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의학석사 논문

The Effect of Apple Juice and OATP2B1 Polymorphism on the Pharmacokinetics and Tolerability of Atenolol in Healthy Volunteers

정상인 자원자에서 사과주스와 OATP2B1의 유전적 다형성이 아테놀올의 약동학적 특성과 내약성에 미치는 영향에 관한 연구

2012년 8월

서울대학교 대학원

임상약리학 전공

전 혜 원

Abstract

The Effect of Apple Juice and OATP2B1 Polymorphism on the Pharmacokinetics and Tolerability of Atenolol in Healthy Volunteers

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Introduction: Fruit juice reduces the plasma concentrations of several beta-blockers likely by inhibiting their OATP2B1-mediated intestinal absorption. The aim of this study was to investigate the effects of apple juice on the pharmacokinetics of atenolol. Twelve healthy Korean volunteers were enrolled in this study based on the genotype of *SLCO2B1* c.1457C> T (*1/*1 (n=6) and *3/*3 (n=6)).

Methods: In a three-phase, one-sequence cross-over study, the pharmacokinetics (PK) of atenolol was evaluated after administration

of 50 mg of atenolol. Each subject received atenolol with 300 mL of water, 1,200 mL and 600 mL of apple juice. Apple juice markedly reduced the systemic exposure of atenolol.

Results: The geometric mean ratios (95% confidence intervals) of apple juice to water phase for AUC_{last} were 0.18 (0.13–0.25, 1,200 mL) and 0.42 (0.30–0.59, 600 mL). In this study, the PK parameters of atenolol responded in a dose–dependent manner to apple juice.

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

Keyword: *apple juice, atenolol, pharmacokinetics, SLCO2B1*

Student number: 2010–21891

Contents

List of Tables

List of Figures

List of abbreviations and symbols

AE	Adverse event
ANOVA	Analysis of variance
AUC	Area under the concentration–time curve
AUC_{last}	AUC from drug administration to the last quantifiable
AUC_{inf}	AUC from the drug admnistration to time infinity
CI	Confidence interval
C_{max}	Maximum observed plasma concentration
CL	Clearance
CL/F	Apparent clearance
DBP	Diastolic blood pressure
ECG	Electrocardiogram
LC–MS/MS	Liquid chromatography–tandem mass spectrometry
OATP	Organic anion–transporting polypeptide
PK	Pharmacokinetics
PD	Pharmacodynamics
SD	Standard deviation

SNP	Single nucleotide polymorphism
LLOQ	Lower limit of quantification
SBP	Systolic Blood Pressure
t_{max}	Observed Time of maximum plasma concentration
$t_{1/2}$	Terminal elimination half-life

INTRODUCTION

Atenolol is a selective beta-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 of hypertension, beta-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]. (Figure 1) Pharmacokinetic parameters of atenolol show 26-38% inter-individual variability [8].

Organic anion-transporting polypeptide (OATP) 2B1 and OATP1A2 are influx transporters located in the luminal membrane of small intestine enterocytes [9]. Fruit juices may decrease 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 bioavailability of celiprolol, in part, due to the OATP2B1-mediated absorption process [17, 18]. At present, OATP2B1 and OATP1A2 are seemed to be related to this interaction. [19]. Recently, genetic variants with nonsynonymous single nucleotide polymorphisms (SNPs), *SLCO2B1* *3 (Ser→Phe (c.1457C>T)) has 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 SLC2B1*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]. 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.

