의학박사 학위논문

Aspirin decreases the systemic exposure to clopidogrel through modulation of P-glycoprotein, but does not alter its antithrombotic activity

아스피린 투약에 의한 P-당단백질 조절을 통한 클로피도그렐 전신약물노출 감소와 항혈전 효과 유지에 관한 연구

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서울대학교 대학원

의과학과 의과학전공

신 동 훈

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이 논문을 의학박사 학위논문으로 제출함

2014년 10월

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ABSTRACT

Introduction: Decreased oral clopidogrel absorption caused by the induction in the intestinal P-glycoprotein (P-gp) expression after aspirin administration was observed in animal models. This study was to evaluate the influence of aspirin co-administration on the pharmacokinetics and pharmacodynamics of clopidogrel in humans.

Methods: A single dose of clopidogrel 75 mg was orally administered before and after 2 and 4 weeks of once-daily 100 mg aspirin administration in 18 healthy volunteers, who were recruited according to the CYP2C19 and PON1 genotypes to control variability caused by metabolizing enzymes for clopidogrel. Plasma concentrations of clopidogrel, its active metabolite H4 and relative platelet inhibition (RPI) were determined for pharmacokinetic and pharmacodynamic assessment. MDR1 mRNA, miR-27a and miR-16 expression in blood were quantified to assess the effect of aspirin on P-gp expression in the peripheral blood.

Results: The P-gp microRNA mir-27a was increased up to 7.67-fold (P=0.004) and the area under the concentration-time curve (AUC) of clopidogrel was lowered by 14% (P>0.05) but the AUC of H4 was not changed, and RPI was increased up to 15% (P=0.002) after aspirin administrations.

Conclusions: These findings indicate that low dose aspirin co-administration may decrease bioavailability of clopidogrel but it does not decrease its efficacy related pharmacodynamics in healthy subjects.

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Keywords: Clopidogrel, Aspirin, Drug interactions, Pharmacokinetics, P-Glycoprotein

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LIST OF ABBREVIATIONS

ABCB1	ATP-binding cassette transporter, subfamily B, member 1
ADP	Adenosine diphosphate
ASA	Acetylsalicylic acid
ATP	Adenosine triphosphate
AUC	Area under the concentration-time curve
$AUC_{0\text{-}24h}$	AUC from 0 to 24 hours
$AUC_{0\text{-}2h}$	AUC from 0 to 2 hours
CI	Confidence intervals
C_{max}	Maximum concentration
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
EM	Extensive metabolizer
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GMR	Geometric mean ratio
ICH	International Conference on Harmonization
m/z	Charge ratio
MDR1	Multidrug resistance protein 1
miR	microRNA
MPA	Maximum platelet aggregation
MR	Metabolic ratio
MRM	Multiple reaction monitoring

mRNA	Messenger RNA
MS/MS	Tandem mass spectrometric
PCR	Polymerase chain reaction
PD	Pharmacodynamics
P-gp	Permeability glycoprotein
PK	Pharmacokinetics
PM	Poor metabolizer
PON1	Paraoxonase-1
PPP	Platelet poor plasma
PRP	Platelet rich plasma
RNA	Ribo nucleic acid
RPI	Relative platelet inhibition
SD	Standard deviation
SNP	Single nucleotide polymorphism
$t_{1/2}$	Terminal elimination half-life
T_{max}	Time to C_{max}
UPLC	Ultra performance liquid chromatography

INTRODUCTION

Clopidogrel is one of the most commonly used drugs to prevent atherothrombotic events in patients with post myocardial infarction or stroke. Although many patients may get benefit from clopidogrel, up to 30% of patients reported no beneficial effect (1), which is associated with the large inter-individual variability in the pharmacokinetics (PK) of clopidogrel (2-10). Drug transporters may affect the clopidogrel's PK, leading to its large inter-individual variability. Clopidogrel is a substrate of the ATP-binding cassette transporter, subfamily B, member 1 (ABCB1) which is also known as multidrug resistance protein 1 (MDR1) or permeability glycoprotein (P-gp) (9). The oral bioavailability of clopidogrel is therefore decreased by P-gp mediated efflux into the intestinal lumen, which negatively affects its antithrombotic action (9).

