Exposure of Fexofenadine, but Not Pseudoephedrine, Is Markedly Decreased by Green Tea Extract in Healthy Volunteers

Shingen Misaka[1,*], Yuko Ono[1,4], R. Verena Taudte[2], Eva Hoier[2], Hiroshi Ogata[1], Tomoyuki Ono[1], Jörg König[2], Hiroshi Watanabe[3], Martin F. Fromm[2] and Kenju Shimomura[1]

Green tea (GT) alters the disposition of a number of drugs, such as nadolol and lisinopril. However, it is unknown whether GT affects disposition of hydrophilic anti-allergic drugs. The purpose of this study was to investigate whether pharmacokinetics of fexofenadine and pseudoephedrine are affected by catechins, major GT components. A randomized, open, 2-phase crossover study was conducted in 10 healthy Japanese volunteers. After overnight fasting, subjects were simultaneously administered fexofenadine (60 mg) and pseudoephedrine (120 mg) with an aqueous solution of green tea extract (GTE) containing (−)-epigallocatechin gallate (EGCG) of ~300 mg or water (control). *In vitro* transport assays were performed using HEK293 cells stably expressing organic anion transporting polypeptide (OATP)1A2 to evaluate the inhibitory effect of EGCG on OATP1A2-mediated fexofenadine transport. In the GTE phase, the area under the plasma concentration-time curve and the amount excreted unchanged into urine for 24 hours of fexofenadine were significantly decreased by 70% ($P < 0.001$) and 67% ($P < 0.001$), respectively, compared with control. There were no differences in time to maximum plasma concentration and the elimination half-life of fexofenadine between phases. Fexofenadine was confirmed to be a substrate of OATP1A2, and EGCG (100 and 1,000 μM) and GTE (0.1 and 1 mg/mL) inhibited OATP1A2-mediated uptake of fexofenadine. On the contrary, the concomitant administration of GTE did not influence the pharmacokinetics of pseudoephedrine. These results suggest that intake of GT may result in a markedly reduced exposure of fexofenadine, but not of pseudoephedrine, putatively by inhibiting OATP1A2-mediated intestinal absorption.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Green tea (GT) and its catechin components interact with hydrophilic drugs, such as nadolol and lisinopril. A combination of fexofenadine and pseudoephedrine is clinically available for treatment of allergic rhinitis.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study evaluated whether pharmacokinetics of fexofenadine and pseudoephedrine are affected when orally administered with an aqueous solution of green tea extract (GTE) in healthy volunteers.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Plasma concentrations and urinary excretions of fexofenadine were markedly decreased when coadministered with GTE, containing ~300 mg of (−)-epigallocatechin gallate. GTE and (−)-epigallocatechin gallate significantly inhibited OATP1A2-mediated fexofenadine uptake. No differences were observed in the pharmacokinetics of pseudoephedrine between water and GTE.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ GT and its catechin supplement reduce oral bioavailability of fexofenadine, but not of pseudoephedrine. The inhibition of intestinal OATP1A2 by (−)-epigallocatechin gallate is a likely mechanism underlying this interaction.

Green tea (GT; *Camellia sinensis*) and its main components, catechins, are perpetrators of clinically relevant food-drug interactions with hydrophilic and nonmetabolized drugs, such as nadolol and lisinopril.[1–4] Among naturally occurring catechins, (−)-epigallocatechin gallate (EGCG) plays a major role in GT–drug interactions.[2,4] Orally ingested EGCG likely inhibits

[1]Department of Bioregulation and Pharmacological Medicine, School of Medicine, Fukushima Medical University School of Medicine, Fukushima, Japan; [2]Institute of Experimental and Clinical Pharmacology and Toxicology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; [3]Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Hamamatsu, Japan; [4]Present address: Department of Disaster and Emergency Medicine, Graduate School of Medicine, Kobe University, Kobe, Japan. *Correspondence: Shingen Misaka (misaka@fmu.ac.jp)

Received February 7, 2022; accepted May 24, 2022. doi:10.1002/cpt.2682

membrane permeation of drugs into enterocytes mediated by uptake transporters expressed in the apical membrane, such as organic anion transporting polypeptide (OATP)s.[1,2,5–7] These findings led to the hypothesis that pharmacokinetics of hydrophilic drugs which are generally categorized as class 3 drugs according to the biopharmaceutics classification system (BCS) and the biopharmaceutics drug disposition classification system (BDDCS), are affected when co-administered with GT.

The H_1 blocker fexofenadine has intensively been investigated with respect to pharmacokinetic characteristics and transporter-mediated drug interactions.[8,9] Due to its hydrophilicity, fexofenadine is not a substrate of CYP enzymes and is largely excreted unchanged with minimal hepatic metabolism.[10] Both uptake and efflux transporters are involved in the disposition of fexofenadine including P-glycoprotein and OATPs.[11–13] Pharmacokinetics after oral administration of fexofenadine are influenced not only by drugs, such as itraconazole and rifampicin,[13–15] but also by concomitant food or beverages, such as grapefruit juice and apple juice.[16–18] To date, however, no studies have examined the effects of GT or catechins on the pharmacokinetics of fexofenadine.

At present, a combined tablet of fexofenadine and pseudoephedrine, a nasal decongestant, is available for the treatment of allergic rhinitis in several countries, such as the United States (Allegra-D) and Japan (Dellegra).[19] It has been reported that the pharmacokinetics of fexofenadine/pseudoephedrine combination formulation are bioequivalent to that of the individual drugs.[20] Of note, pseudoephedrine is also hydrophilic and a weak base compound, and is classified as BCS and BDDCS class 3 drug.[21,22] Indeed, pseudoephedrine is metabolized in the liver to a minor extent and is mainly excreted unchanged in the urine.[23] However, contrarily to fexofenadine, orally administered pseudoephedrine is nearly completely absorbed from the gastrointestinal tract.[23] Previous studies showed a negligible food effect on pseudoephedrine pharmacokinetics in humans.[24] Nevertheless, it remains to be elucidated whether plasma concentrations of pseudoephedrine are influenced by concomitant beverages as it is the case for other hydrophilic drugs such as fexofenadine.

Taking into account the anti-allergic potential of GT and catechins,[25] the opportunity of drinking GT during the treatment with anti-allergic drugs will arise in expectation of its beneficial effect on allergic symptoms. Therefore, the objective of this study was to assess whether GT catechins affect the pharmacokinetics of fexofenadine and pseudoephedrine in humans using a commercially available EGCG-concentrated green tea extract (GTE). We also performed *in vitro* assays to investigate possible molecular mechanisms of the interaction between GTE and fexofenadine.