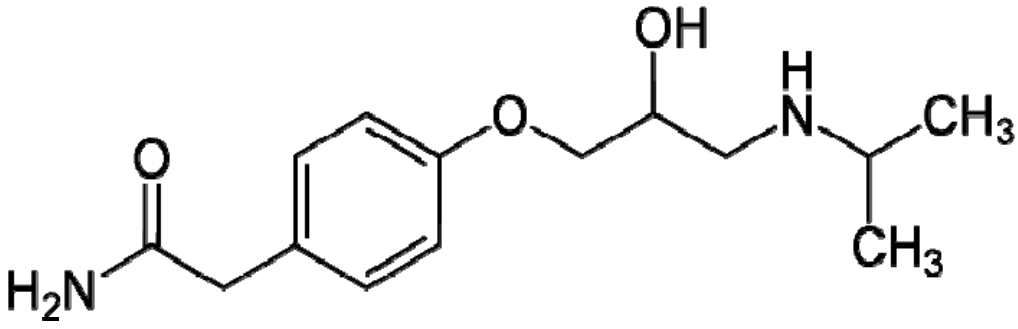

Figure 1 Structure of Atenolol

MATERIALS AND METHODS

Subjects

Korean male volunteers aged 20 to 50 years with body mass indexes 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 of atenolol was administered with 300 mL of soft mineral water. In period 2, the drug was taken with 300 mL of apple juice, and, additionally, the participants took 150 mL of apple juice every 0.5 hours for 3 hours (a total of 1,200 mL) which was identical to the previous juice–drug interaction study [11]. In period 3, 300 mL of apple juice was administered as in period 2, and the participants also received 100 mL every 0.5 hours for 1.5 hours (a total of 600 mL). Each period was separated by a minimum 7–day wash–out interval. For 4 hours 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 hours after dosing. Each sample was collected in a heparinized tube, centrifuged at 2,000 *g* for 10 minutes and stored below −70℃ 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 minutes 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) and the allele detection of 3 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 which is useful to detect genetic biomarker candidates, determine drug responders [23]. The polymorphisms were chosen for the panel as known drug metabolizing enzymes and transporters. DMET plus chip utilizes

molecular-inversion probe technology to develop a multiplex genotyping assay that can simultaneously test more than 1,936 markers in 225 genes of drug metabolizing enzymes and transporters [24, 25].

Quantification of plasma concentration of atenolol

Plasma atenolol concentration was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The high performance liquid chromatography (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 μm analytical column (100 × 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→145.100 for atenolol and m/z 260.190→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. The solutions were diluted and used to spike blank matrices to obtain calibration standard solutions. The standard curve for atenolol was 5 – 2,000 ng/mL, 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 of plasma. After centrifugation for 5 minutes at 9,900 *g*, 4 μL of supernatant was injected into the LC–MS/MS. The concentrations were quantified by the internal standard method. The calibration curve was obtained using a 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. The accuracy of the analysis ranged from 99 – 102%, and the precision was lower than 5%.

Pharmacokinetic analysis

Pharmacokinetic parameters (C_{max}, AUC_{last}, T_{max} and half-life) were calculated using Phoenix 6.1 (Pharsight Corporation, Mountain View,

CA, USA) by noncomparmetal 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_{last}) was estimated using linear up/log down method. The elimination rate constant (k_e) was estimated by linear regression of the log–linear portion of the plasma concentration–time curve. The half–life ($t_{1/2}$) was calculated by ln 2/ k_e.

Statistical analysis

The values of pharmacokinetic parameters, except T_{max}, were presented as geometric means with 95% confidence intervals. The T_{max} was given as a median with a range. The pharmacokinetic parameters (C_{max}, AUC_{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. Hemodynamic values (systolic and diastolic blood pressures) were compared using 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).

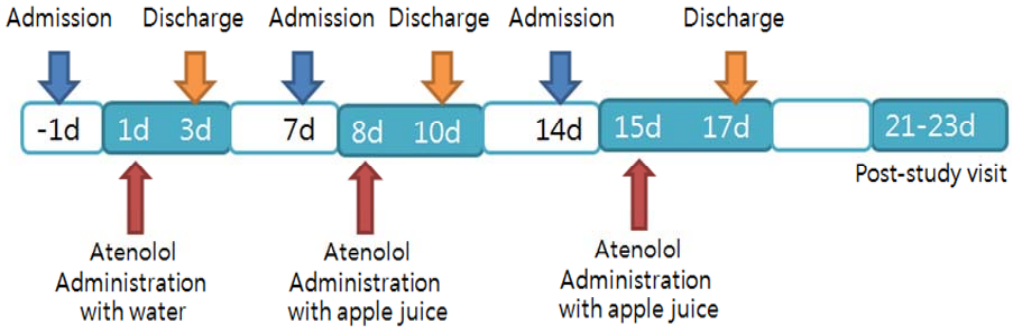

Figure 2 Study design

RESULTS

Pharmacokinetics

Twelve healthy male Korean volunteers participated the study (6 with *SLCO2B1* c.1457C> T *1/*1 and 6 with *3/*3). Three subjects withdrew consent before the third period (1 in the *SLCO2B1* *1/*1 group and 2 in the *SLCO2B1* *3/*3 group). The systemic exposure of atenolol was inversely proportionate to the total amount of apple juice received (Figures 3 and 4, and Table 1).

