Apple juice greatly reduces systemic exposure to atenolol

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Atenolol is an antihypertensive drug, of which negligible amounts are metabolized.
- Fruit juices may decrease the oral absorption of drugs by inhibiting intestinal drug transporters, as demonstrated *in vitro* and *in vivo*.

WHAT THIS STUDY ADDS

- The pharmacokinetic characteristics of atenolol were determined according to the *SLCO2B1* genotype after apple juice administration in healthy Korean volunteers.
- Apple juice ingestion markedly reduced the systemic exposure to atenolol, but genetic variations in *SLCO2B1* were unlikely to contribute substantial variability to the pharmacokinetics of atenolol.

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AIM

Fruit juice reduces the plasma concentrations of several β -adrenoceptor blockers, likely by inhibiting OATP2B1-mediated intestinal absorption. The aim of this study was to investigate the effects of apple juice on the pharmacokinetics of atenolol.

METHODS

Twelve healthy Korean volunteers with genotypes of *SLCO2B1* c.1457C>T (*1/*1 ($n = 6$) and *3/*3 ($n = 6$)) were enrolled in this study. In a three-phase, one-sequence crossover study, the pharmacokinetics (PK) of atenolol was evaluated after administration of 50 mg atenolol. Subjects received atenolol with either 300 ml water, 1200 ml apple juice or 600 ml apple juice.

RESULTS

Apple juice markedly reduced the systemic exposure to atenolol. The geometric mean ratios (95% confidence intervals) of apple juice : water were 0.18 (0.13, 0.25, 1200 ml) and 0.42 (0.30, 0.59, 600 ml) for the $AUC(0, t_{last})$. In this study, the PK parameters of atenolol responded in a dose-dependent manner to apple juice.

CONCLUSIONS

Apple juice markedly reduced systemic exposure to atenolol. The genetic variation of *SLCO2B1* c.1457C>T had a minimal effect on the pharmacokinetics of atenolol when the drug was administered with water or apple juice.

Introduction

Atenolol is a selective β_1 -receptor blocking agent and is one of the most widely prescribed drugs used in the treatment of angina pectoris and hypertension [1–4]. As a first

line therapy for hypertension, β -adrenoceptor blockers have proven to be safe and effective in reducing mortality and morbidity [3, 5]. The bioavailability of atenolol is approximately 40–50%. Less than 5% of the drug is bound to plasma proteins and renal excretion eliminates

approximately 90% of the absorbed drug in its unchanged form [6, 7]. Atenolol is a hydrophilic compound, and only negligible amounts of the drug are metabolized [7]. The pharmacokinetic parameters of atenolol show 26–38% inter-individual variability [8].

Organic anion-transporting polypeptides OATP2B1 and OATP1A2 are influx transporters located in the luminal membranes of small intestine enterocytes [9]. Fruit juices may decrease the oral absorption of drugs by inhibiting intestinal drug transport, as demonstrated by *in vitro* and *in vivo* studies [10–15]. Orange juice reduced the mean peak plasma concentration ($C_{\max}$) and area under the plasma concentration–time curve (AUC) of atenolol by 49% and 40%, respectively [16]. Apple juice reduced the exposure of fexofenadine to a mean of 75%, and grapefruit juice decreased the bioavailability of celiprolol, in part due to the OATP2B1-mediated absorption process [17, 18]. At present, OATP2B1 and OATP1A2 are considered to be related to this interaction [19]. Recently, genetic variants with non-synonymous single nucleotide polymorphisms (SNPs), *SLCO2B1* *3 (Ser→Phe (c.1457C>T)) have been shown to alter the systemic exposure of substrate drugs compared with the wild-type genotype [17, 18, 20]. In a Japanese population, an allele frequency of 30.9% of the *SLCO2B1**3 was reported [21]. In a Korean population, the allele frequency of the variant was 25.5% in healthy volunteers (our unpublished data), which is similar to that of the Japanese population and greater than that of the Finnish population (2.1%) [22]. Atenolol is thought to be a substrate of OATP1A2 [10]. However the frequency of non-synonymous SNPs of *SLCO1A2* has been reported to be very low in the Asian population [23]. Although atenolol is one substrate of OATP1A2, we hypothesized that atenolol could also be transported by OATP2B1, as is observed for hydrophilic β -adrenoceptor blockers that are only metabolized weakly (e.g. celiprolol or talinolol) [18, 24]. Therefore, we investigated whether atenolol absorption is affected by (i) apple juice ingestion and (ii) genetic variants in the gene coding for OATP2B1 (*SLCO2B1*) in humans.