METHODS

Subjects

Ten healthy nonsmoking Japanese volunteers (8 men and 2 women) participated in this study with an age range of 21–45 years and body mass index of 18.4–26.0 kg/m^2. All volunteers provided a written informed consent for study participation. The volunteers were ascertained to be healthy by medical history, physical examination, and routine laboratory tests. They were prohibited from consuming GT and fruit products, including apple, grapefruit, and orange juices, for 7 days before each trial day. The participants were genotyped for *SLCO2B1**3 (c.*1457C>T*) single nucleotide variation (SNV), as stated in **Methods S1**.

Clinical study design

The study protocol was approved by the ethics committee of the Fukushima Medical University (approval number: RK29037) and was registered at the UMIN Clinical Trials Registry (UMIN000032828). The study was conducted in compliance with the principles of the Declaration of Helsinki. In a single-center, open-label, randomized 2-way crossover study with a washout period of 2 weeks, the participants ingested a single oral dose of 2 tablets of fexofenadine (30 mg × 2) and (+)-pseudoephedrine (sustained-release formulation, 60 mg × 2; Dellegra; LTL Pharma, Tokyo, Japan) simultaneously with 150 mL of an aqueous solution of a commercial GTE (Sunphenon-EGCG, Taiyo Kagaku, Yokkaichi, Japan) or with 150 mL of water after overnight fasting. The GTE contained 92.5% (w/w) of EGCG, and 325 mg of which was dissolved in water with stirring. Subjects had a snack at 1 hour and a standardized meal at 4 hours after administration. In each study period, 5 mL venous blood samples were collected from an indwelling catheter placed in an antecubital vein or by direct venipuncture into EDTA-treated tubes at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 hours after administration. Blood samples were immediately centrifuged at 2,000 *g* for 10 minutes at 4°C. Urine was collected during periods of 0–4, 4–8, and 8–24 hours after administration. Plasma and urine samples were stored at −80°C until analysis.

Determinations of drug concentration in plasma and urine

The concentrations of fexofenadine and pseudoephedrine in plasma and urine were determined using ultra-performance liquid chromatography (UPLC) with fluorescence detection (UPLC, Waters, Milford, MA), as summarized in **Methods S1**. Diphenhydramine and phenylephrine were used as internal standards for fexofenadine and pseudoephedrine, respectively. The limit of quantification for fexofenadine and pseudoephedrine was both 10 ng/mL. The inter-day coefficients of variation of fexofenadine and pseudoephedrine were 9.9% and 7.3%, respectively.

Transport assays

To test whether GTE and EGCG affect OATP1A2- and OATP2B1-mediated uptake of fexofenadine, we performed *in vitro* transport assays using OATP1A2- and OATP2B1-stably expressing HEK cells and the respective HEK-VC vector control cells. Sulfobromophthalein (BSP) was used as a typical substrate for OATP2B1. The transporter-expressing cells and the respective vector control cells were cultured according to previous studies.[1,26] The experimental and quantitation methods of fexofenadine in the *in vitro* samples using liquid chromatography–tandem mass spectrometry (Thermo Fisher Scientific, Waltham, MA) are described in **Methods S1**.

Chemical binding assays

To examine the possibility of direct chemical binding between fexofenadine and EGCG in the gastrointestinal tract, fexofenadine (400 µg/mL, based on the maximum concentration when a 60-mg dose was dissolved in 150 mL) was incubated in the presence or absence of EGCG (1 mg/mL) in saline at 37°C for 1, 2, and 24 hours. Bortezomib (20 µg/mL) was used as a positive control for the chemical interaction with EGCG. The chromatographic separation of the samples was achieved by UPLC (Waters) system, as stated in detail in **Methods S1**.

Pharmacokinetics

The peak plasma concentration (C_{max}), time to C_{max} (T_{max}), area under the concentration-time curve ($AUC_{0-\infty}$), AUC_{0-8}, AUC_{0-24}, and elimination half-life ($t_{1/2}$), were calculated by noncompartmental analysis using WinNonlin software (version 5.1; Certara, Princeton, NJ). The

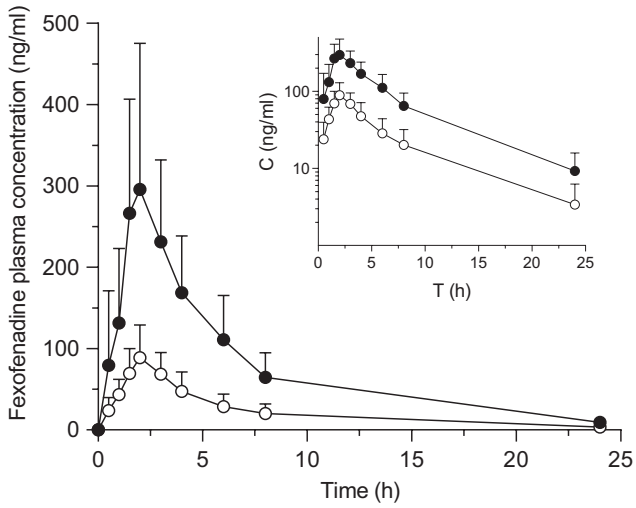

Figure 1 Plasma concentration profile of fexofenadine after oral administration of fexofenadine (60 mg) with 150 mL of an aqueous solution of (−)-epigallocatechin gallate (EGCG)-concentrated green tea extract (○), or water (●) in 10 healthy volunteers. Data are expressed as the arithmetic mean ± SD. The inset is the log-concentration vs. time profile.

renal clearance was obtained from the equation $CL_{renal} = A_e/AUC_{0-24}$, in which A_e is the amount of fexofenadine excreted into urine up to 24 hours.

Statistical analysis

In vitro data are expressed as mean ± standard error mean. Clinical pharmacokinetic data are expressed as geometric means and coefficient of variation (geoCV, %) unless otherwise noted. The number of subjects was deemed to be sufficient to detect a potentially clinically meaningful effect size of 35% difference in $AUC_{0-\infty}$ of fexofenadine between 2 phases with a power of 80% (α-level 5%) based on the previous pharmacokinetic data of fexofenadine in healthy Japanese subjects.[27] Effects of GTE on pharmacokinetics of test drugs were accepted if the 90% confidence interval (CI) of the geometric mean ratios (GMRs) did not fall within the bioequivalence boundary of 0.8–1.25. Correlations were examined using Pearson's correlation coefficients. *In vitro* data were analyzed by one-way analysis of variance and Tukey's multiple comparison test. Pharmacokinetic parameters were analyzed by a paired *t*-test. Statistical analyses were performed using GraphPad Prism software (version 8.4; GraphPad Software, San Diego, CA). Differences were regarded as statistically significant when P values were < 0.05.