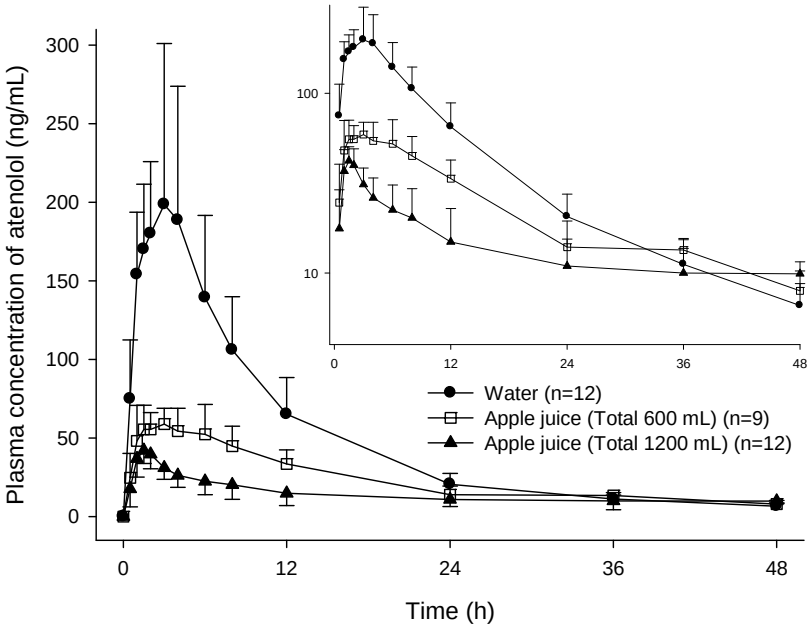

Figure 3 Plasma atenolol concentration-time profiles (arithmetic mean ± standard deviation) after oral administration of 50 mg atenolol with water or apple juice. (inset shows log-linear scale)

Table 1 Pharmacokinetic variables of a single 50 mg dose of atenolol in 12 healthy volunteers during the water, 1,200 mL apple juice, and 600 mL apple juice phases

	Water	Apple juice (1,200 mL)	Apple juice (600 mL)	Geometric mean ratio		
				Apple juice 1,200 mL/ Water	Apple juice 600 mL/ Water	Apple juice 1,200 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)	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_{last} (ng*h/mL)	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)	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_{last} (ng*h/mL)	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	9.48	11.6	-	-	-

	(6.66, 13.9)	(6.55, 13.7)	(7.76, 17.3)			
SLCO2B1 *3/*3 (n=6)						
T_{max} (h)	2.50 [1.50-4.00]	1.50 [1.00-2.00]	2.30 [1.50-6.00]	-	-	-
C_{max} (ng/mL)	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_{last} (ng*h/mL)	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, 9 subjects completed the study. (*SLCO2B1* *1/*1 (n=5), *SLCO2B1* *3/*3 (n=4))

* $P < 0.05$

** $P < 0.001$

The effect of *SLCO2B1* polymorphism on the pharmacokinetics of atenolol

Genetic variation (*SLCO2B1 *3/*3*) did not affect the pharmacokinetic parameters of atenolol in any of the 3 periods (Figures 4 and 5, Table 1). In period 1 (water phase), lower C_{max} and AUC_{last} were seen in *3/*3 group, but the difference between the two genotypes was not significant (P=0.870 and 0.909, respectively). The mean half-life was 10.2 hours in *3/*3 group, which was similar to that in *1/*1 group (9.6 hours), and that of median T_{max} was equal to the *3/*3 group as 2.5 hours in water period (Table 1). The pharmacokinetic parameters of atenolol exhibited moderate inter-individual variability in that C_{max} and AUC_{last} of atenolol ranged from 100 to 400 ng/mL and 1,500 to 3,500 ng*hr/mL, respectively (Figure 4). There were no significant differences in half-lives among periods (Figure 3). Interestingly, there were no remarkable relationships between genetic variations and systemic exposure of 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. Haplotype analysis included the SNPs *SLCO2B1* 1457 C>T, 935 G>A, 282 G>A on the basis of the linkage disequilibrium observed between position,

possible functional relevance from 48 Korean population data. There was negligible linkage disequilibrium and one possible block was detected which could occur over 5% (Figure 6, Table 2) However, there were no meaningful genetic factors which affected the pharmacokinetics of atenolol. We compared possible wild haplotype carrier (GGC) or variant haplotype carrier (AAG) between C_{max} and AUC_{last}, no obvious relationship was identifed. (Figure 7)