Metabolizing enzymes may also contribute to the large inter-individual variability of clopidogrel in its PK and pharmacodynamics (PD). Clopidogrel is absorbed as an inactive prodrug, which is then converted to H4, an active thiol metabolite, by the gut and hepatic metabolizing enzymes. H4 irreversibly binds to the P2Y12 receptor of platelets and inhibits their aggregation induced by adenosine diphosphate (ADP) over the life span of the platelet (11). Bioactivation of absorbed clopidogrel to H4 plays a key role in the PD of clopidogrel, in which various metabolizing enzymes are involved including the cytochrome P450 (CYP) isoenzyme CYP1A2, CYP2C9, CYP2C19, CYP3A4 and paraoxonase-1 (PON1) (2, 4, 6-8, 10, 12, 13), of which

CYP2C19 and CYP3A4 are thought to play the most important role *in vivo* (13-16). Therefore, inhibition of those bioactivation enzymes for clopidogrel results in lower H4 exposures, which can be affected by the genetic variations in those enzymes. Single nucleotide polymorphisms (SNPs) in *CYP2C19*, such as *2 or *3 alleles are nonfunctional, resulting in the lower exposure to H4, thereby a lower antithrombotic effect (4, 6, 8, 10, 12, 17). By contrast, in the study performed by Bouman *et al.*, PON1 SNP Q192R (rs662) was associated with higher PON1 enzyme activity and the higher exposure to H4, leading to a greater antithrombotic effect of clopidogrel (2) although the role of PON1 is still controversial (4, 7, 18, 19).

Drugs co-administered with clopidogrel can also affect its PK and PD. For example, low-dose aspirin enhances the antithrombotic action of clopidogrel by blocking the formation of thromboxane A2, which is why aspirin is frequently co-administered with clopidogrel to prevent thrombotic events.1, 20 However, prolonged use of aspirin induced the expression of P-gp in the rat intestine, resulting in the reduced systemic exposure to clopidogrel.5 Aspirin also induced *in vitro* P-gp expression in humans such as epithelial colorectal cells5, prostate cancer cells21 and T lymphoma cells22, and the antithrombotic effect of clopidogrel may be offset by co-administered aspirin.

Based on these understandings, the objective of the present study was to investigate the effect of co-administered aspirin on the systemic exposure of clopidogrel and its active metabolite and its antithrombotic effect in humans. To this end, the PK of clopidogrel and its active metabolite, and platelet aggregation were compared between before, 2 and 4 weeks after once-daily

aspirin was administration in 18 healthy male volunteers (**Figure 1**). The usual daily maintenance doses were used in the present study, i.e., 75 mg and 100 mg for clopidogrel and aspirin, respectively, to reproduce interaction in the typical clinical setting. Furthermore, to control variability caused by individual's genotypes in the metabolizing enzymes for clopidogrel, the same number of subjects were recruited by CYP2C19 and PON1 genotype groups.

MATERIALS AND METHODS

Clinical Study Design

An open label, 3-period, single sequence clinical study was performed (**Figure 1**). A day before clopidogrel administration in period 1, subjects were admitted to the Clinical Trials Center at Seoul National University Hospital. After overnight fast, a single dose of 75 mg clopidogrel was orally administered with 250 mL of water. Subjects remain fasted until 4 hours after clopidogrel administration. Serial blood samples for PK and PD were taken over 24 hours and subjects were discharged from the hospital. From the day of discharge, multiple daily doses of 100 mg aspirin were administered for 4 weeks at every morning. At 2 and 4 weeks after aspirin dosing (period 2 and 3 respectively), subjects underwent the same in-hospital procedures as period 1.

For plasma concentration of clopidogrel and its active metabolite (H4) analysis, serial blood samples were collected using a heparinized tube at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h post dose. For analysis of ADP-induced platelet aggregation, blood samples were collected using a tube containing 1/10 volume of 3.2% trisodium citrate at 0, 4, and 12 h post dose. To investigate the effect of aspirin on intestinal P-gp expression, the levels of mRNA and microRNAs (miR-27a, miR-451) were determined in whole blood at 1, 2, 3 and 4 weeks after aspirin administration.