RESULTS

All participants completed the study. None of the subjects experienced any adverse events related to the drugs. Three individuals were genotyped as *SLCO2B1* c.1457CC (*1/*1) carriers, 5 individuals as *SLCO2B1* c.1457CT (*1/*3) carriers, and 2 individuals as *SLCO2B1* c.1457TT (*3/*3) carriers.

Fexofenadine pharmacokinetics

Plasma concentration-time profiles and pharmacokinetic parameters of fexofenadine are shown in **Figure 1** and **Table 1**. There were no apparent differences in pharmacokinetic parameters of fexofenadine stratified for *SLCO2B1* *3 SNV in water (control) phase (data not shown). Fexofenadine C_{max}, AUC_{0-8}, $AUC_{0-\infty}$, and A_e in GTE phase were significantly decreased by 70% ($P < 0.001$), 71% ($P < 0.001$), 70% ($P < 0.001$), and 67% ($P < 0.001$), respectively, compared with control phase (**Figures 1**, **2a–c**, **Table 1**). The GMR (GTE/water) for C_{max} and $AUC_{0-\infty}$ of fexofenadine were 0.296 (90% CI, 0.197–0.396) and 0.296 (90% CI, 0.225–0.366), respectively. Changes in pharmacokinetic parameters for each individual with *SLCO2B1* *3 SNV are shown in **Figure S1**. The decrease in fexofenadine $AUC_{0-\infty}$ by GTE was negatively correlated with fexofenadine $AUC_{0-\infty}$ in control phase ($r = -0.9249$, $P < 0.001$) (**Figure S2a**). There were no differences in T_{max} and $t_{1/2}$ of fexofenadine between control and GTE phases. CL_{renal} of fexofenadine of

Table 1 Pharmacokinetic parameters of fexofenadine after oral administration with GTE or water in healthy volunteers

	Water phase (control)		GTE phase	
Fexofenadine	Geometric mean	Geo CV(%)	Geometric mean	Geo CV(%)
C_{max} (ng/mL)	278.7	48.3	82.6	47.1
GMR (90% CI)			0.296	(0.197–0.396)
T_{max} (h)	2.0	(1.5–6.0)	2.0	(1.5–3.0)
AUC_{0-8} (h ng/mL)	1131.7	47.5	323.6	45.7
GMR (90% CI)			0.286	(0.214–0.357)
$AUC_{0-\infty}$ (h ng/mL)	1765.0	44.8	521.9	50.6
GMR (90% CI)			0.296	(0.225–0.366)
$t_{1/2}$ (h)	5.2	31.4	5.7	48.7
GMR (90% CI)			1.096	(0.927–1.265)
A_e (mg)	6.0	35.0	2.0	27.3
GMR (90% CI)			0.335	(0.251–0.418)
CL_R (mL/min)	59.2	27.2	68.5	33.5
GMR (90% CI)			1.158	(1.074–1.242)

Fexofenadine (60 mg) was orally administered with water (150 mL), or an aqueous solution of EGCG-concentrated green tea extract (150 mL) in 10 healthy Japanese volunteers. The T_{max} values are expressed as median (range).
A_e, amount excreted unchanged into urine over 24 hours; AUC, area under the plasma concentration-time curve; CI, confidence interval; CL_R, renal clearance; C_{max}, peak plasma concentration; CV, coefficient of variation; GMR, geometric mean ratio; GTE, green tea extract; T_{max}, time to C_{max}; $t_{1/2}$, terminal half-life.

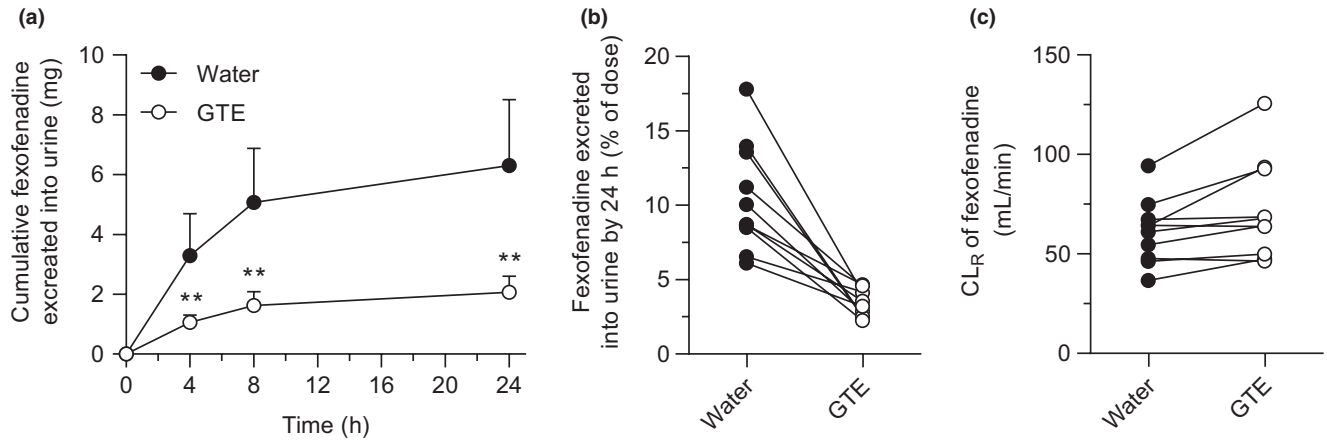

Figure 2 Urinary excretion of fexofenadine after oral administration of fexofenadine (60 mg) with 150 mL of an aqueous solution of (−)-epigallocatechin gallate (EGCG)-concentrated green tea extract (○), or water (●) in 10 healthy volunteers. (a) Cumulative urinary excretion (mg) of fexofenadine over 24 hours after administration, (b) % of dose, and (c) renal clearance (CL_R) of fexofenadine. Data are expressed as the arithmetic mean ± SD. **, $P < 0.01$ with respect to water phase. GTE, green tea extract.

this study was comparative to that of the previous study.[28] A slight but statistically significant ($P = 0.017$) increase in CL_{renal} of fexofenadine was observed by co-administration with GTE (**Figure 2c**).