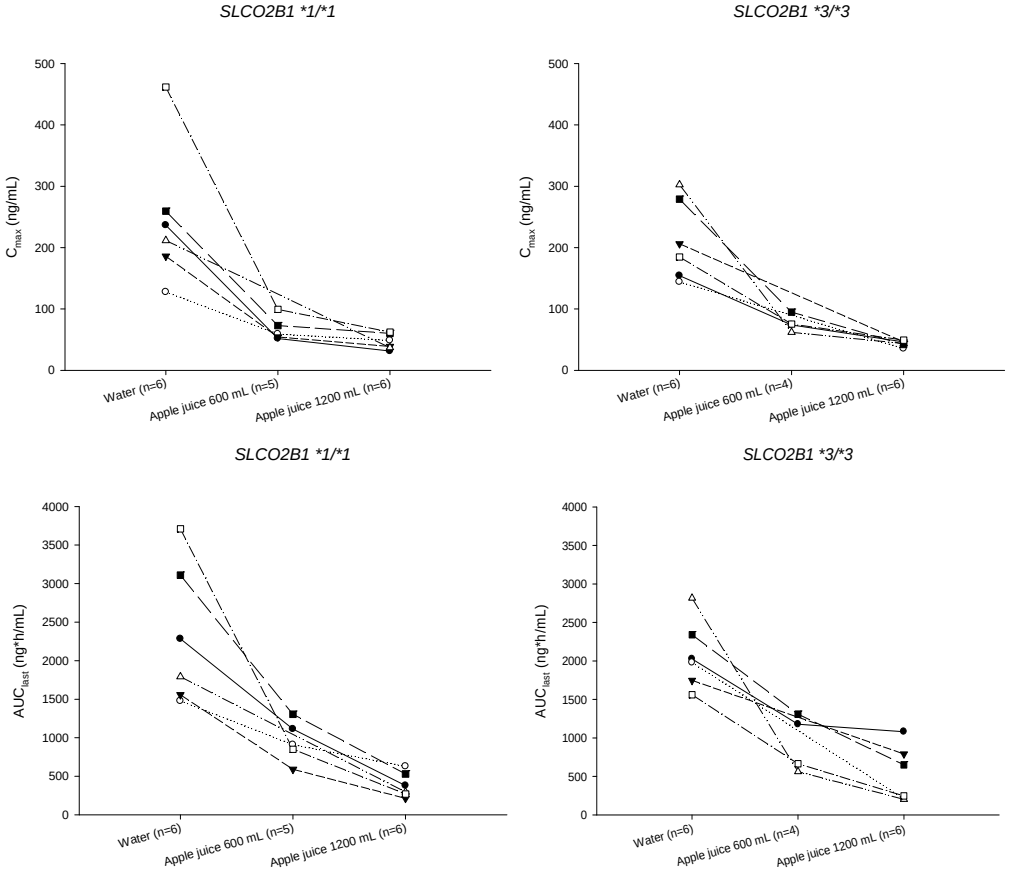

Figure 4 Individual C_{max} (upper panel) and AUC_{last} (lower panel) values of atenolol in 12 healthy volunteers according to the *SLCO2B1* genotype The volunteers ingested 300 mL of soft mineral water, a total of 600 mL, and 1,200 mL of apple juice with 50 mg of atenolol

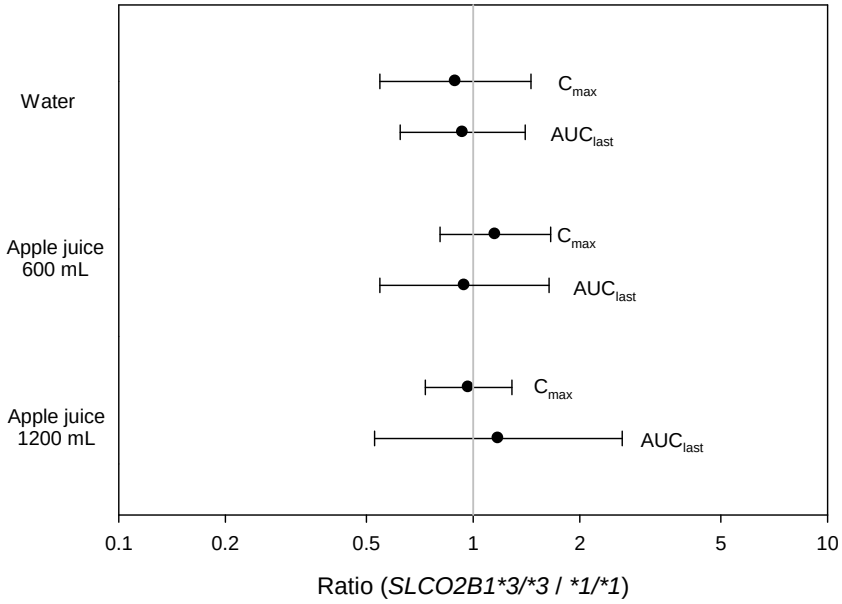

Figure 5 Geometric mean ratios and 95% confidence intervals (*SLCO2B1*3/*3* to **1/*1*) for C_{max} and AUC_{last} of atenolol after ingestion of water, 600 mL of apple juice, and 1,200 mL of apple juice

