# Effects of Grapefruit Juice and Orange Juice on the Intestinal Efflux of P-Glycoprotein Substrates

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***Purpose.*** The aim of this study is to investigate the effects of 50% ethyl acetate extracts of grapefruit juice (GFJ) and orange juice (OJ) on the transport activity of P-glycoprotein (P-gp) in the rat small intestine.

***Methods.*** The efflux of P-gp substrates from rat everted sac in the absence or presence of verapamil, GFJ, OJ or erythromycin was measured. Rhodamine123, fexofenadine and saquinavir were used as P-gp substrates. P-gp expression levels in the rat jejunum and ileum were determined by Western blot analysis.

***Results.*** The efflux of rhodamine123 from the everted sac increased from the apex of the jejunum to the low ileum and the expression of P-gp in the ileum was 2.31-fold higher than that in the jejunum. Verapamil and the 50% GFJ and OJ extracts inhibited the efflux from the intestine of all three drugs tested. Erythromycin decreased the efflux of rhodamine123 and fexofenadine, but did not affect the efflux of saquinavir in the intestine.

***Conclusions.*** GFJ and OJ extracts inhibited the efflux of P-gp substrates from the small intestine. Therefore, they may enhance the oral bioavailability of P-gp substrates by increasing absorption in the small intestine.

**KEY WORDS:** grapefruit juice; orange juice; P-glycoprotein (P-gp); intestinal efflux; fexofenadine; saquinavir.

## INTRODUCTION

Orally ingested grapefruit juice (GFJ) has been reported to increase the bioavailability of a wide variety of drugs, which are known to be substrates of cytochrome P450 (CYP) 3A4, such as dihydropyridine calcium channel antagonists (1,2), terfenadine (3), saquinavir (4), cyclosporin (5), and midazolam (6). This effect has been attributed to the selective down-regulation of the expression of CYP3A4 protein in the small intestine (1,7).

Unlike GFJ, orange juice (OJ) does not affect the oral bioavailability of CYP3A4 substrates such as felodipine (8) and pranidipine (2), suggesting that OJ does not affect CYP3A4 protein activity.

On the other hand, we recently reported that GFJ and OJ inhibited the efflux of vinblastine, a P-glycoprotein (P-gp) substrate from cell lines in which P-gp was overexpressed (9–11). P-gp acts as an ATP-dependent drug secretory pump to reduce intracellular concentrations of cytotoxic drugs, and is involved in multidrug resistance in tumor cells. In the intestine, P-gp is expressed on the apical membrane and acts as an efflux pump to limit the absorption of xenobiotics. P-gp also contributes to the elimination of many drugs into the intestinal lumen (12). Therefore, inhibitory effects of GFJ and OJ on the intestinal P-gp would be expected to enhance the bioavailability of drugs and could be another mechanism leading to adverse drug reactions, in addition to CYP3A4-inhibition by GFJ.

Fexofenadine, (Hoechst Marion Roussel, New Jersey) a non-sedating histamine $H_1$-receptor antagonist, which does not undergo significant metabolic biotransformation, is eliminated by biliary excretion and possibly direct intestinal secretion (13). Fexofenadine was found to be a substrate of P-gp, since its plasma concentration was increased 5-fold in mice lacking *mdr1a*-encoded P-gp and it was transported from the basal side to the apical side in the L-MDR1 cell line, which overexpresses P-gp (14). Thus, intestinal activity of P-gp may greatly affect the pharmacokinetics of fexofenadine. Indeed, erythromycin, a P-gp inhibitor has been reported to increase the steady-state plasma levels of fexofenadine (product information Moechst Marion Roussel).

Saquinavir, (Roche, London, United Kingdom) a HIV protease inhibitor, is also extruded from human and rat intestinal tissue by P-gp (15), so that inhibition of P-gp is expected to increase the poor oral bioavailability (4%) of saquinavir.

In this study, we chose fexofenadine and [$^{14}$C] saquinavir as P-gp substrates along with a typical P-gp substrate, rhodamine123, to investigate the effects of 50% extracts of GFJ and OJ, as well as erythromycin, on the efflux of these P-gp substrates from rat everted small intestine.

## MATERIALS AND METHODS

### Materials and Animals

Rhodamine123 was purchased from Acros Organics (New Jersey). Verapamil hydrochloride was from Nacalai Tesque Inc. (Kyoto, Japan). Erythromycin was from Sigma Chemical Co. (Missouri). Fexofenadine hydrochloride was a gift from Hoechst Marion Roussel Inc. (New Jersey). [$^{14}$C]Saquinavir (41.3 μCi/mg) was supplied by Roche Products Ltd. (London, United Kingdom). GFJ and OJ were purchased from the Dole Food Company Inc. (Sapporo, Japan). Mouse monoclonal antibody C219 (Mab C219) was purchased from TFB Inc. (Tokyo, Japan), monoclonal anti-β-actin from Sigma Chemical Co. (Missouri), and peroxidase-conjugated sheep affinity purified antibody to mouse IgG from ICP Pharmaceuticals Inc. (Ohio). The enhanced luminol reagent and oxidizing reagent were from NEN Life Science Products Inc. (Massachusetts). All other reagents used were of the highest purity available.