Methods

Subjects

Korean male volunteers aged 20 to 50 years with a body mass index in the range of 17 to 28 kg m⁻² were enrolled in this study if deemed healthy by physical examination, medical history, laboratory results, 12-lead electrocardiogram and vital signs. The subjects who had ingested fruit juice within 7 days prior to drug administration were excluded. Written informed consent was obtained from all subjects prior to the study. Twelve healthy male volunteers were selected based on genotyping for *SLCO2B1* (*SLCO2B1* *1/*1 ($n = 6$) and *3/*3 ($n = 6$)).

Study design

The study was a single-dose, randomized, open-label, one-sequence, three-period crossover design. All subjects were hospitalized the night before drug administration. After overnight fasting, each individual received a single oral dose of atenolol (Tenormin, AstraZeneca Japan, Osaka, Japan). The study drug was administered with water or apple juice (100% Martinelli's Gold Medal apple juice, CA, USA). In period 1, 50 mg atenolol was administered with 300 ml soft mineral water. In period 2, the drug was taken with 300 ml apple juice, and the participants consumed an additional 150 ml apple juice every 0.5 h for 3 h (for a total of 1200 ml), which was identical to the protocol used for the previous juice–drug interaction study [11]. In period 3, 300 ml apple juice was administered as in period 2 and the participants also consumed an additional 100 ml every 0.5 h for 1.5 h (for a total of 600 ml). Each period was separated by a wash-out interval of at least 7 days. For 4 h after drug administration, subjects were required to remain in a seated position. After this time period, standardized lunches were provided and the subjects were allowed to drink water. Blood samples were collected before dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36 and 48 h after dosing. Each sample was collected in a heparinized tube, centrifuged at 2000 *g* for 10 min and stored below –70°C until the assay was performed. For safety evaluation, systolic and diastolic blood pressures were measured after the subjects had been sitting for a minimum of 5 min using an automatic oscillometric blood pressure monitor prior to each pharmacokinetic sampling (Dash 5000, General Electric Healthcare, Finland). This study was approved by the Institutional Review Board of Seoul National University Hospital and registered at ClinicalTrials.gov (identifier: NCT01445964).

Genotyping

Blood samples were collected from each subject and DNA was extracted using a purification kit (QIAamp DNA blood mini kit, QIAGEN, Hilden, Germany). *SLCO2B1* *3 (1457C>T, rs2306168, assay ID: C_16193013_20). The allele detection of three other common variants (935G>A, rs12422149, assay ID: C_3101331_10; 282 G>A, rs2712807, assay ID: C_1786365_10; 1175 C>T, rs1621378, assay ID: C_8750223_30) was performed by TaqMan allelic discrimination assays on an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

For the 12 enrolled subjects, genetic variants regarding pharmacokinetics were evaluated using the DMET plus panel (Affymetrix, Santa Clara, California, USA). The DMET plus panel is a targeted single nucleotide polymorphism (SNP) chip that is useful in identifying genetic biomarker candidates and drug responders [25]. The polymorphisms that were chosen for the panel are known as drug-metabolizing enzymes and transporters. The DMET plus chip utilizes molecular-inversion probe technology to develop a multiplex genotyping assay that can simulta-

neously test more than 1936 markers in 225 genes associated with drug metabolizing enzymes and transporters [26, 27].