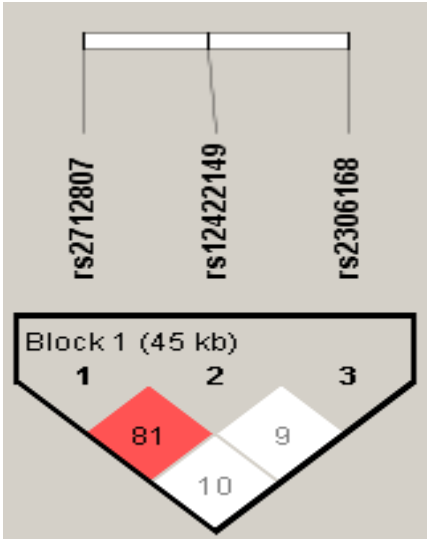

Figure 6 Haplotype analysis included the SNPs *SLCO2B1* 1457 C>T, 935 G>A, 282 G>A on the basis of the linkage disequilibrium observed between position, possible functional relevance (nonsynonymous, promoter region) from 48 Korean population data

Table 2 Possible haplotypes and frequencies of *SLCO2B1*

SLCO2B1 haplotypes	
Haplotype	Frequency
AAC	0.272
AGC	0.208
GGC	0.185
GGT	0.128
AAT	0.108
AAT	0.071

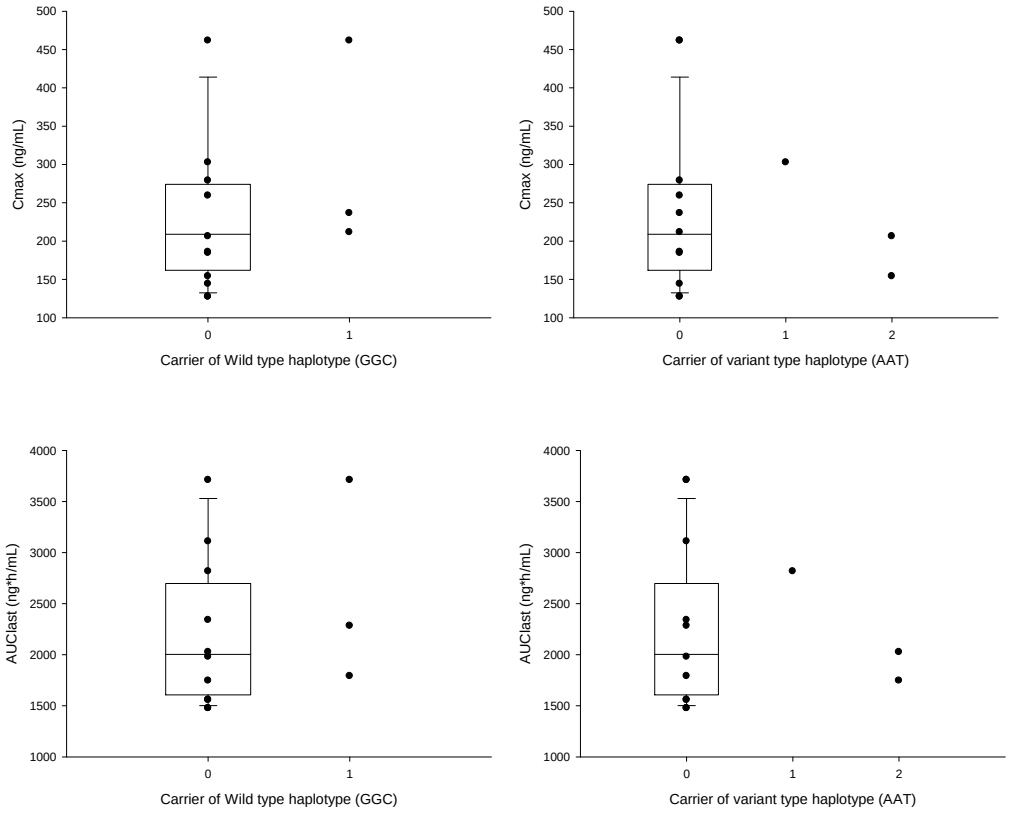

Figure 7 Individual C_{max} (upeer panel) and AUC_{last} (lower panel) values of atenolol in 12 healthy volunteers according to the number of *SLCO2B1* haplotypes. The wild type haplotype was GGC and the variant was AAT