Male Sprague-Dawley rats (200 ~ 250 g) were purchased from Seac Yoshitomi Ltd. (Fukuoka, Japan).

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**ABBREVIATIONS:** CYP3A4, cytochrome P450 3A4; GFJ, grapefruit juice; Mab C219, mouse monoclonal antibody C219; OJ, orange juice; P-gp, P-glycoprotein.

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## Methods

### *Extraction of GFJ and OJ*

An aliquot of 200 ml of GFJ or OJ was mixed with 400 ml of ethyl acetate and shaken vigorously for 10 min by hand. The organic phase was separated and evaporated to dryness. The residue was dissolved in 1 ml of dimethylsulfoxide (DMSO) and added to 400 ml of Krebs-Henseleit buffer to make 50% GFJ or 50% OJ extract. Final DMSO concentration was 0.25%.

### *Determination of Drug Concentrations*

The concentration of rhodamine123 was assayed by two methods. A Shimadzu RF-1500 spectrofluorophotometer (Kyoto, Japan) was used when the effect of verapamil was studied and a high-performance liquid chromatography (HPLC) system was used when the effects of juice extracts and erythromycin were studied. The results obtained with the two detection methods were similar (data not shown).

The HPLC system consisted of a pump (LC-6A, Shimadzu, Kyoto, Japan), a fluorescence detector (RF-550, Shimadzu, Kyoto, Japan), and a chromatointegrator (C-R6A, Shimadzu, Kyoto, Japan).

The separation of rhodamine123 was carried out by using a reverse-phase column (Cosmosil, Nacalai Tesque Inc., Kyoto, Japan) (150 × 4.6 mm I.D., particle size 5 μm). The mobile phase consisted of acetonitrile and 0.05 M ammonium dihydrogen phosphate buffer (pH 4.0) (35: 65, v/v) and was pumped at a rate of 1.0 ml/min. Detection was done at wavelengths of 500 nm for excitation and 550 nm for emission.

The concentration of fexofenadine was determined using a reversed-phase RP-Select B column (Cica-Merck, Missouri) (150 × 4.6 mm I.D., particle size 5 μm). The mobile phase was 0.01 M phosphate buffer (pH 2.5), methanol and acetonitrile (52: 48: 1.5, v/v/v) and was pumped at a flow rate of 1.0 ml/min. Detection was done at wavelengths of 230 nm for excitation and 280 nm for emission.

To determine the concentration of [$^{14}$C]saquinavir, samples were mixed with scintillation fluid (Clearsol I, Nacalai Tesque Inc., Kyoto, Japan), and the radioactivity was measured by a liquid scintillation counter (LS6500, Beckman Instruments Inc., California).

### *Efflux of Rhodamine123, Fexofenadine and Saquinavir from Everted Rat Intestine*

A Sprague-Dawley rat (200 ~ 250 g) was anesthetized with ether. The whole small intestine was excised and flushed with 50 ml of ice-cold saline. A section of small intestine 10-cm in length was removed from the oral terminal of the duodenum. A 50-cm length of intestine was cut from the duodenal end and sequentially divided into five segments each with a length of 10 cm. These five segments were designated as sites 1, 2, 3, 4 and 5 from the apex of the region used. After a 10-cm segment had been removed from the distal end of the whole small intestine, another segment with a length of 10 cm was cut above the end and designated as site 6 in this study. We considered sites 1, 2 and 3 to be jejunal segments and sites 4, 5 and 6, ileal segments. Site 3 and site 6 were regarded as typical examples of jejunum and ileum, respectively. Each segment was everted and one end was ligated to make a sac. Rhodamine123 (150 μM), fexofenadine (150 μM) or [$^{14}$C]saquinavir (20 μM) was dissolved in oxygenated Krebs-Henseleit buffer (118 mM NaCl, 4.80 mM KCl, 0.59 mM MgSO4, 0.96 mM $KH_2PO_4$, 23.8 mM NaHCO3, 2.54 mM CaCl2 and 5.55 mM D-glucose) (pH7.4) and used as a donor phase. The sacs were placed into 10 ml of preoxygenated Krebs-Henseleit buffer preincubated at 37°C with or without inhibitor. Verapamil (300 μM), 50% GFJ extract, 50% OJ extract and erythromycin (300 μM and 1mM) were used as inhibitors. After incubation for 15 min, 1 ml of the donor phase was injected into the serosal side. An aliquot of 0.5 ml was taken as a sample from the mucosal (receptor) phase at 30, 60, 90 and 120 min and replaced with the same volume of blank buffer. Throughout the experiment, the receptor phase was maintained at 37°C and gas (5% $CO_2$/95% $O_2$) was circulated continuously.