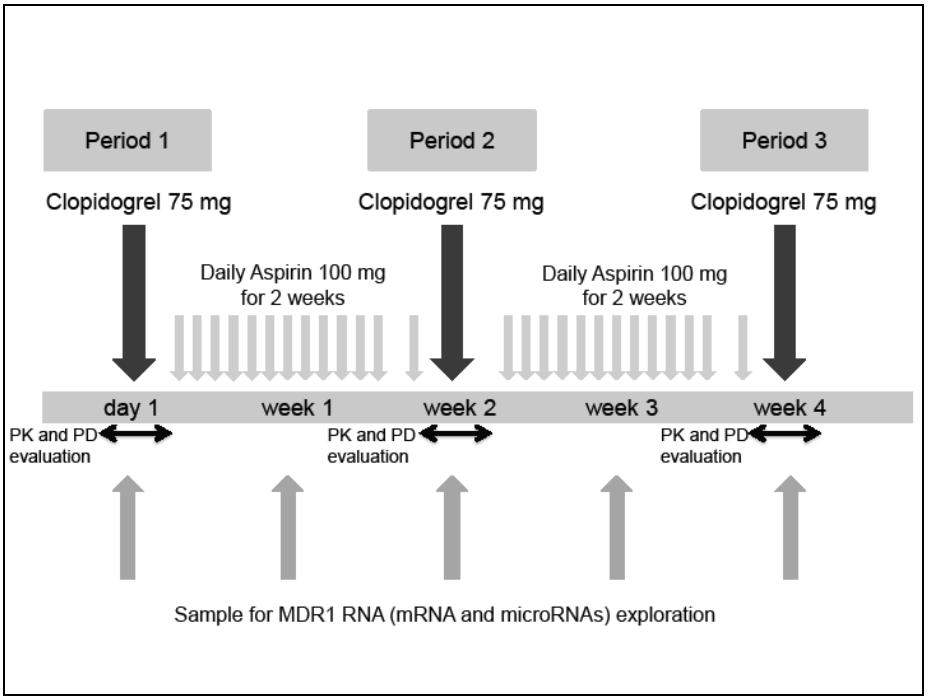

Figure 1. Study flowchart.

This clinical study (NCT01503658) was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, Korea, and conducted accordance with the principles of the Declaration of Helsinki and ICH Good Clinical Practice. Every subjects participated were informed about the study protocol and every possible adverse events before written consent.

Determination of Plasma Concentrations

Plasma concentration of clopidogrel and H4 were determined using the ultra performance liquid chromatography-tandem mass spectrometric (UPLC-MS/MS) method. A 400 μL of plasma sample was spiked with 100 μL of 1% formic acid in water, 30 μL of internal standard (clopidogrel-d4, 3 μg/mL in

50% methanol) and 1.25 mL of ethyl acetate was added into each sample for liquid-liquid extraction. After being vortexed for 10 minutes at 1,500 rpm and centrifuged for 10 minutes at 14,000 rpm, respectively, 1 mL of organic layer was transferred to a polypropylene conical tube and evaporated under nitrogen concentrator for 40 minutes. The residue was reconstituted in 100 μL of 1:1 (v/v) acetonitrile:water and 2 μL was used for analysis. The analytes were separated on a Kinetex C8 (2.6 μm particle size, 2.1 × 100 mm; Phenomenex, CA, USA) at 20℃ under the gradient elution for 4 minutes. The mobile phase consisted of a mixture of 0.1% formic acid in distilled water and 0.1% formic acid in acetonitrile. Detection of precursor to product ion transition was achieved using Xevo TQ (Waters, MA, USA) employing electrospray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The precursor to product ion pairs at mass to charge ratio (*m/z*) were 322.09 to 154.96 for clopidogrel, 504.21 to 155.02 for H4 and 326.09 to 216.06 for clopidogrel-d4. The calibration curves were linear over the range of 0.02 – 10 ng/mL for clopidogrel and 0.1 – 50 ng/mL for H4.

Measurement of Platelet Aggregation

Maximum platelet aggregation (MPA) in response to 5 μM/L of ADP was measured by light transmittance aggregometry using Chrono-log model 700 Aggregometer (Chrono-log, Havertown, PA). Platelet rich plasma (PRP) and platelet poor plasma (PPP) were obtained after differential centrifugation of citrated blood samples. After incubating 495 μL of PRP in the aggregometer

at 37℃ for 10 minutes 5 μL ADP was added. PPP (500 μL) was used as a reference. Platelet aggregation was recorded for 5 minutes and MPA was calculated as the maximal change of light transmission from baseline. Relative platelet inhibition (RPI) was calculated as [(pre-dose MPA on day 1 - MPA at each time point) / pre-dose MPA on day 1] X 100%.