Pseudoephedrine pharmacokinetics

Plasma concentration-time profiles and pharmacokinetic parameters of pseudoephedrine are shown in **Figure 3** and **Table 2**. No differences were observed in any pharmacokinetic parameters of pseudoephedrine, including AUC, T_{max}, $t_{1/2}$, and CL_{renal} between phases, whereas C_{max} was slightly decreased by GTE ($P = 0.048$; **Figure S1**). Urinary excretion of pseudoephedrine in GTE phase was nearly superimposed on those in water phase (**Figure 4a–c**). The change in pseudoephedrine $AUC_{0-\infty}$ by GTE was not correlated with pseudoephedrine $AUC_{0-\infty}$ in control phase ($r = -0.3548$, $P = 0.3144$; **Figure S2b**).

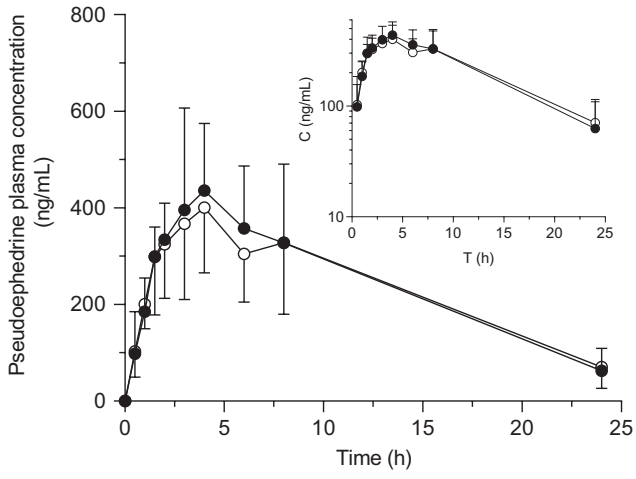

Figure 3 Plasma concentration profile of pseudoephedrine after oral administration of pseudoephedrine (120 mg) with 150 mL of an aqueous solution of (−)-epigallocatechin gallate (EGCG)-concentrated green tea extract (○), or water (●) in 10 healthy volunteers. Data are expressed as the arithmetic mean ± SD. The inset is the log-concentration vs. time profile.

Inhibition of fexofenadine uptake by EGCG and GTE

Cellular accumulation of fexofenadine (10 μM) in HEK-OATP1A2 cells was 22.14-fold higher than that in vector control (PQX) cells ($P < 0.01$) at pH 7.3. Moreover, OATP1A2-mediated fexofenadine uptake was nearly completely inhibited in the presence of EGCG (100 μM and 1 mM) or GTE (0.1 and 1 mg/mL; $P < 0.01$; **Figure 5a**). On the other hand, only slight but significant uptake of fexofenadine (10 μM) mediated by OATP2B1 was observed in HEK-OATP2B1 cells compared with vector control cells at both pH 6.3 and pH 7.3 (**Figure S3**), which is in accordance with recent papers.[29,30] However, due to low uptake ratio for OATP2B1-mediated fexofenadine transport, inhibition studies at both pH conditions revealed no consistent effect for both EGCG and GTE (data not shown). We used BSP as a typical substrate of OATP2B1, and found that EGCG (100 μM and 1 mM) and GTE (0.1 and 1 mg/mL) significantly reduced OATP2B1-mediated uptake of BSP at both pH 6.3 and pH 7.3 ($P < 0.01$; **Figure 5b,c**).

Chemical binding between fexofenadine and EGCG

It has been reported that GT catechins chemically interacted with various kinds of drugs including bortezomib, a proteasome inhibitor (**Figure S4a**).[31–34] Accordingly, we investigated whether EGCG also directly interacts with fexofenadine *in vitro*. As shown in the representative chromatograms (**Figure S4b,c**), the bortezomib peak found in 0 hour was gradually decreased, and 2 new peaks appeared in the presence of EGCG over 24 hours compared with bortezomib alone. Bortezomib concentration was significantly decreased by co-incubation with EGCG ($P < 0.01$; **Figure S4d**), indicating that EGCG interacted with bortezomib molecule and enhanced its degradation. By contrast, EGCG did not reduce fexofenadine concentration in an aqueous solution through 24 hours at 37°C (**Figure S4e**).

DISCUSSION

In this study, we report that a single concomitant ingestion of an aqueous solution (150 mL) of GTE containing about 300 mg of EGCG (~4.4 mM), significantly decreases plasma

Table 2 Pharmacokinetic parameters of pseudoephedrine after oral administration with GTE or water in healthy volunteers

	Water phase (control)		GTE phase	
Pseudoephedrine	**Geometric mean**	**Geo CV (%)**	**Geometric mean**	**Geo CV (%)**
C_{max} (ng/mL)	474.8	34.6	407.3	27.4
GMR (90% CI)			0.858	(0.757–0.958)
T_{max} (h)	4.0	(1.5–6.0)	3.5	(1.5–8.0)
AUC_{0-24} (h ng/mL)	5396.5	37.7	5309.6	36.6
GMR (90% CI)			0.984	(0.931–1.037)
$AUC_{0-\infty}$ (h ng/mL)	5965.8	43.1	6107.8	42.4
GMR (90% CI)			1.024	(0.906–1.142)
$t_{1/2}$ (h)	6.5	31.1	7.7	31.6
GMR (90% CI)			1.174	(0.908–1.439)
A_e (mg)	68.7	21.0	63.2	20.4
GMR (90% CI)			0.920	(0.846–0.994)
CL_R (mL/min)	212.1	53.3	198.3	50.2
GMR (90% CI)			0.935	(0.813–1.058)

Pseudoephedrine (120 mg) was orally administered with water (150 mL), or an aqueous solution of EGCG-concentrated green tea extract (150 mL) in 10 healthy Japanese volunteers. The T_{max} values are expressed as median (range).
A_e, amount excreted unchanged into urine over 24 hours; AUC, area under the plasma concentration-time curve; CI, confidence interval; CL_R, renal clearance; C_{max}, peak plasma concentration; CV, coefficient of variation; GMR, geometric mean ratio; GTE, green tea extract; T_{max}, time to C_{max}; $t_{1/2}$, terminal half-life.

concentrations and urinary excretion of fexofenadine when compared with water (control) in healthy volunteers (**Figures 1**, **2**). In contrast, no substantial differences were observed in the pharmacokinetics of pseudoephedrine between GTE and control phases (**Figures 3**, **4**). Fexofenadine and pseudoephedrine have common properties in terms of high hydrophilicity and negligible oxidative metabolism in the body, whereas the oral bioavailability of fexofenadine (35%) is considerably lower than that of pseudoephedrine (≈100%).[23,35] In addition, whereas fexofenadine is mainly eliminated by hepato-biliary elimination, pseudoephedrine is mostly excreted into urine. Previous clinical studies imply that GT and its main component, EGCG, may reduce the intestinal absorption of hydrophilic drugs with a relatively low bioavailability, including nadolol and lisinopril.[2–4] Therefore, taken together with data showing that GTE did not alter the apparent $t_{1/2}$ of fexofenadine, it is suggested that EGCG in the GTE mainly inhibits the intestinal absorption of fexofenadine.