### *Western Blot Analysis*

The crude membrane fractions from the rat intestine were prepared as previously described (16).

Protein concentration was determined according to Lowry's method (17) using bovine serum albumin as a standard. A 10 μg aliquot of protein was loaded in each lane, electrophoresed on 7.5% SDS-PAGE by the method of Laemmli (18) and transferred to a 0.2 μm pore size Immobilon Transfer Membrane (Millipore Co., MA, USA). For immunoblotting, the membranes were blocked with 5% nonfat powdered milk in T-TBS buffer (20 μM Tris-HCl, 135 mM NaCl, 0.1% Twin-20) at 4°C for 2 h. The blots were incubated with MAb C219 or anti-β-actin monoclonal for 2 h, then with peroxidase-conjugated sheep affinity-purified antibody to mouse IgG as a secondary antibody for 40 min, and washed five times with 1% nonfat powdered milk in T-TBS buffer. All washing and incubation steps were performed at ambient temperature. P-gp and β-actin were detected with the enhanced chemiluminescence (ECL) system according to the manufacturer's instructions (NEN Life Science Products Inc., Massachusetts). The expression levels were quantified with Quick One software (Bio-Rad Laboratories, California) for Macintosh.

## Statistics

The regional inter-site differences in the efflux of rhodamine123 were evaluated with ANOVA followed by Bonferroni's *t*-test. All other statistical analyses were performed by using Student's *t*-test. All data are expressed as the mean ± SEM.

## RESULTS

### Regional Differences in the Efflux of Rhodamine123

Regional differences in the efflux of rhodamine123 are shown in Fig. 1. After 150 μM rhodamine123 was applied to the serosal side, the time-course of the transfer to the mucosal side was followed for 2 h. The efflux of rhodamine123 increased with the distance from the oral to the anal terminal of the small intestine.

### Expression of P-gp along the Rat Intestine

The rat intestinal crude membrane fractions from site 3 (jejunum) and site 6 (ileum) were evaluated by Western blot

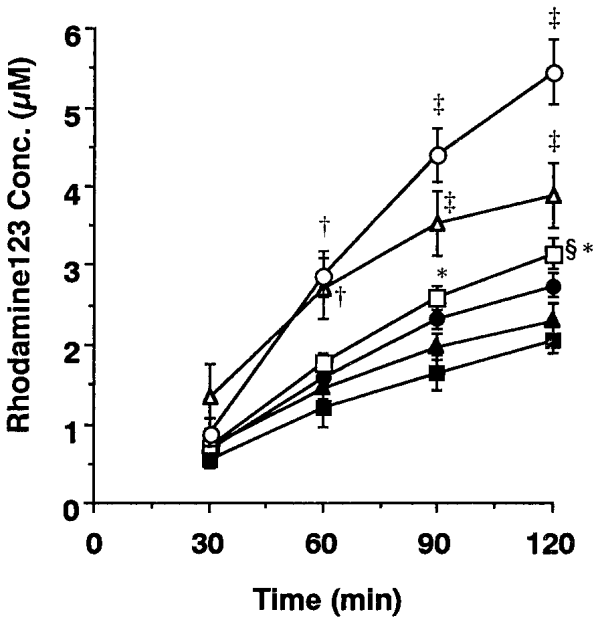


**Fig. 1.** The time courses of rhodamine123 efflux from site 1 (■), site 2 (▲), site 3 (●), site 4 (□), site 5 (△) and site 6 (○) of rat everted small intestine. Rhodamine123 (150 μM) was applied to the serosal side of an everted intestine and samples were taken from the mucosal side at the indicated time points. Each value represents the mean ± SEM ($n$ = 5 ~7). ‡; $P < 0.05$, from all the other groups; †; $P < 0.01$, from site 1, 2, 3 and 4; *; $P < 0.05$, from site 1; §; $P < 0.01$, from site 2.

analysis to determine P-gp expression (Fig. 2). The bands in the molecular weight range between 152 to 159 kDa were considered to represent immunoreactive P-gp. Anti-β-actin monoclonal was used as a total protein loading control. The relative level of P-gp protein in the ileum was 2.31-fold higher than that in the jejunum.

### Effect of Verapamil on Rhodamine123 Efflux

Figure 3 shows the effect of 300 μM verapamil on rhodamine123 efflux from rat everted small intestine. Verapamil significantly decreased the efflux rate of rhodamine123 at all the sites except site 1. The inter-site difference of the efflux also disappeared in the presence of verapamil.