Quantification of plasma concentrations of atenolol

Plasma atenolol concentration was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The high performance liquid chromatography system (HPLC, Agilent 1100 series, Agilent Technologies, Wilmington, DE, USA) was connected to a mass spectrometer (API 4000 Quadrupole, Applied Biosystems, Foster City, USA) equipped with a Turbo Spray. The chromatographic column was a Luna Phenylhexyl 3 μ m analytical column (100 $\times$ 2.0 mm inner diameter, Phenomenex, Torrance, USA). Atenolol was monitored in the positive mode and quantifications were conducted in the multiple reaction monitoring (MRM) mode. The mass transitions were m/z 267.288 $\rightarrow$ 145.100 for atenolol and m/z 260.190 $\rightarrow$ 116.200 for the internal standard.

Stock solutions of atenolol and propranolol (internal standard, Sigma-Aldrich, Buchs, Switzerland) were extracted from the samples by protein precipitation using acetonitrile and prepared in methanol at approximately 1 mg ml⁻¹. The solutions were diluted and used to spike blank matrices to obtain calibration standard solutions. The standard curve for atenolol was 5–2000 ng ml⁻¹ and each standard solution was prepared by adding a working standard solution to a blank matrix. Quality control samples were prepared by adding separate stock solutions of atenolol to the blank matrix. The internal standard (propranolol) solution was added to 1 ml plasma. After centrifugation for 5 min at 9900 g, 4 μ l of supernatant was injected into the LC-MS/MS. The concentrations were quantified by the internal standard method. The calibration curve was obtained using least square linear regression analysis by fitting the peak area ratio against the internal standard and nominal concentration (x) of the analyte to the equation $y = ax + b$ (weighting $1/x^2$). The quantification limit of atenolol was 5 ng ml⁻¹. The accuracy of the analysis ranged from 99–102%, and the precision was lower than 5%.

Pharmacokinetic analysis

Pharmacokinetic parameters ($C_{\max}$, $AUC(0, t_{\text{last}})$, $t_{\max}$ and half-life ($t_{1/2}$)) were calculated using Phoenix 6.1 (Pharsight Corporation, Mountain View, CA, USA) for non-compartmental analysis. The maximal plasma concentration ($C_{\max}$) and the time to reach $C_{\max}$ ($t_{\max}$) were identified from the observed values. The area under the plasma concentration–time curve (AUC) from dosing to the last measurable concentration ($AUC(0, t_{\text{last}})$) was estimated using the linear up/log down method. The elimination rate constant (λ_z) was estimated by linear regression of the log-linear portion of the plasma concentration–time curve. $t_{1/2}$ was calculated by $\ln 2/\lambda_z$.

Statistical analysis

The values of pharmacokinetic parameters, except $t_{\max}$, are presented as geometric means with 95% confidence intervals. $t_{\max}$ is given as a median with a range. The pharmacokinetic parameters ($C_{\max}$, $AUC(0, t_{\text{last}})$ and $t_{1/2}$) were logarithmically transformed before statistical analysis. Statistical comparisons were conducted using a mixed model with period and genotype as fixed effects and subject as a random effect. Haemodynamic values (systolic and diastolic blood pressures) were compared using a repeated-measures analysis of variance (ANOVA). P values of less than 0.05 were considered statistically significant. Statistical analysis was performed using SAS 9.2 (SAS Institute, Cary, NC).

Results

Twelve healthy male Korean volunteers participated in the study (six with *SLCO2B1* c.1457C>T *1/*1 and six with *3/*3). Three subjects withdrew consent before the third period (one in the *SLCO2B1* *1/*1 group and two in the *SLCO2B1* *3/*3 group). The systemic exposure of atenolol was inversely proportional to the total amount of apple juice received (Figures 1 and 2, and Table 1).

