*[1] showed that coadministration of GFJ and FEX resulted in a more pronounced decrease in FEX AUC (52%) when compared with GFJ given 2 hours before FEX (38%). When GFJ was given 4 hours before FEX, the effect on FEX AUC was abolished, indicating that within 4 hours of GFJ ingestion, inhibition of OATP1A2 by competitive or other mechanism(s) had subsided. Interestingly, the initial absorption rate (first three time points) of FEX seemed to be unaltered when GFJ was given 2 hours before FEX, yet the AUC was reduced significantly. These data are intriguing, because they are suggestive of multiple mechanisms operating in the absorption of FEX. A more thorough investigation of this phenomenon will further our understanding of the mechanisms of absorption of FEX and the mechanisms of FEX-GFJ interaction. However, because FEX is not transported by OATP1B1, 1B3, or 2B1, it has yet to be determined whether GFJ can inhibit these OATP isoforms *in vivo*.

As Glaeser *et al.*[1] have stressed, it is important to broaden the knowledge base of intestinal transporters, by identifying the transporters functionally expressed in the small intestine, their spatial localization (apical vs. basolateral), their gradient along the length of the intestine, their substrate specificity, and their role in *in vivo* transport of drugs. Historically, considerable attention has been directed toward the role of and interplay between intestinal metabolism and drug efflux in drug absorption and drug interactions. Only recently have influx transporters been included in this focus. Glaeser *et al.*[1] have shown that GFJ has the poten-

tial to simultaneously modify OATP1A2 and CYP3A activity. This, together with the possibility that GFJ may inhibit P-gp mediated efflux of drugs, makes it difficult to predict whether a GFJ-drug interaction will occur and the magnitude of such an interaction. An understanding of the specific components causing the transporter-mediated interactions and the mechanisms by which they produce their effect could help predict the magnitude of GFJ-drug interactions. Because these data are not currently available, the best approach to rapidly address GFJ-transporter interactions is to assess the potential of widely used GFJ products to inhibit, *in vivo*, the intestinal and hepatic transporters important in drug absorption and hepatic extraction. To accomplish this goal, it is important to identify selective substrates of various transporters that can be used in *in vivo* studies. Once these have been identified, such studies can be used to determine categories of drugs that are likely to be susceptible to GFJ interactions as proposed by the FDA for CYP3A substrates. This categorization will increase our ability to predict GFJ-drug interactions. Until such categorization has been achieved, GFJ consumption should be avoided for as long as 4 hours before administration of narrow-therapeutic-index drugs whose absorption is mediated by intestinal OATP1A2. Further research will be needed to determine whether GFJ significantly inhibits other transporters functionally expressed in the small intestine.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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