### Effects of 50% GFJ Extract and 50% OJ Extract on Rhodamine123 Efflux

Figure 4 and Figure 5 show the effect of 50% GFJ extract or 50% OJ extract on the efflux of rhodamine123 from everted small intestine, respectively. Incubation of intestine with 50% GFJ extract or 50% OJ extract resulted in a significant reduction of the efflux rate of rhodamine123 in the ileum (sites 4, 5 and 6), but not the jejunum (sites 1, 2 and 3). The inhibitory potencies of 50% GFJ extract and 50% OJ extract on the efflux of rhodamine123 appeared to be weaker than that of verapamil.

### Effect of Erythromycin on Rhodamine123 Efflux

Figure 6 shows the efflux of rhodamine123 from site 3 (jejunum) and site 6 (ileum) in the absence or the presence of erythromycin (300 μM). Erythromycin significantly reduced the efflux rate of rhodamine123 from the jejunum and the ileum.

### Effects of Verapamil, 50% GFJ Extract, 50% OJ Extract and Erythromycin on the Efflux of Fexofenadine

Figure 7 shows the efflux of fexofenadine from site 3 (jejunum) and site 6 (ileum) in the absence or presence of verapamil (300 μM), 50% GFJ extract, 50% OJ extract and erythromycin (1 mM). Verapamil, 50% GFJ extract and 50% OJ extract significantly reduced the efflux rate of fexofenadine from the jejunum and the ileum, while erythromycin only decreased the efflux rate from the ileum. Inhibition of the efflux of fexofenadine by 50% GFJ extract and 50% OJ extract was weaker than that by verapamil, while erythromycin only slightly inhibited the efflux of fexofenadine from the ileum. The efflux of fexofenadine from the ileum was a little

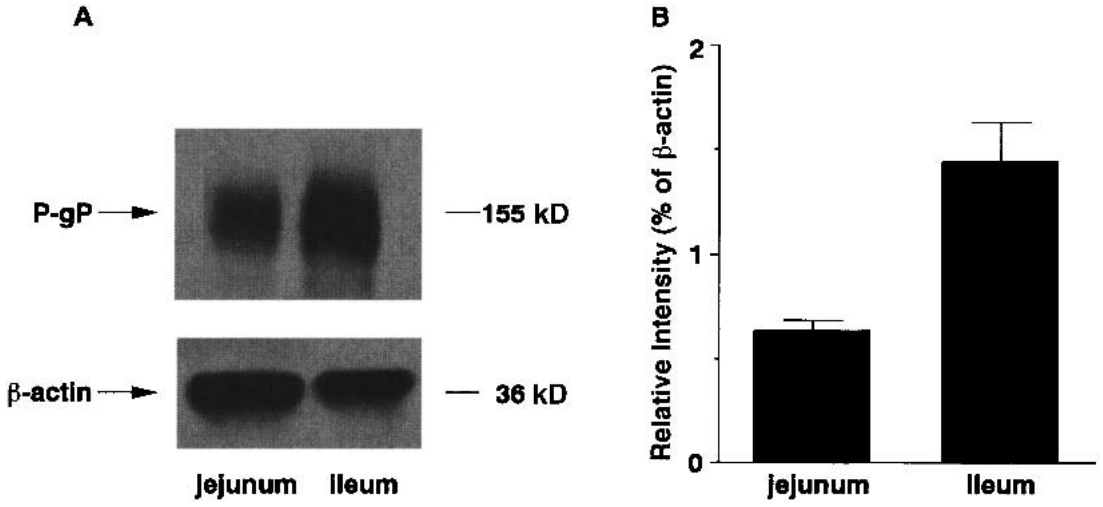


**Fig. 2.** P-gp expressions in intestinal epithelial cells of rat jejunum and ileum. Proteins (10 μg) derived from crude membrane fractions of site 3 and site 6 were incubated with C219 MAb or anti-β-actin monoclonal. Peroxidase-conjugated sheep affinity-purified antibody to mouse IgG was used as a secondary antibody. P-gp and β-actin were detected with the ECL system (A). The relative content of each point was quantified with Quick One software. (B). Each value represents the mean ± SEM ($n$ = 3).