Genetic variation (*SLCO2B1* *3/*3) did not affect the pharmacokinetic parameters of atenolol in any of the three periods (Figures 2 and 3, Table 1). During period 1 (water phase), lower $C_{\max}$ and $AUC(0, t_{\text{last}})$ were observed in the *3/*3 group, but the difference between the two genotypes was not significant ($P = 0.870$ and 0.909 , respectively). The mean $t_{1/2}$ was 10.2 h in the *3/*3 group, which was similar to that in the *1/*1 group (9.6 h). The median $t_{\max}$ was equal to

Figure 1

Plasma atenolol concentration–time profiles (arithmetic mean $\pm$ standard deviation) after the oral administration of 50 mg atenolol with water or apple juice. (inset shows log-linear scale). $\bullet$, water ($n = 12$); $\square$, apple juice (total 600 ml) ($n = 9$); $\blacktriangle$, apple juice (total 1200 ml) ($n = 12$)

Figure 2

Individual C_{max} (A, C) and $AUC(0,t_{last})$ (B, D) values of atenolol in 12 healthy volunteers according to *SLCO2B1* genotype (*1/*1 A, B and *3/*3 C, D). The volunteers ingested 300 ml soft mineral water, a total of 600 ml, and 1200 ml apple juice with 50 mg atenolol

that observed in the *3/*3 group: 2.5 h during the water period (Table 1). The pharmacokinetic parameters of atenolol exhibited moderate inter-individual variability in that the C_{max} and $AUC(0,t_{last})$ of atenolol ranged from 100 to 400 ng ml⁻¹ and 1500 to 3500 ng ml⁻¹ h, respectively (Figure 2). There were no significant differences in $t_{1/2}$ among periods (Figure 1). Interestingly, there were no remarkable relationships between certain genotypes and systemic exposure to atenolol. Therefore, we chose to explore other common variants, *SLCO2B1* G>A (rs12422149, R312Q) and *SLCO2B1* 282 G>A (rs2712807, -546A/G), alone or in combination. However, there was no meaningful genetic factor that affected the pharmacokinetics of atenolol.

Using the DMET Plus, a commercial SNP chip, pharmacokinetic-related genetic polymorphisms were screened in each participant to identify the effect of other influx or efflux transporters. The nonsynonymous SNPs of several genes demonstrating more than 0.05 minor allele frequencies were studied due to the translation of these genes to transporter proteins, such as OATP1A2, peptide transporter 1 (PEPT1, SLC15A1), apical sodium-dependent bile acid cotransporter (ASBT, SLC10A2) and multi-drug resistance protein 1 (MDR1, ABCB1) [28–31]. However, there was no obvious relationship between these SNPs and atenolol pharmacokinetic parameters, possibly due to the relatively small number of subjects ($n = 12$, Table 2). All subjects were wild-type for

Table 1

Pharmacokinetic variables of a single 50 mg dose of atenolol in 12 healthy volunteers during the water, 1200 ml apple juice and 600 ml apple juice phases