The effect of other polymorphisms on the pharmacokinetics of atenolol

Using the commercial SNP chip, the DMET Plus, pharmacokinetics-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 chosen for study because of the translation of these genes to transporter proteins, such as OATP1A2, peptide transporter 1 (*PEPT1, SLC15A1*), apical sodium-dependent bile acid cotransporter (*ASBT, SLCO10A2*) and multidrug resistance protein 1 (*MDR1, ABCB1*).[26-29] However, there was no obvious relationship between these SNPs and atenolol pharmacokinetic parameters, possibly due to the relatively small numbers of subjects (n=12, Table 3). Genotypes of all of the subjects were determined as wild-type in OATP1A2 (Table 4).

Table 3 *SLCO2B1, SLC15A1, SLC10A2* and *ABCB1* genotypes of the volunteers

	SLCO2B1			*SLC15A1*			*SLC 10A2*	*ABCB1*
	c.1457C>T	935 G>A	−282 G>A	rs2274828	rs4646227	rs2297322	rs188096	rs2032582
	p.Ser486Phe	p.Arg312Gln	−	c.1348G>A	c.1256G>C	c.350G>A	c.511T>G	c.2677T>G
	rs2306168	rs12422149	rs2712807	p.Val450Ile	p.Gly419Ala	p.Ser117Asn	p.Ala171=	p.Ser893Ala
R002	C/C	G/A	G/A	G/G	G/G	A/A	G/T	G/G
R003	C/C	G/G	A/A	G/G	C/G	G/G	G/T	G/G
R004	C/C	G/G	A/A	A/G	G/G	A/G	G/T	G/T
R005	C/C	G/A	G/A	G/G	G/G	G/G	G/T	G/G
R006	C/C	G/A	A/A	G/G	G/G	A/G	G/G	T/T
R007	C/C	G/G	G/A	A/G	G/G	G/G	G/G	G/T
R008	T/T	A/A	A/A	G/G	G/G	A/G	G/G	G/G
R010	T/T	G/G	G/G	G/G	G/G	G/G	G/T	G/T

R011	T/T	A/A	A/A	G/G	G/G	A/G	G/T	G/G
R012	T/T	G/A	G/A	G/G	G/G	G/G	G/G	No
R013	T/T	G/G	A/A	G/G	G/G	A/G	G/T	G/T
R014	T/T	G/G	G/G	G/G	G/G	G/G	G/T	G/T

Table 4 *SLC15A1, SLC10A2* and *ABCB1* genotypes of the volunteers

ID	*SLCO 2B1*				*SLCO1A2*			
	c.1457C>T	c.38T>C	c.516A>C	c.1063A>G	c.968T>C	c.841A>G	c.502C>T	c.382A>T
	p.Ser486Phe	p.Ile13Thr	p.Glu172Asp	p.Ile355Val	p.Leu323Pro	p.Ile281Val	p.Arg168Cys	p.Asn128Tyr
	rs2306168	rs10841795	rs11568563	rs45628437	rs11568579	rs11568551	rs11568564	rs11568567
R002	C/C	T/T	A/A	A/A	T/T	A/A	C/C	A/A
R003	C/C	T/T	A/A	A/A	T/T	A/A	C/C	A/A
R004	C/C	T/T	A/A	A/A	T/T	A/A	C/C	A/A
R005	C/C	T/T	A/A	A/A	T/T	A/A	C/C	A/A
R006	C/C	T/T	A/A	A/A	T/T	A/A	C/C	A/A
R007	C/C	T/T	A/A	A/A	T/T	A/A	C/C	A/A
R008	T/T	T/T	A/A	A/A	T/T	A/A	C/C	A/A
R010	T/T	T/T	A/A	A/A	T/T	A/A	C/C	A/A

R011	T/T	T/T	A/A	A/A	T/T	A/A	C/C	A/A
R012	T/T	T/T	A/A	A/A	T/T	A/A	C/C	A/A
R013	T/T	T/T	A/A	A/A	T/T	A/A	C/C	A/A
R014	T/T	T/T	A/A	A/A	T/T	A/A	C/C	A/A

Pharmacodynamics

Systolic and diastolic blood pressures decreased with treatment and recovered to pre-dose levels approximately 24 hours after drug administration was discontinued. When comparing each period using repeated measures analysis of variance (ANOVA), apple juice had effect on systolic blood pressure but not diastolic blood pressure. For systolic blood pressure, the difference between water and apple juice 1,200 mL phases was only 2.8 mmHg, therefore it was considered not to have clinical meaning. (Figure 8).