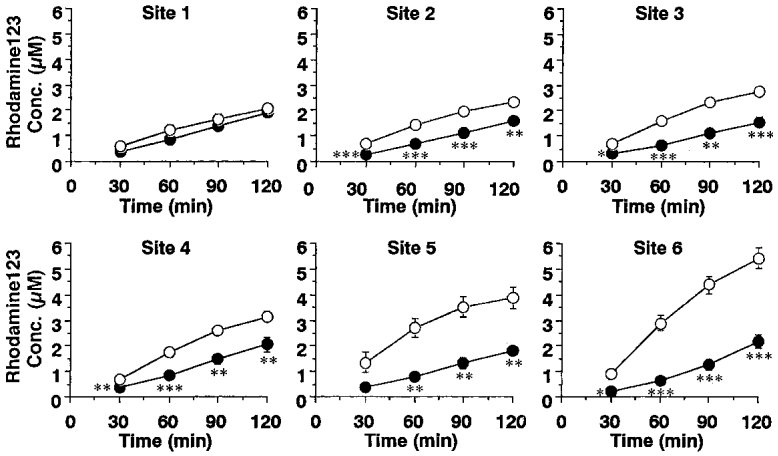


**Fig. 3.** Rhodamine123 efflux from rat everted small intestine of different regions in the absence (○) or presence (●) of verapamil. Rhodamine123 (150 μM) was applied to the serosal side and verapamil (300 μM) was applied to the mucosal side of the everted intestine. Samples were taken from the mucosal side at the indicated time points. Each value represents the mean ± SEM ($n = 5 \sim 7$). *; $P < 0.05$; **; $P < 0.01$, ***; $P < 0.001$, from the control.

higher than that from the jejunum although the difference was not statistically significant.

### Effects of Verapamil, 50% GFJ Extract, 50% OJ Extract and Erythromycin on the Efflux of [$^{14}$C]Saquinavir

Figure 8 shows the efflux of [$^{14}$C]saquinavir from site 3 (jejunum) and site 6 (ileum) in the absence or the presence of verapamil (300 μM), 50% GFJ extract, 50% OJ extract and erythromycin (1 mM). Incubation of the intestine with verapamil resulted in a significant reduction in the efflux rate of [$^{14}$C]saquinavir from the jejunum and the ileum, while 50% GFJ extract and 50% OJ extract only slightly inhibited the efflux of [$^{14}$C]saquinavir from the ileum. Erythromycin did not affect [$^{14}$C]saquinavir efflux.

## DISCUSSION

The rat-everted intestine is regarded as a reliable system to investigate drug transport across the gastrointestinal tract. The activity of P-gp assessed by the everted sac method *in vitro* was reported to correspond well with that assessed *in vivo* (19).

Drug efflux mediated by P-gp is reported to increase from the proximal to the distal intestine in mice, rats and humans (20,21). These observations are in agreement with the finding that the expression of *MDR1* mRNA increases from duodenum to ileum (20,22). In our study, as shown in Fig. 1, site-dependent efflux of rhodamine123 was observed in rat everted intestine. Western blot analysis showed that the expression of P-gp in the ileum was also higher than that in the

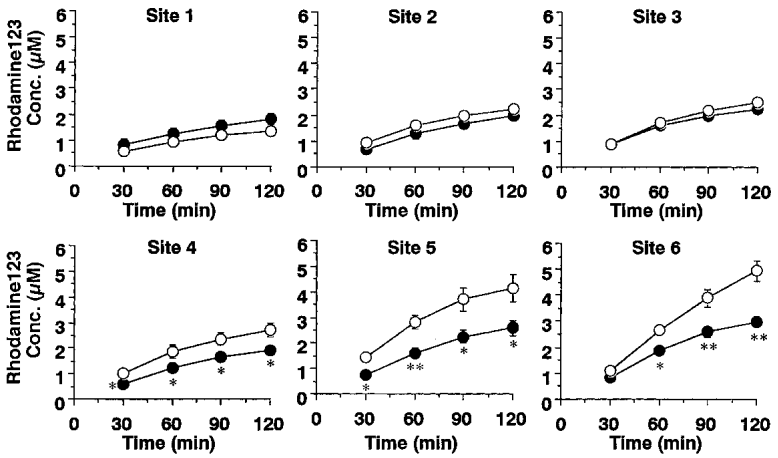


**Fig. 4.** Rhodamine123 efflux from rat everted small intestine of different regions in the absence (○) or presence (●) of 50% GFJ extract. Rhodamine123 (150 μM) was applied to the serosal side and 50% GFJ extract was applied to the mucosal side of the everted intestine. Samples were taken from the mucosal side at the indicated time points. GFJ extract preparation was as described in *Materials and methods*. Each value represents the mean ± SEM ($n = 5, 6$). *; $P < 0.05$; **; $P < 0.01$, from the control.