	Water	Apple juice (1200 ml)	Apple juice (600 ml)	Apple juice 1200 ml/water	Geometric mean ratio	
					Apple juice 600 ml/water	Apple juice 1200 ml/ apple juice 600 ml
Total (n = 12)						
t _{max} (h)	2.50 [1.00–4.00]	1.50 [1.00–2.00]	2.00 [1.00–6.00]	–	–	–
C _{max} (ng ml ⁻¹)	216 (183, 254)	44.3 (37.6, 52.2)	68.0 (56.7, 81.5)	0.21 (0.17, 0.25)**	0.32 (0.26, 0.38)**	0.65 (0.54, 0.79)**
AUC(0,t _{last}) (ng ml ⁻¹ h)	2,110 (1,635, 2,723)	389.7 (302.0, 503.0)	885.3 (662.6, 1183)	0.18 (0.13, 0.25)**	0.42 (0.30, 0.59)**	0.44 (0.31, 0.62)**
t _{1/2} (h)	9.93 (7.33, 13.5)	10.5 (7.73, 14.2)	12.8 (9.14, 17.9)	–	–	–
SLCO2B1 *1/*1 (n = 6)						
t _{max} (h)	2.50 [1.00–4.00]	1.50 [1.00–2.00]	2.00 [1.00–4.00]	–	–	–
C _{max} (ng ml ⁻¹)	228 (168, 310)	44.9 (33.1, 61.1)	64.2 (46.7, 88.3)	0.20 (0.15, 0.26)**	0.28 (0.21, 0.38)**	0.70 (0.52, 0.94)*
AUC(0,t _{last}) (ng ml ⁻¹ h)	2,182 (1,568, 3,036)	359.0 (258.0, 499.4)	902.9 (633.7, 1,287)	0.16 (0.11, 0.24)**	0.41 (0.27, 0.63)*	0.40 (0.26, 0.60)**
t _{1/2} (h)	9.64 (6.66, 13.9)	9.48 (6.55, 13.7)	11.6 (7.76, 17.3)	–	–	–
SLCO2B1 *3/*3 (n = 6)						
t _{max} (h)	2.50 [1.50–4.00]	1.50 [1.00–2.00]	2.25 [1.50–6.00]	–	–	–
C _{max} (ng ml ⁻¹)	204 (168, 247)	43.6 (36.0, 52.9)	75.2 (59.4, 95.2)	0.21 (0.16, 0.29)**	0.37 (0.27, 0.51)**	0.58 (0.42, 0.79)*
AUC(0,t _{last}) (ng ml ⁻¹ h)	2,040 (1,284, 3,241)	423.1 (266.2, 672.2)	859.1 (493.3, 1496)	0.21 (0.11, 0.38)**	0.42 (0.21, 0.83)*	0.49 (0.25, 0.97)*
t _{1/2} (h)	10.2 (5.78, 18.1)	11.5 (6.52, 20.4)	14.1 (7.43, 26.7)	–	–	–

The data are presented as the geometric mean (95% confidence interval) except for t_{max} , which is given as the median [minimum–maximum].

Apple juice 600 ml: In total, nine subjects completed the study. (*SLCO2B1* *1/*1 (n = 5), *SLCO2B1* *3/*3 (n = 4)).

* $P < 0.05$. ** $P < 0.001$.

Figure 3

Geometric mean ratios and 95% confidence intervals (*SLCO2B1* *3/*3 to *1/*1) for C_{max} and AUC(0, t_{last}) of atenolol after the ingestion of water, 600 ml apple juice, and 1200 ml apple juice

OATP1A2, based on an analysis of the *SLCO1A2* *2, *3, *5, *6, *7 variants, and two more nonsynonymous SNPs (data not shown) [23].

Systolic and diastolic blood pressures decreased with treatment and recovered to pre-dose levels approximately 24 h after drug treatment was discontinued. When comparing each period using repeated-measures analysis of variance (ANOVA), apple juice had an effect on systolic blood pressure but not on diastolic blood pressure. For systolic blood pressure, the difference between water and apple juice 1200 ml phases was only 2.8 mmHg. It was therefore considered not to have clinical meaning

(Figure 4). Treatment-related adverse events were all mild in intensity, with diarrhoea being the most frequently reported (12 of 33 total adverse events).

Discussion

Membrane transporters, which are mainly expressed in the intestines, can affect the uptake process of many endogenous and exogenous anions, including many small molecular drugs. Because the transport of β -adrenoceptor blockers across the intestinal epithelium may be mediated by the solute carrier OATP2B1, we evaluated the effect of apple juice and genetic polymorphisms of *SLCO2B1*, the gene that encodes OATP2B1 [18, 24]. In a previous study, the ingestion of orange juice three times a day for 4 days decreased the systemic exposure to atenolol by 40% compared with administration of the drug with water [32]. In the present study, the ingestion of 600 ml and 1200 ml apple juice reduced the systemic exposure to atenolol in a dose-dependent manner. To the best of our knowledge, this is the first study to report that the AUC of atenolol decreased by 82% after the ingestion of 1200 ml apple juice. Due to the apple juice–atenolol interaction observed in patients consuming either 600 or 1200 ml apple juice, no obvious changes in pharmacodynamic outcomes were observed in healthy volunteers.

