Major Active Components in Grapefruit, Orange, and Apple Juices Responsible for OATP2B1-Mediated Drug Interactions

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ABSTRACT: We aimed to explore the major active components in grapefruit juice (GFJ), orange juice (OJ), and apple juice (AJ) that are responsible for OATP2B1-mediated drug interactions, by means of *in vitro* studies using *Xenopus* oocytes expressing OATP2B1 with a typical OATP2B1 substrate, estrone-3-sulfate. All three juices inhibited OATP2B1-mediated estrone-3-sulfate uptake with half-maximum inhibition (IC_{50}) values of 0.222% (GFJ), 0.807% (OJ), and 2.27% (AJ). Eight major flavonoids (naringin, naringenin, hesperidin, hesperetin, phloridzin, phloretin, quercetin, and kaempferol) contained in the juices inhibited OATP2B1-mediated estrone-3-sulfate uptake with IC_{50} values of 4.63, 49.2, 1.92, 67.6, 23.2, 1.31, 9.47, and 21.3 μM, respectively. When the concentration–IC_{50} ratios ($[C]/IC_{50}$) of these flavonoids in GFJ, OJ, and AJ were calculated, values of $[C]/IC_{50} \geq 100$ were obtained for naringin in GFJ and hesperidin in OJ. No flavonoid in AJ showed a ratio higher than unity. However, significant inhibition of OATP2B1 was observed with a mixture of phloridzin, phloretin, hesperidin, and quercetin at the concentrations present in AJ. In conclusion, our results indicate that naringin and hesperidin are the major OATP2B1 inhibitors in GFJ and OJ, respectively, whereas a combination of multiple components appears to be responsible for OATP2B1 inhibition by AJ. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:280–288, 2013

Keywords: Organic anion-transporting polypeptide transporters; OATP2B1; grapefruit juice; orange juice; apple juice; flavonoid; interaction; food interactions; intestinal absorption; intestinal secretion/transport

INTRODUCTION

Concomitant drug and fruit juice (FJ) intake may causes interactions that alter the oral bioavailability, and consequently the efficacy or toxicity, of a drug. Since the first reported instance of grapefruit juice (GFJ) interaction with felodipine, the scientific literature is now replete with information regarding the effects of GFJ and its components on drug absorption, both *in vitro* and *in vivo*.[1] GFJ produces irreversible mechanism-based inhibition (MBI) of cytochrome p450 3A4 (CYP3A4)-catalyzed drug metabolism during the intestinal absorption process, resulting in clinically significant increases in oral drug bioavailability.[2,3] On the basis of these and subsequent studies, bergamottin and 6′,7′-dihydroxybergamottin, two of the major furanocoumarins in GFJ, have been identified as mechanism-based inhibitors of CYP3A4.[4–6]

On the contrary, more recently, it was reported that GFJ can reduce the oral absorption of drugs.[7] The putative mechanism is a reduction in absorptive transport of drugs through the inhibition of organic anion transporting polypeptide (OATP) by GFJ.[7–10] The clinical concern in this case is potential loss of efficacy of medications, which may be particularly serious for drugs having a narrow therapeutic index or possessing a steep concentration–response relationship. As a result of extensive work on OATP-mediated GFJ—drug interaction, several OATP substrates (e.g., fexofenadine, celiprolol, talinolol, and aliskiren), whose bioavailabilities are reduced by GFJ ingestion, have been identified.[7,11–13] Interestingly, a reduction in the bioavailability of OATP substrates (e.g., fexofenadine, atenolol, celiprolol, and aliskiren) has also been

Abbreviations used: AJ, apple juice; FJ, fruit juice; GFJ, grapefruit juice; GI, gastrointestinal; MBI, mechanism-based inhibition; OATP, organic anion transporting polypeptide; OJ, orange juice.

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observed after OJ and/or AJ ingestion.[7,14–18] We demonstrated that the bioavailability of fexofenadine is significantly decreased in individuals with the OATP2B1 c.1457C>T allele, and is also decreased in the presence of AJ.[14] These findings imply that AJ interacts with fexofenadine via inhibition of the OATP2B1-mediated absorption process. Although contributions of OATP1A2 to FJ–drug interactions cannot be ruled out, OATP2B1 is the most likely candidate for the key determinant of such interactions because intestinal expression of OATP2B1 is much higher than that of OATP1A2.[9,19–23] Several *in vitro* investigations on the effect of GFJ and its components on OATP2B1-mediated drug transport have been performed to explore the major active components in GFJ.[8–10,14] At present, naringin, the main constituent flavonoid of GFJ, is thought to be a major inhibitor of OATP2B1-mediated drug absorption in GFJ and to be primarily responsible for altered absorption kinetic parameters of drugs.[8–10,24–26] However, the nature and importance of other FJ components responsible for OATP2B1-mediated drug interactions remain obscure.