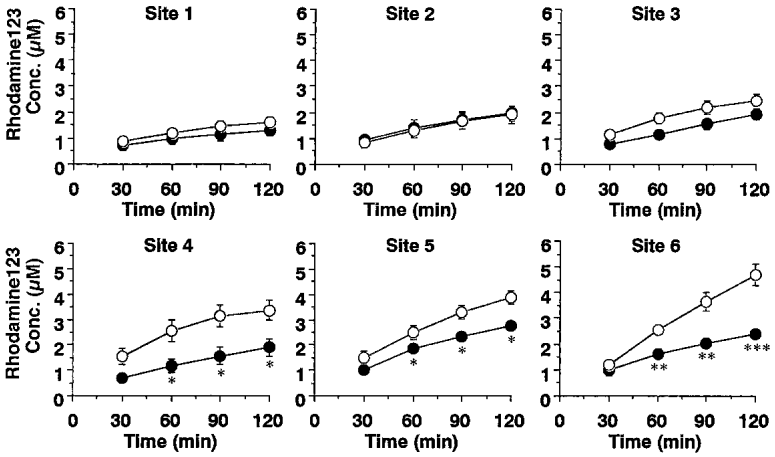


**Fig. 5.** Rhodamine123 efflux from rat everted small intestine of different regions in the absence (○) or presence (●) of 50% OJ extract. Rhodamine123 (150 μM) was applied to the serosal side and 50% OJ extract was applied to the mucosal side of everted intestine. Samples were taken from the mucosal side at the indicated time points. OJ extract preparation was as described in *Materials and Methods*. Each value represents the mean ± SEM ($n = 5 \sim 7$). *; $P < 0.05$, **; $P < 0.01$, ***; $P < 0.001$, from the control.

jejunum (Fig. 2). These results were consistent with previous reports (20–22). The site-dependent efflux of rhodamine123 may reflect the regional difference in the functional expression of P-gp in the small intestine. Indeed, the region-dependent portions of the efflux of rhodamine123 were attenuated by adding verapamil, a P-gp inhibitor, to the mucosal surface of the everted sacs (Fig. 3). In other words, the inhibitory effect of verapamil was more potent in the ileum.

Incubation of everted intestine with GFJ or OJ extract resulted in a significant reduction of the efflux of rhodamine123 from the ileum, where P-gp was more abundant (Fig. 4 and Fig. 5). We employed ethyl acetate extracts of the juices, because large amounts of components that inhibit P-gp are extracted from GFJ with this organic reagent (9). Indeed, the efflux of rhodamine123 was inhibited by these juice extracts. These results are in good accordance with our previous findings that there are several P-gp inhibitors in GFJ and OJ (9–11,23). However, the inhibitory effects of GFJ and OJ extracts on the efflux of rhodamine123 were observed only in the ileum, but not in the jejunum (Fig. 4 and Fig. 5), while verapamil inhibited the efflux at all the sites except site 1 (Fig. 3). A possible explanation is that the P-gp-inhibitory effects of GFJ and OJ are weaker than that of 300 μM verapamil, so

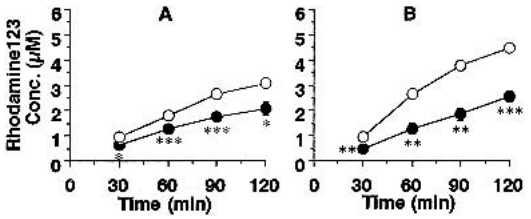


**Fig. 6.** Rhodamine123 efflux from site 3 (Fig. 5A) and site 6 (Fig. 5B) in the absence (○) or presence (●) of erythromycin. Rhodamine123 (150 μM) was applied to the serosal side and erythromycin (300 μM) was applied to the mucosal side of the everted intestine. Samples were taken from the mucosal side at the indicated time points. Each value represents the mean ± SEM ($n = 8$). *; $P < 0.05$, **; $P < 0.01$, ***; $P < 0.001$, from the control.

that GFJ and OJ extracts could not significantly inhibit the efflux in the jejunum, where P-gp expression is relatively low. Unlike the post-translational down-regulation of CYP3A4 by GFJ, the inhibitory effects of GFJ and OJ on P-gp seemed to be competitive in nature, because the extracts evoked the inhibition within 2 h.

Most GFJ-drug interactions have been believed to be evoked by metabolic inhibition of CYP3A4, based on pharmacokinetic studies of typical CYP3A4 substrates such as dihydropyridine calcium channel antagonists (1,2). However, GFJ was also reported to affect intestinal absorption of certain drugs whose oral bioavailabilities are limited by P-gp-mediated efflux in the small intestine (4,5). Therefore, the influence of P-gp inhibition on the GFJ-drug interaction can not be ignored. In contrast to our results, Soldner *et al.* reported that P-gp-mediated drug transport was activated by a low concentration of GFJ (24). This discrepancy may have resulted from the difference in the concentration of the juice used in the two studies. The level of 0.05% to 5% GFJ used by Soldner *et al.* might be too dilute to exert an inhibitory effect.

OJ was reported not to affect the oral bioavailabilities of several drugs (2,8), such as felodipine, which is almost completely absorbed from the small intestine and extensively metabolized by intestinal and hepatic CYP3A4, resulting in low bioavailability (25). This seems reasonable, since OJ was reported not to inhibit CYP3A4 (11). On the other hand, OJ extract inhibited the efflux of rhodamine123 in the rat everted ileum to the same extent as GFJ extract in this study, suggesting that OJ might affect the oral bioavailability of a drug which is extensively excreted by P-gp in the small intestine. However, OJ was reported not to elevate the concentration of cyclosporine after oral administration in humans (26). This discrepancy may be explained by possible differences in the composition and/or concentration of P-gp inhibitors in the OJ used.