Genotyping and Quantification of mRNA and microRNA

Genotypes of CYP2C19 and PON1 were performed using TaqMan Allelic Discrimination Assays on the AB 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA). Genomic DNA was extracted from 200 μL of peripheral whole blood of each volunteer, using a QIAamp DNA Blood Mini Kit (QIAGEN). A total of 10 μL of PCR reaction mixture was prepared with 5 μL of 2X TaqMan Genotyping Master mix, 0.5 μL of 20X Drug Metabolism Genotyping Assay Mix, 3.5 μL of DNase-free water, and 1 μL of genomic DNA. Among *CYP2C19* SNPs, *CYP2C19*2* G681A (rs 4244285), *CYP2C19*3* G636A (rs4986893) were analyzed because of the rare allele frequency of the other SNPs in Koreans (23). Genotyping of *CYP2C19* SNPs and *PON1* Q192R (rs662) SNP were performed with validated TaqMan genotyping assays purchased from Applied Biosystems. PCR reactions were carried out as follows: initial denaturation at 95℃ for 10 min, and 50 cycles on denaturation at 92℃ for 15 seconds and anneal/extension at 60℃ for 1 min. The allelic discrimination results were determined after the amplification by performing an end - point read. 7500 Real-Time PCR System software

version 2.0.1 and 2.0.6 (Applied Biosystems, Foster City, CA, USA) were used for the analysis.

For quantitative analysis of MDR1 mRNA expression in blood, total RNA was extracted from whole blood using PAXgene TM RNA Kit (PreAnalytiX, Hombrechtikon, Switzerland) according to the manufacturer's instructions. The complementary DNA was synthesized from 1 μg of total RNA by QuantiTect Rev. Transcription Kit (Qiagen, Hilden, Germany). The 20 μL PCR reaction contained 10 μL QuantiTect SYBR Green PCR Master Mix (Qiagen), 6 μL nuclease-free water, 2 μL 10X QuantiTect Primer assay for *MDR1* gene (Qiagen) and 2 μL cDNA template. Amplification was performed by CFX96TM Real-Time System (Bio-Rad, CA, USA) under the following conditions: initial denaturation at 95℃ for 15 minutes, and 40 cycles of denaturation for 15 seconds at 94℃, annealing for 30 seconds at 55℃, and elongation for 30 seconds at 70℃. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression was used as an internal control.

For quantitative analysis of miR-27a and miR-16 expression in serum, total RNA was extracted by miRNeasy mini kit (Qiagen) according to the manufacturer's instructions. One hundred microliters of serum were used for the RNA extraction. To validate the RNA extraction efficiency, a synthetic microRNA of *Caenorhabditis elegans* (syn-cel-miR-39) was spiked in the sample after homogenization in QIAzol Lysis Reagent (Qiagen). The complementary DNA was prepared by miScript Reverse Transcription Kit (Qiagen). The 20 μL PCR reaction contained 10 μL miScript SYBR Green

PCR Master Mix (Qiagen), 4 μL nuclease-free water, 2 μL 10X miScript Primer assay, 2 μL 10X miScript Universial Primer and 2 μL cDNA template. Amplification was performed by CFX96TM Real-Time System under the same PCR condition above. MiR-16 was used as an internal control.

Pharmacokinetic and Pharmacodynamic Analysis

Plasma concentration of clopidogrel and H4 were analyzed by noncompartmental analysis using Phoenix® WinNonlin® software version 1.3 (Certara, St. Louis, MO, USA). $AUC_{0\text{-}2h}$, $AUC_{0\text{-}24h}$ of clopidogrel and $AUC_{0\text{-}24h}$ of H4 after clopidogrel administrations were calculated using the linear up, log down trapezoidal method. $AUC_{0\text{-}2h}$ was used in the present study because most of the reduction in clopidogrel exposure by co-administered aspirin was observed during the first two hours after clopidogrel administration. Observed concentrations and times were used to estimate the C_{max} and T_{max} of clopidogrel and H4. The T_{max} reflects the rate of absorption and elimination of clopidogrel but it depends on the PK sampling time. The apparent terminal elimination rate constant (λz) was estimated from a regression of log transformed plasma concentration of clopidogrel and H4 versus time over the terminal log-linear disposition portion of the concentration-time profiles and $t_{1/2}$ was calculated as $t_{1/2} = \ln 2 / \lambda z$. The metabolic ratios were calculated as $AUC_{0\text{-}24h,\ H4}$ / $AUC_{0\text{-}24h,\ clopidogrel}$. RPI was evaluated before and 4 and 12 hours after clopidogrel administrations to evaluate PD change. RPIs before clopidogrel administrations in periods 2 and 3 were thought to show the effect