Regarding the molecular mechanisms for reduced exposure of fexofenadine after oral administration, it is well known that the intestinal absorption of fexofenadine is highly dependent on transporter-mediated uptake and/or efflux, and that inhibition of the relevant transporters by co-administered drugs or food could result in a significant impact on its pharmacokinetics.[9] OATP1A2 and OATP2B1 are reported to be expressed in enterocytes,[36–39] and fexofenadine is a substrate of both transporters with a kinetic metabolite value of 6.4 μM for OATP1A2, and kinetic metabolite values for high- and

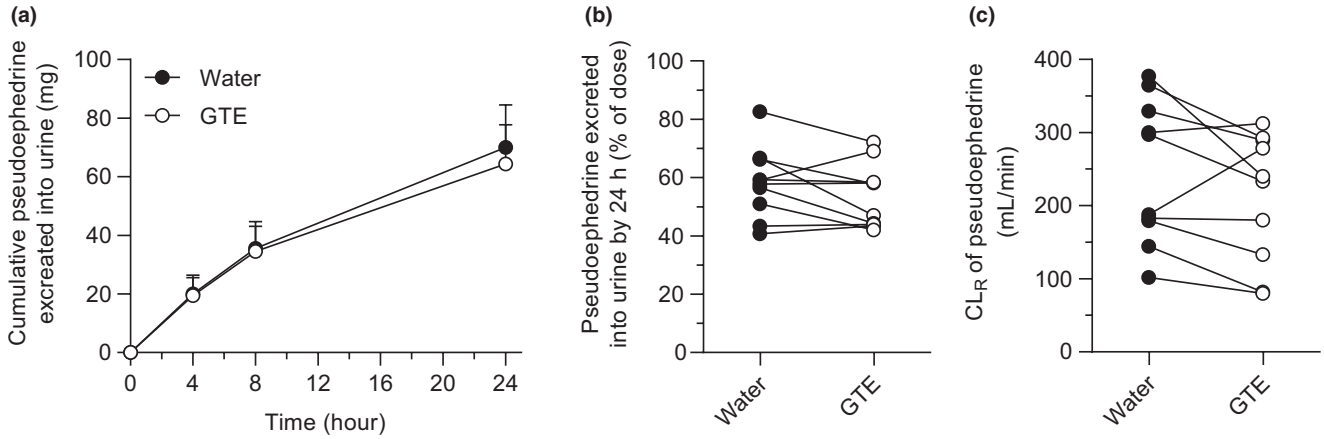

Figure 4 Urinary excretion of pseudoephedrine after oral administration of pseudoephedrine (120 mg) with 150 mL of an aqueous solution of (−)-epigallocatechin gallate (EGCG)-concentrated green tea extract (GTE; ○), or water (●) in 10 healthy volunteers. (a) Cumulative urinary excretion (mg) of pseudoephedrine over 24 hours after administration, (b) percent of dose, and (c) renal clearance (CL_R) of pseudoephedrine. Data are expressed as the arithmetic mean ± SD.

low-affinity binding sites of 0.14 μM and 885 μM, respectively, for OATP2B1.[11,40] EGCG is also a substrate of OATP1A2, and competitively inhibits OATP1A2-mediated nadolol transport with inhibition constant value of ~20 μM.[2,5] In the present study, we confirmed significant OATP1A2-mediated uptake of fexofenadine, and which was potently inhibited by GTE used in the clinical study and EGCG (**Figure 5a**). In addition, EGCG inhibits OATP2B1-mediated uptake of estrone-3-sulfate with half-maximal inhibitory concentration values ranging from 7.1–101 μM.[5,6] This was confirmed in BSP transport assay for OATP2B1 (**Figure 5b,c**). On the other hand, only slight uptake of fexofenadine was observed in HEK-OATP2B1 cells at pH 6.3 and pH 7.3, which is in accordance with recently published data.[29,30,41] Due to low uptake ratio (HEK-OATP2B1/HEK-Co), we found no consistent effects of GTE and EGCG on OATP2B1-mediated fexofenadine transport. As mentioned above, the subjects orally ingested the GTE aqueous solution containing ~4.4 mM of EGCG, and therefore the concentration of EGCG in the gastrointestinal tract may be sufficient to inhibit the uptake transport of fexofenadine by OATP1A2 into enterocytes, which may lead to a reduction in the oral bioavailability. It is noted that intestinal expression of OATP1A2 is still controversial. However, recent highly sensitive proteomic analyses reported the expression of OATP1A2 in human jejunum and ileum samples.[37,39] Moreover, Hirvensalo *et al*. recently demonstrated that intestinal OATP1A2 and P-glycoprotein play a role in the pharmacokinetics of celiprolol in humans,[42] further supporting the molecular mechanism underlying fexofenadine-GT interaction through OATP1A2.

A second possible mechanism is a direct chemical interaction between fexofenadine and EGCG. Indeed, various drugs, including aripiprazole, bortezomib, cetirizine, and sunitinib, has been reported to interact with EGCG.[31–34] In line with a previous study by Golden *et al*.,[31] our data show that EGCG interacted with bortezomib and significantly enhanced its degradation over 24 hours at 37°C (**Figure S3**). In contrast, EGCG did not reduce but rather stabilized fexofenadine in solution presumably by the anti-oxidative properties, suggesting that EGCG is unlikely to chemically interact with fexofenadine. Another possibility is the osmotic effects of co-ingested beverages on fexofenadine pharmacokinetics. Fruit juices containing a high amount of nonabsorbed carbohydrates, such as apple juice, show volume-dependent interaction with fexofenadine.[16,27,43] In such cases, water volume in intestinal lumen may be increased by an osmotic gradient,[44] and the luminal drug concentration is decreased, which may result in a reduction in intestinal absorption with a tendency of delay in the absorption rate (T_{max}).[27,43] In this study, in order to avoid osmotic effects of GT components, we used an aqueous solution of EGCG-concentrated GTE with water volume of 150 mL. These solutions are reported to be even more hypotonic than GT infusion.[45] Indeed, we found no prolongation in T_{max} of fexofenadine in GTE phase, indicating