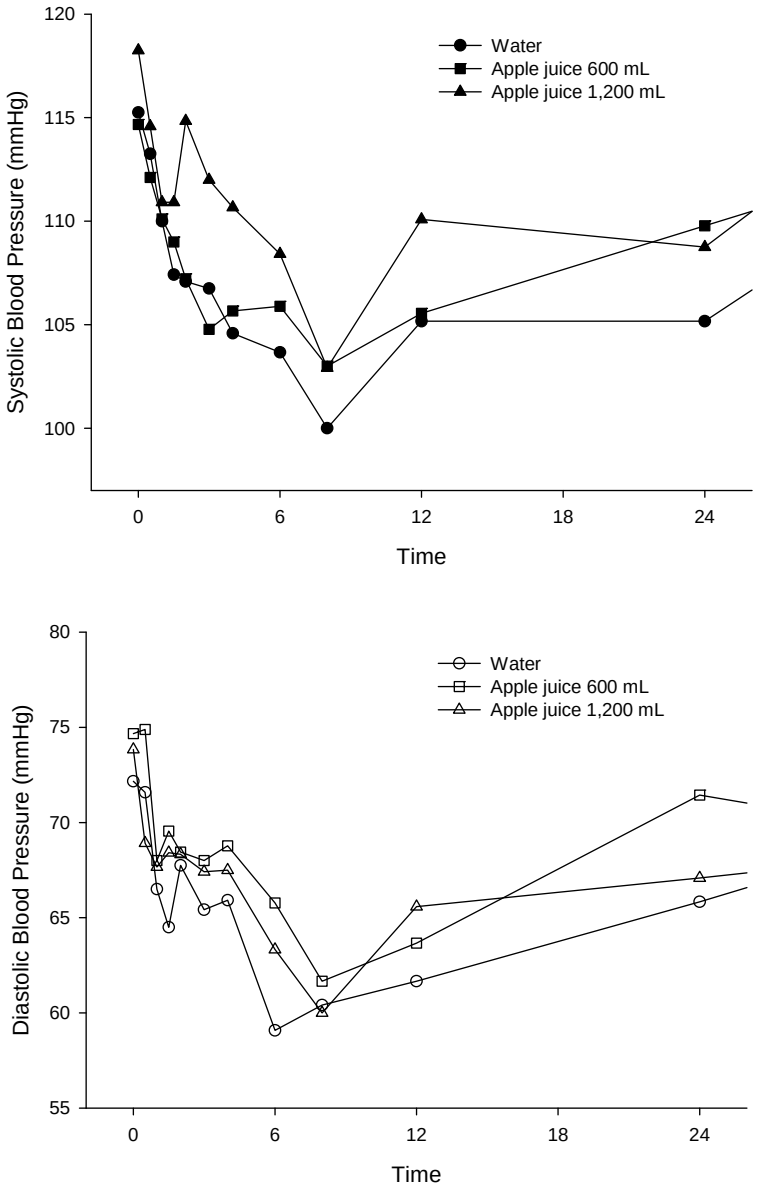

Figure 8 Mean systolic (upper panel) and diastolic (lower panel) blood pressure (mmHg) after atenolol administration

Tolerability

A total of 33 adverse events (AEs) were reported in 12 volunteers. (Table 5) Treatment-related adverse events were all mild in intensity, with diarrhoea being the most frequently reported (12 of 26 total adverse events).

Table 5 Adverse events (AEs) reported after atenolol administration, The number in parentheses is the number of AEs judged to be related to the study drug

	Water (n=12)	Apple juice (1,200 mL, n=12)	Apple juice (600 mL, n=9)
Number of AEs	4 (4)	27 (20)	2 (2)
Gastrointestinal disorders			
Abdominal distension	.	3 (3)	.
Abdominal pain	.	1 (1)	.
Diarrhoea	1 (1)	9 (8)	2 (2)
Dyspepsia	.	1 (1)	.
Flatulence	.	3 (3)	.
Nausea	.	1 (1)	.
General disorders and administration site conditions			
Feeling abnormal	1 (1)	.	.
Feeling hot	.	1 (1)	.
Hyperhidrosis	.	1 (1)	.
Pain	.	1 (0)	.
Pyrexia	.	1 (0)	.
Respiratory, thoracic and mediastinal disorders			
Cough	.	1 (0)	.
Oropharyngeal pain	.	1 (0)	.
Rhinorrhoea	.	1 (0)	.
Nervous system disorders			

Headache	1 (1)	1 (1)	.
Migraine	1 (1)	.	.