With minimal biotransformation and without prolonging the $t_{1/2}$ of atenolol, apple juice seems to affect intestinal drug uptake rather than the efflux transporters [28]. Although fruit juices are known to be associated with

Table 2
SLC15A1, *SLC10A2* and *ABCB1* genotypes of the volunteers

Participant	rs2274828 c.1348G>A p.Val450Ile	<i>SLC15A1</i> rs4646227 c.1256G>C p.Gly419Ala	rs2297322 c.350G>A p.Ser117Asn	<i>SLC10A2</i> rs188096 c.511T>G p.Ala171=	<i>ABCB1</i> rs2032582 c.2677T>G p.Ser893Ala
R002	G/G	G/G	A/A	G/T	G/G
R003	G/G	C/G	G/G	G/T	G/G
R004	A/G	G/G	A/G	G/T	G/T
R005	G/G	G/G	G/G	G/T	G/G
R006	G/G	G/G	A/G	G/G	T/T
R007	A/G	G/G	G/G	G/G	G/T
R008	G/G	G/G	A/G	G/G	G/G
R010	G/G	G/G	G/G	G/T	G/T
R011	G/G	G/G	A/G	G/T	G/G
R012	G/G	G/G	G/G	G/G	No
R013	G/G	G/G	A/G	G/T	G/T
R014	G/G	G/G	G/G	G/T	G/T

diminished oral bioavailability through the inhibition of intestinal uptake transporters, there was no obvious effect of *SLCO2B1* genotypes on the pharmacokinetics of atenolol, in contrast to the results reported for celiprolol and fexofenadine [17, 18]. Therefore, we expect that the minimal effect of *SLCO2B1* on transport activity was due to the substrate specificity of OATP2B1. One possible mechanism of the reduced exposure to atenolol is a potential indirect effect on OATP function resulting from enhanced intestinal fluid volume due to the non-specific osmotic effects of solutes in apple juice [11]. The pharmacokinetic parameters of atenolol in the apple juice phase displayed less inter-individual variability than those in the water phase, regardless of the *SLCO2B1* genotype that was present. The temporary ingestion of excessive amounts of sugar water may alter OATP function or the acidity of the gastrointestinal environment, which can influence the absorption of a drug [24]. Therefore, further evaluation regarding the effects of variable pH and the amount of apple juice ingested on the transport activity of OATPs *ex vivo* is needed to confirm this hypothesis.

In previous studies, systemic exposure to aliskiren, fexofenadine and montelukast, which are substrates of OATP2B1 or other uptake transporters, were decreased by the ingestion of grapefruit juice and apple juice [10, 33, 34]. The molecular weight of atenolol (266.3) is less than that of fexofenadine (501.7), aliskiren (551.8) and montelukast (586.2). The smaller size would allow for paracellular absorption and transport through other membrane carriers as well [35].

Another possible mechanism for decreased atenolol concentrations with the co-administration of apple juice relies on the indirect effect on uptake transporters mediated by the apple juice itself. For example, atenolol may form a molecular complex with apple juice in the intestine

[11, 36]. In the present study we conducted a candidate gene approach as part of a pharmacogenomic study to examine the effect of *SLCO2B1* c.1457C>T and apple juice on the pharmacokinetics of atenolol. Further research is needed to confirm which transporters are related to the absorption of atenolol.

Decreases in mean systolic and diastolic blood pressures were observed at all time points after atenolol administration. The intervals of change did not differ significantly among the various treatment conditions. Notably, the results of this study are limited by the relatively small sample size and all participating subjects were healthy volunteers.

In conclusion, our results indicate that apple juice ingestion markedly reduced the systemic exposure to atenolol, but genetic variations in *SLCO2B1* are unlikely to contribute substantial variability to the pharmacokinetics of atenolol. Further research is needed to identify the specific mechanism relating apple juice ingestion and its influence on the systemic exposure to atenolol.

Competing Interests

The authors do not have any conflicts of interest to disclose.

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Figure 4

Mean systolic (A) and diastolic (B) blood pressure (mmHg) after atenolol administration. —●—, water; —■—, apple juice 600 ml; —▲—, apple juice 1200 ml

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