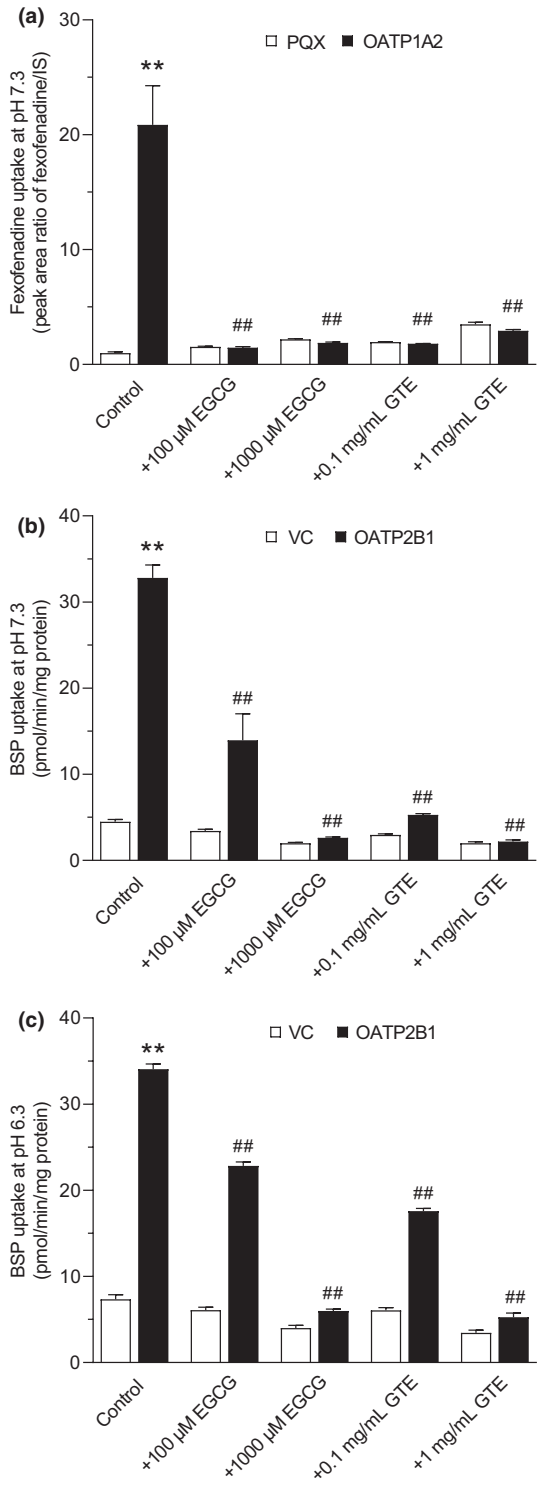

Figure 5 *In vitro* uptake assays of fexofenadine in HEK-OATP1A2 cells or sulfobromophthalein (BSP) in HEK-OATP2B1 cells (black bars), and in the respective HEK control cells (PQX) for OATP1A2 or (VC) for OATP2B1 (white bars). Cellular accumulation of 10 μM fexofenadine at pH 7.3 (a), or 1 μM BSP at pH 7.3 (b) and pH 6.3 (c) after 10 minutes incubation was measured by liquid chromatography–tandem mass spectrometry (LC–MS/MS) (fexofenadine) or liquid scintillation counting (BSP) in the presence or absence of (–)-epigallocatechin gallate (EGCG at 100 and 1,000 μM) or EGCG-concentrated green tea extract (GTE at 0.1 and 1 mg/mL) used in the clinical study. Data are presented as mean ± SEM. (n = 6). $^{**}P<0.01$, vs. uptake into HEK293 control cells (PQX or VC); $^{\#\#}P<0.01$, vs. uptake into HEK293-OATP1A2 or HEK-OATP2B1 cells without inhibitors.

that GTE used in this study has different interaction mechanisms from high-volume fruit juices.

In contrast to fexofenadine, the plasma concentration-time profile and cumulative urinary excretions of pseudoephedrine were similar in both phases, suggesting that EGCG had no impact on pseudoephedrine pharmacokinetics (**Figures 3**, **4**). Due to high solubility and low permeability, pseudoephedrine was provisionally classified as BCS class 3 drug.[21] However, subsequent studies have demonstrated that the permeability of pseudoephedrine is dependent on pH in the gastrointestinal tract.[46,47] Pseudoephedrine was shown to exhibit a relatively low permeability at the proximal regions of intestine (pH 6.5), which increases with its transition to distal regions (pH 7.5), possibly accounting for its excellent intestinal absorption. In addition, the molecular size may be another factor contributing to the lack of interaction between GTE and pseudoephedrine. Compared with the molecular weights of nadolol (309.4 g/mol), lisinopril (405.5 g/mol), and fexofenadine (501.7 g/mol), the molecular weight of pseudoephedrine (165.2 g/mol) is much smaller, which offers advantages of (i) higher concentration at the surface of the intestinal epithelium; and (ii) easier passive diffusion through the plasma membrane or the paracellular pathway.[48] Although drug transporters responsible for membrane trafficking of pseudoephedrine in the intestine remains to be investigated, the differences in physicochemical and pharmacokinetic properties may explain the distinct effects of GTE on the intestinal absorption of fexofenadine and pseudoephedrine.

A limitation of this study is that all volunteers were Japanese, so it remains unclear whether ethnic differences effect pharmacokinetic interactions between GT and fexofenadine. In relation to ethnicity, there was no influence of the *SLCO2B1**3 SNV on fexofenadine pharmacokinetics, although we confirmed that this polymorphism is common in Japanese population.[18] Fexofenadine disposition is influenced by multiple transporters, such as OATP1Bs (*SLCO1B1* and *SLCO1B3*) and P-glycoprotein (*ABCB1*), in addition to OATP1A2 and OATP2B1.[13,49,50] For example, *ABCB1* c.*2677G>T/A* and *SLCO1B1* c.*521T>C* (OATP1B1*5) polymorphisms might contribute to pharmacokinetic differences of fexofenadine,[40,41] however, we did not plan to identify these genotypes in the subjects because of our working hypothesis that GTE could mainly affect intestinal absorption process of test drugs. Finally, we did not differentiate the plasma concentrations and urinary excretions between fexofenadine enantiomers. It would be worth investigating the influence of EGCG on the pharmacokinetics of each stereoisomer of fexofenadine in the future.