Fexofenadine was progressively excreted from rat everted intestine (Fig. 7). This efflux was potently inhibited

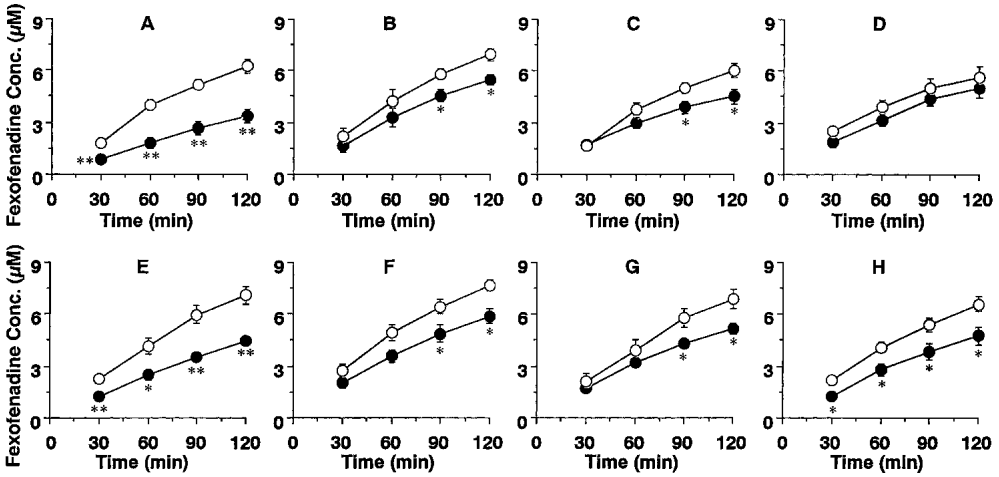


**Fig. 7.** The effects of 300 μM verapamil (A, E), 50% GFJ extract (B, F), 50% OJ extract (C, G) and 1 mM erythromycin (D, H) on the efflux of fexofenadine. Panels A-D and E-H show the effects of inhibitors at site 3 and site 6, respectively. (○) and (●) indicate the concentration of fexofenadine on the mucosal side in the absence and presence of inhibitors, respectively. Fexofenadine (150 μM) was applied to the serosal side and inhibitors were applied to the mucosal side of the everted intestine. Samples were taken from the mucosal side at the indicated time points. GFJ and OJ extracts were prepared as described in *Materials and methods*. Each value represents the mean ± SEM ($n = 4 \sim 6$). *; $P < 0.05$, **; $P < 0.01$, from the control.

by a P-gp inhibitor, verapamil (Fig. 7A and E), suggesting that P-gp acts as an absorption barrier for fexofenadine in the small intestine, and a part of the unchanged fexofenadine recovered in feces might have resulted from direct elimination from the intestine. In that case, a P-gp inhibitor may decrease the intestinal excretion of fexofenadine and so improve the bioavailability. However, fexofenadine has been reported to possess a wide therapeutic index, it has a minimally effective plasma concentration in the range of 15 ng/ml, and its safety has been established at steady-state plasma concentrations of up to 4677 ng/ml (27). Indeed, coadministration of erythromycin did not increase the QT interval (28). Therefore, cardiovascular adverse events may be unlikely even when fexofenadine is taken concomitantly with P-gp inhibitors. In contrast to rhodamine123, the efflux activity of fexofenadine was not statistically significantly different between the jejunum and the ileum (Fig. 7), although the efflux of fexofenadine from the ileum tended to be higher than that from the jejunum. These data suggest that P-gp-independent transporters may possibly be involved in the transport of fexofenadine in the small intestine.

The regional variation of [$^{14}$C]saquinavir efflux was quite similar to that of rhodamine123 (Fig. 8). Moreover, verapamil significantly decreased the efflux of [$^{14}$C]saquinavir and re-