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 beta-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, 30]. In a previous study, ingestion of orange juice three times a day for 4 days decreased the systemic exposure of atenolol by 40% compared to administration of the drug with water [31]. In the present study, ingestion of 600 mL and 1,200 mL of apple juice reduced the systemic exposure of 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 1,200 mL of apple juice ingestion. Because of the great extent of the apple juice-atenolol interaction in both 600 and 1,200 mL of apple juice and a wide use of apple juice, although no obvious changes in pharmacodynamic outcomes were observed in healthy volunteers.

With minimal biotransformation and without prolonging the half-life of atenolol, the apple juice seems to affect the intestinal drug uptake rather than the efflux transporters [26]. Although fruit juices are known to be associated with diminished oral bioavailability through inhibition of intestinal uptake transporters, there was no obvious effect of *SLCO2B1* genotypes on pharmacokinetics of atenolol unlike those of celiprolol and fexofenadine [17, 18]. Therefore we estimated minimal effect of *SLCO2B1* on the transporting activity was observed probably by substrate specificity of OATP2B1. One possible mechanism of the reduced exposure of atenolol is a potential indirect effect on OATP function resulting from enhanced intestinal fluid volume by the nonspecific osmotic effects of solutes in apple juice [11]. Also, pharmacokinetic parameters of atenolol in the apple juice phase represented smaller 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 the OATP function or the acidity of the gastrointestinal environment, which can influence the absorption of a drug [30]. Therefore, further evaluation regarding the effects of variable pH and the amount of apple juice ingested on the transporting activity of OATPs in *ex vivo* is

needed to confirm this hypothesis.

In previous studies, systemic exposures of aliskiren, fexofenadine and montelukast, which are substrates of OATP2B1 or other uptake transporters, were decreased by grapefruit juice and apple juice ingestion [10, 32, 33]. The molecular weight of atenolol (266.3) is smaller than that of fexofenadine (501.7), aliskiren (551.8) and montelukast (586.2). The smaller size would allow paracellular absorption and transport through other membrane carriers as well [34]. Another possible mechanism for decreased atenolol concentrations with co-administration of apple juice relies on the indirect effect on uptake transporters caused by the apple juice itself. For example, the unexpected formation of a molecular complex with atenolol in the intestine might occur [11, 35].

Decreases in mean systolic and diastolic blood pressures were observed in all periods after atenolol administration. The intervals of change among the periods did not show significant differences. The result of this study is 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 of atenolol, but genetic variations in

SLCO2B1 are unlikely to contribute substantial variability in the pharmacokinetics of atenolol. Further research is needed to identify the specific mechanism relating apple juice ingestion and its influence on the systemic exposure of atenolol.

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Abstract in Korean

정상인 자원자에서 사과주스와 OATP2B1의 유전적 다형성이 아테놀올의 약동학적 특성과 내약성에 미치는 영향에 관한 연구

서론: 사과주스는 여러 베타 차단제의 혈중 약물농도를 감소시키며, 사과주스의 OATP2B1 수송체를 매개하는 위장관 흡수의 억제가 이러한 감소의 기전으로 보고되었다. 본 연구는 사과주스가 아테놀올의 약동학에 미치는 영향을 탐구하는 것을 목적으로 하였다.

방법: 건강한 한국인 자원자를 대상으로 유전형을 분석하였으며, *SLCO2B1* c.1457C> T 단일염기변이에 대하여 총 12명 (*1/*1 6 명, *3/*3 6 명)이 참여하였다. 본 연구는 3-시기, 단일 순서군, 교차 설계로, 각 시기별로 아테놀올 50 mg 투여 후 아테놀올의 약동학적 특성 및 내약성을 평가하였다. 피험자들은 투약시 물 300 mL 또는 사과주스 1200 mL 또는 600 mL 를 섭취하였다.

결과: 아테놀올의 시간-농도 곡선하 면적 (AUC_{last})의 경우 물 300 mL 를 투여했을 때와 비교시 기하평균비 (95% 신뢰구간)는 각각 사과주스 1200 mL 투여시 0.18 (0.13-0.25), 600 mL 투여시 0.42 (0.30-0.59) 였다.

결론: 아테놀올의 약동학 파라미터는 사과주스 투여량에 비례하여 감소하는

양상이었다. 사과주스는 아테놀올의 체내 노출을 현저하게 감소시켰으며, *SLCO2B1* c.1457C> T 의 유전형이 아테놀올의 약동학적 특성 및 내약성에 미치는 영향은 미미하였다.

중심단어: 사과주스, 아테놀올, 약동학, SLCO2B1

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