In conclusion, we show for the first time that co-administration of GTE markedly reduces exposure to fexofenadine in humans by inhibiting OATP1A2-mediated intestinal absorption, whereas the pharmacokinetics of pseudoephedrine are unlikely to be influenced by co-administration of GTE. Considering that brewed GT contains more catechins and flavonoids in addition to EGCG, we propose that GT should not be taken during anti-allergic therapy using, at least, fexofenadine.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

ACKNOWLEDGMENTS

The authors thank Dr. Kazuo Minamikawa, Dr. Akihiro Inano, Ms. Hazuki Ito, and Ms. Momoka Ito for support on conducting the clinical trial.

FUNDING

This study was partly supported by JSPS KAKENHI Grant Number 17K17983 and 17K08949.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

S.M., Y.O., R.T., J.K., H.W., M.F., and K.S. wrote the manuscript. S.M., J.K., H.W., M.F., and K.S. designed the research. S.M., Y.O., R.T., E.H., T.O., H.O., and K.S. performed the research. S.M., Y.O., R.T., J.K., H.W., M.F., and K.S. analyzed the data.

© 2022 The Authors. *Clinical Pharmacology & Therapeutics* published by Wiley Periodicals LLC on behalf of American Society for Clinical Pharmacology and Therapeutics.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

1. Misaka, S. *et al*. Green tea ingestion greatly reduces plasma concentrations of nadolol in healthy subjects. *Clin. Pharmacol. Ther.* **95**, 432–438 (2014).
2. Abe, O. *et al*. Role of (–)-epigallocatechin gallate in the pharmacokinetic interaction between nadolol and green tea in healthy volunteers. *Eur. J. Clin. Pharmacol.* **74**, 775–783 (2018).
3. Misaka, S. *et al*. Effects of single green tea ingestion on pharmacokinetics of nadolol in healthy volunteers. *Br. J. Clin. Pharmacol.* **86**, 2314–2318 (2020).
4. Misaka, S. *et al*. Impact of green tea catechin ingestion on the pharmacokinetics of lisinopril in healthy volunteers. *Clin. Transl. Sci.* **14**, 476–480 (2021).
5. Roth, M., Timmermann, B.N. & Hagenbuch, B. Interactions of green tea catechins with organic anion-transporting polypeptides. *Drug Metab. Dispos.* **39**, 920–926 (2011).
6. Iijima, R., Watanabe, T., Ishiuchi, K., Matsumoto, T., Watanabe, J. & Makino, T. Interactions between crude drug extracts used in Japanese traditional Kampo medicines and organic anion-transporting polypeptide 2B1. *J. Ethnopharmacol.* **214**, 153–159 (2018).
7. Fuchikami, H., Satoh, H., Tsujimoto, M., Ohdo, S., Ohtani, H. & Sawada, Y. Effects of herbal extracts on the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab. Dispos.* **34**, 577–582 (2006).
8. Paśko, P., Rodacki, T., Domagała-Rodacka, R., Palimonka, K., Marcinkowska, M. & Owczarek, D. Second generation H1 – antihistamines interaction with food and alcohol – a systematic review. *Biomed. Pharmacother.* **93**, 27–39 (2017).
9. Akamine, Y. & Miura, M. An update on the clinical pharmacokinetics of fexofenadine enantiomers. *Expert Opin. Drug Metab. Toxicol.* **14**, 429–434 (2018).
10. Wu, C.Y. & Benet, L.Z. Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm. Res.* **22**, 11–23 (2005).
11. Cvetkovic, M., Leake, B., Fromm, M.F., Wilkinson, G.R. & Kim, R.B. OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab. Dispos.* **27**, 866–871 (1999).
12. Ma, J.D., Tsunoda, S.M., Bertino, J.S. Jr., Trivedi, M., Beale, K.K. & Nafziger, A.N. Evaluation of in vivo P-glycoprotein phenotyping