In the present study, we aimed to explore the major causal components of OATP2B1-mediated drug interactions involving GFJ, OJ, and AJ, by means of *in vitro* studies using OATP2B1-expressing *Xenopus* oocytes together with an OATP2B1 substrate, estrone-3-sulfate. Our results indicated that naringin and hesperidin are the predominant inhibitors of OATP2B1 in GFJ and OJ, respectively. On the contrary, the significant interaction of AJ with OATP2B1 can be explained in terms of a combined effect of multiple flavonoid components of AJ.

MATERIALS AND METHODS

Materials

[^{3}H]Estrone-3-sulfate ammonium salt was purchased from PerkinElmer Life Sciences (Boston, Massachusetts). GFJ, OJ, and AJ (Tropicana^{TM}; 100% pure at normal strength) were purchased from a supermarket in Kanazawa city (Kanazawa, Japan). All other compounds and reagents were obtained from Sigma–Aldrich Company (St. Louis, Missouri), Invitrogen (Carlsbad, California), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Nacalai Tesque, Inc. (Kyoto, Japan), or Applied Biosystems (Foster City, California).

Uptake Experiments in *Xenopus laevis* Oocytes

Preparation of oocytes, *in vitro* synthesis of OATP2B1 complementary RNA (cRNA), and uptake experiments were conducted as described previously.[9,27,28] In brief, the construct pGEMHE containing OATP2B1 complementary DNA was used to synthesize cRNA *in vitro*. For standard experiments, defolliculated oocytes were injected with 50 nL of the cRNA solution (1 μg/μL) or water, and then incubated for 3 days at 18°C in modified Barth's saline [MBS; 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, and 10 mM HEPES, pH 7.4] containing 50 μg/mL gentamicin (MBS).

For the uptake studies, oocytes expressing OATP2B1 were preincubated in uptake buffer [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, and 10 mM 2-(N-morpholino)ethanesulfonic acid, pH 6.5] for 10 min at 25°C. To initiate uptake, the uptake buffer was completely replaced with [^{3}H]estrone-3-sulfate solution (uptake buffer, pH 6.5), and the oocytes were incubated for the designated time at 25°C. The uptake was terminated by washing the oocytes three times with ice-cold MBS. For the inhibition studies, oocytes expressing OATP2B1 were preincubated in uptake buffer (pH 6.5) for 10 min at 25°C. To initiate uptake, the uptake buffer was completely replaced with [^{3}H]estrone-3-sulfate solution (uptake buffer in the absence or presence of GFJ, OJ, AJ, various flavonoids, or D-mannitol, pH 6.5), and the oocytes were incubated for 15 min at 25°C. Meanwhile, the GFJ, OJ, and AJ solution was composed of uptake buffer excluding NaCl (pH 6.5). The uptake was terminated by washing the oocytes three times with ice-cold MBS. To determine the uptake of [^{3}H]estrone-3-sulfate, the oocytes were solubilized in 50 μL of 5% sodium dodecyl sulfate. One milliliter of scintillation fluid was added to the sample and the radioactivity was determined with a liquid scintillation counter (LSC 5100; Aloka, Tokyo, Japan) after incubation for 6–12 h at room temperature.

Uptake (nL/oocyte) was calculated as the cell-to-medium ratio by dividing the uptake amount by the initial concentration of estrone-3-sulfate in the uptake medium. Uptake rate (nL/min per oocyte) was determined as uptake of estrone-3-sulfate over a specified time. OATP2B1-mediated uptake rates were obtained after subtraction of the uptake by water-injected oocytes from that by OATP2B1 cRNA-injected oocytes.

Inhibition kinetic parameters were estimated by means of nonlinear least-squares analysis using the MULTI program or KaleidaGraph (Synergy Software, Reading, PA). The inhibitory effect of an inhibitor on estrone-3-sulfate transport was expressed as percentage of control, and the inhibitor concentration giving half-maximum inhibition (IC_{50}) was obtained by applying the following equation:

$$\%\ \text{of control} = \frac{100 \times IC_{50}}{IC_{50} + [I]} \quad (1)$$

where [I] is inhibitor concentration (μM).