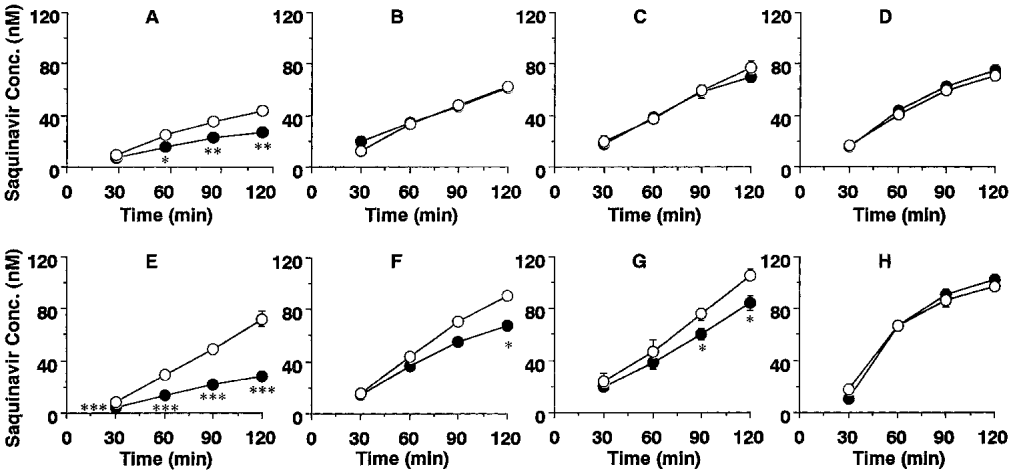


**Fig. 8.** The effects of 300 μM verapamil (A, E), 50% GFJ extract (B, F), 50% OJ extract (C, G) and 1 mM erythromycin (D, H) on the efflux of [$^{14}$C]saquinavir. Panels A-D and E-H show the effects of inhibitors at site 3 and site 6, respectively. (○) and (●) indicate the concentration of [$^{14}$C]saquinavir on the mucosal side in the absence and the presence of inhibitors, respectively. [$^{14}$C]Saquinavir (20 μM) was applied to the serosal side and inhibitors were applied to the mucosal side of the everted intestine. Samples were taken from the mucosal side at the indicated time points. Preparation of GFJ and OJ extracts was described in *Materials and methods*. Each value represents the mean ± SEM ($n = 6$). *; $P < 0.05$, **; $P < 0.01$, ***; $P < 0.001$, from the control.

sulted in disappearance of the regional difference (Fig. 7A and E). These results suggest that [$^{14}$C]saquinavir was excreted, at least in part, by P-gp in rat intestine. GFJ and OJ extracts decreased the efflux of saquinavir in rat ileum, which might result from P-gp inhibition (Fig. 8B, C, F and G). Although Eagling *et al.* (29) reported that the inhibitory effects of GFJ components on the P-gp-mediated transport of saquinavir were quite weak, they only investigated the effects of GFJ components, but not those of GFJ extract. In fact, the inhibition of P-gp efflux from Caco-2 cell line by GFJ ethyl acetate extract and by some GFJ components has been reported (9,10). With regard to saquinavir, it is necessary to consider the inhibitory effects of GFJ on CYP3A4-mediated metabolism, because saquinavir is metabolized by CYP3A4. However, OJ extract, which is devoid of metabolic inhibitors unlike GFJ extract (11), inhibited the efflux of saquinavir to the same extent as GFJ extract. Therefore, the inhibitory effects of GFJ and OJ extracts on the efflux of saquinavir observed in this study may not be attributed to the inhibition of the metabolism of saquinavir.

Erythromycin inhibited the efflux of rhodamine123 from the jejunum and the ileum (Fig. 6), in accordance with the findings of Takano *et al.* (30) and Yumoto *et al.* (19) that erythromycin is a P-gp inhibitor. Indeed, erythromycin also reduced the efflux of fexofenadine (Fig. 7D and H). Therefore, the known interaction of erythromycin and fexofenadine (product information, Hoechst Marion Roussel) might be a result of the inhibition of intestinal P-gp by erythromycin. However, erythromycin did not affect the efflux of [$^{14}$C]saquinavir (Fig. 8D and H). A possible explanation of this result is that erythromycin is a weak inhibitor of P-gp, and saquinavir is transported not only by P-gp, but also by other mechanisms. The roles of other active transporters, such as MRP2, remain to be investigated.

In this study, the contribution of P-gp to drug absorption was smaller in the jejunum than in the ileum in rats, while many drugs are mainly absorbed in the jejunum in humans. However, the contribution of P-gp to drug absorption in rats does not precisely reflect that in humans. To estimate quantitatively the contribution of P-gp to drug absorption in humans, it will be necessary to investigate the effects of P-gp inhibitors on the pharmacokinetics of each drug in humans *in vivo*.

In conclusion, the efflux of rhodamine123 is regarded as a functional expression of P-gp in the rat everted intestine. The efflux of fexofenadine and that of [$^{14}$C]saquinavir were also mediated, at least in part, by P-gp. The 50% extracts of GFJ and OJ decreased the efflux of rhodamine123, as well as those of fexofenadine and [$^{14}$C]saquinavir, in the rat intestine by inhibiting P-gp. Erythromycin inhibited the efflux of rhodamine123 and fexofenadine, but not that of [$^{14}$C]saquinavir from the rat intestine. Coadministration of GFJ, OJ or erythromycin may possibly alter the pharmacokinetics of drugs whose bioavailability is limited by P-gp-mediated intestinal efflux.

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