probes: a need for validation. *Clin. Pharmacokinet.* **49**, 223–237 (2010).
13. Kusuhara, H. *et al*. Effect of coadministration of single and multiple doses of rifampicin on the pharmacokinetics of fexofenadine enantiomers in healthy subjects. *Drug Metab. Dispos.* **41**, 206–213 (2013).
14. Shon, J.H. *et al*. Effect of itraconazole on the pharmacokinetics and pharmacodynamics of fexofenadine in relation to the MDR1 genetic polymorphism. *Clin. Pharmacol. Ther.* **78**, 191–201 (2005).
15. Hamman, M.A., Bruce, M.A., Haehner-Daniels, B.D. & Hall, S.D. The effect of rifampin administration on the disposition of fexofenadine. *Clin. Pharmacol. Ther.* **69**, 114–121 (2001).
16. Dresser, G.K. *et al*. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin. Pharmacol. Ther.* **71**, 11–20 (2002).
17. Bailey, D.G., Dresser, G.K., Leake, B.F. & Kim, R.B. Naringin is a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) in grapefruit juice. *Clin. Pharmacol. Ther.* **81**, 495–502 (2007).
18. Imanaga, J. *et al*. The effects of the SLCO2B1 c.1457C > T polymorphism and apple juice on the pharmacokinetics of fexofenadine and midazolam in humans. *Pharmacogenet. Genomics* **21**, 84–93 (2011).
19. Mansfield, L.E. Once-daily immediate-release fexofenadine and sustained-release pseudoephedrine combination: a new treatment option for allergic rhinitis. *Expert. Opin. Pharmacother.* **7**, 941–951 (2006).
20. Howard, D.R., Haribhakti, R., Kittner, B. & Agrawala, P. Single-dose and steady-state bioequivalence of fexofenadine and pseudoephedrine combination tablets compared with individual formulations in healthy adults. *Curr. Med. Res. Opin.* **21**, 769–776 (2005).
21. Takagi, T., Ramachandran, C., Bermejo, M., Yamashita, S., Yu, L.X. & Amidon, G.L. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. *Mol. Pharm.* **3**, 631–643 (2006).
22. Benet, L.Z., Broccatelli, F. & Oprea, T.I. BDDCS applied to over 900 drugs. *AAPS J.* **13**, 519–547 (2011).
23. Kanfer, I., Dowse, R. & Vuma, V. Pharmacokinetics of oral decongestants. *Pharmacotherapy* **13**, 116S–128S (1993).
24. Wecker, M.T., Graves, D.A., Amsel, L.P., Hinsvark, O.N. & Rotenberg, K.S. Influence of a standard meal on the absorption of controlled-release pseudoephedrine capsules. *J. Pharm. Sci.* **76**, 29–31 (1987).
25. Li, Q.S., Wang, Y.Q., Liang, Y.R. & Lu, J.L. The anti-allergic potential of tea: a review of its components, mechanisms and risks. *Food Funct.* **12**, 57–69 (2021).
26. Mandery, K. *et al*. Influence of the flavonoids apigenin, kaempferol, and quercetin on the function of organic anion transporting polypeptides 1A2 and 2B1. *Biochem. Pharmacol.* **80**, 1746–1753 (2010).
27. Luo, J. *et al*. The pharmacokinetic exposure to fexofenadine is volume-dependently reduced in healthy subjects following oral administration with apple juice. *Clin. Transl. Sci.* **9**, 201–206 (2016).
28. Russell, T., Stoltz, M. & Weir, S. Pharmacokinetics, pharmacodynamics, and tolerance of single- and multiple-dose fexofenadine hydrochloride in healthy male volunteers. *Clin. Pharmacol. Ther.* **64**, 612–621 (1998).
29. Bednarczyk, D. & Sanghvi, M.V. Organic anion transporting polypeptide 2B1 (OATP2B1), an expanded substrate profile, does it align with OATP2B1's hypothesized function? *Xenobiotica* **50**, 1128–1137 (2020).
30. Morita, T. *et al*. Citrus fruit-derived flavanone glycoside narirutin is a novel potent inhibitor of organic anion-transporting polypeptides. *J. Agric. Food Chem.* **68**, 14182–14191 (2020).
31. Golden, E.B. *et al*. Green tea polyphenols block the anticancer effects of bortezomib and other boronic acid-based proteasome inhibitors. *Blood* **113**, 5927–5937 (2009).
32. Ge, J. *et al*. Interaction of green tea polyphenol epigallocatechin-3-gallate with sunitinib: potential risk of diminished sunitinib bioavailability. *J. Mol. Med.* **89**, 595–602 (2011).
33. Ohata, T. *et al*. Drug-tea polyphenol interaction (II) complexation of piperazine derivatives with green tea polyphenol. *Thermochim. Acta* **653**, 1–7 (2017).
34. Ikeda, H. *et al*. Drug-tea polyphenol interaction (III) incompatibility between aripiprazole Oral solution and green tea. *Chem. Pharm. Bull.* **70**, 230–234 (2022).
35. Lappin, G. *et al*. Pharmacokinetics of fexofenadine: evaluation of a microdose and assessment of absolute oral bioavailability. *Eur. J. Pharm. Sci.* **40**, 125–131 (2010).
36. Glaeser, H. *et al*. Intestinal drug transporter expression and the impact of grapefruit juice in humans. *Clin. Pharmacol. Ther.* **81**, 362–370 (2007).
37. Couto, N. *et al*. Quantitative proteomics of clinically relevant drug-metabolizing enzymes and drug transporters and their intercorrelations in the human small intestine. *Drug Metab. Dispos.* **48**, 245–254 (2020).
38. Drozdzik, M. *et al*. Protein abundance of clinically relevant drug transporters in the human liver and intestine: a comparative analysis in paired tissue specimens. *Clin. Pharmacol. Ther.* **105**, 1204–1212 (2019).
39. Akazawa, T., Uchida, Y., Miyauchi, E., Tachikawa, M., Ohtsuki, S. & Terasaki, T. High expression of UGT1A1/1A6 in monkey small intestine: comparison of protein expression levels of cytochromes P450, UDP-glucuronosyltransferases, and transporters in small intestine of Cynomolgus monkey and human. *Mol. Pharm.* **15**, 127–140 (2018).
40. Shirasaka, Y., Mori, T., Murata, Y., Nakanishi, T. & Tamai, I. Substrate- and dose-dependent drug interactions with grapefruit juice caused by multiple binding sites on OATP2B1. *Pharm. Res.* **31**, 2035–2043 (2014).
41. Won, C.S. *et al*. A modified grapefruit juice eliminates two compound classes as major mediators of the grapefruit juice-fexofenadine interaction: an *in vitro*-in vivo "connect". *J. Clin. Pharmacol.* **53**, 982–990 (2013).
42. Hirvensalo, P. *et al*. Pharmacogenomics of celiprolol – evidence for a role of P-glycoprotein and organic anion transporting polypeptide 1A2 in celiprolol pharmacokinetics. *Clin. Transl. Sci.* **15**, 409–421 (2021).
43. Dresser, G.K., Kim, R.B. & Bailey, D.G. Effect of grapefruit juice volume on the reduction of fexofenadine bioavailability: possible role of organic anion transporting polypeptides. *Clin. Pharmacol. Ther.* **77**, 170–177 (2005).
44. Grimm, M. *et al*. Gastric emptying and small bowel water content after administration of grapefruit juice compared to water and isocaloric solutions of glucose and fructose: a four-way crossover MRI pilot study in healthy subjects. *Mol. Pharm.* **15**, 548–559 (2018).
45. Adeli, F. *et al*. Comparative *in vitro* study of the effectiveness of green tea extract and common storage media on periodontal ligament fibroblast viability. *Eur J Dent* **10**, 408–412 (2016).
46. Fairstein, M., Swissa, R. & Dahan, A. Regional-dependent intestinal permeability and BCS classification: elucidation of pH-related complexity in rats using pseudoephedrine. *AAPS J.* **15**, 589–597 (2013).
47. Wolk, O. *et al*. Segmental-dependent intestinal drug permeability: development and model validation of in silico predictions guided by in vivo permeability values. *J. Pharm. Sci.* **108**, 316–325 (2019).
48. Camenisch, G., Alsenz, J., van de Waterbeemd, H. & Folkers, G. Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight. *Eur. J. Pharm. Sci.* **6**, 317–324 (1998).
49. Niemi, M., Kivisto, K.T., Hofmann, U., Schwab, M., Eichelbaum, M. & Fromm, M.F. Fexofenadine pharmacokinetics are associated with a polymorphism of the SLCO1B1 gene (encoding OATP1B1). *Br. J. Clin. Pharmacol.* **59**, 602–604 (2005).
50. Vanhove, T., Bouillon, T., de Loor, H., Annaert, P. & Kuypers, D. Fexofenadine, a putative in vivo P-glycoprotein probe, fails to predict clearance of the substrate tacrolimus in renal recipients. *Clin. Pharmacol. Ther.* **102**, 989–996 (2017).