of aspirin on platelet aggregation. RPIs at 4 hours after clopidogrel administrations were calculated to evaluate the maximal aspirin and clopidogrel interaction effect on platelet aggregation (24). RPIs at 12 hours after clopidogrel administrations were calculated to see the maintenance of those effects.

Statistical Analysis

Sample size was estimated to detect a 20% difference in the $AUC_{0–24h}$ of clopidogrel between before and after aspirin administrations with a 80% power and a significance level 5%. The within subject variability of clopidogrel was assumed to be 33.51% (coefficient of variation) (25). Every individual PK and PD parameters are presented as arithmetic mean and standard deviation. General linear mixed effects model were used to compare $AUC_{0\text{-}2h}$, $AUC_{0\text{-}24h}$, C_{max}, $t_{1/2}$, metabolic ratio and RPI between treatment periods and genotype groups. GMR and its 90% confidence intervals were estimated for PK parameters and mean difference and its 90% confidence intervals were estimated for PD parameter. Changes in serum mRNA and microRNAs over 4 weeks after aspirin administration were analyzed using a general linear mixed effect model. Statistical significance were determined if the *p*-values were less than 0.05. Statistical analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Effects of Aspirin on Clopidogrel Pharmacokinetics

After 2 and 4 weeks of once-daily aspirin 100 mg administrations (periods 2 and 3, respectively), the exposure to clopidogrel was lowered by 14-19% from baseline (i.e., without aspirin administrations, period 1) although it failed to reach statistical significance (**Table 1**; **Figure 2** and **Figure 3**). The geometric mean ratio (GMR) for the $AUC_{0\text{-}2h}$ for clopidogrel after and before aspirin administration was 0.86 both at weeks 4 and 2, and the GMR for the clopidogrel C_{max} were 0.82 and 0.81, respectively. The T_{max} of clopidogrel was not changed after aspirin administration. In contrast, the exposure to H4 was similar before and after aspirin administration.

Table 1. Comparison of pharmacokinetic and pharmacodynamic parameters of clopidogrel after a oral administration of clopidogrel 75 mg in each period

		Period			GMR[1] (90% CI) *P*-value	
		1 (N=18)	2 (N=18)	3 (N=18)	Period 2 / Period 1	Period 3 / Period 1
Clopidogrel	AUC_{0-2h} (μg*h/L)	2.20 (1.88)	1.90 (1.71)	1.96 (1.81)	0.86 (0.73, 1.00) 0.093	0.86 (0.74, 1.01) 0.117
	AUC_{0-24h} (μg*h/L)	3.11 (2.43)	2.52 (2.06)	2.59 (2.32)	0.83 (0.50, 1.38) 0.538	0.82 (0.49, 1.35) 0.5
	C_{max} (μg*h/L)	2.43 (2.17)	2.08 (1.84)	2.11 (2.06)	0.82 (0.64, 1.04) 0.176	0.81 (0.63, 1.03) 0.14
	$t_{1/2}$ (h)	4.77 (5.30)	4.49 (2.90)	3.24 (1.67)	1.15 (0.79, 1.67) 0.541	0.87 (0.60, 1.26) 0.522
H4	AUC_{0-24h} (μg*h/L)	11.84 (5.85)	11.52 (5.33)	13.21 (8.47)	1.01 (0.88, 1.16) 0.915	1.09 (0.94, 1.26) 0.318
	C_{max} (μg*h/L)	10.93 (5.92)	11.54 (6.20)	11.18 (6.78)	1.09 (0.9, 1.34) 0.452	1.00 (0.82, 1.22) 0.981
	$t_{1/2}$ (h)	0.58 (0.20)	0.54 (0.18)	0.65 (0.21)	0.95 (0.83, 1.07) 0.453	1.14 (1.00, 1.29) 0.094
Metabolic Ratio[2]		13.23 (32.26)	13.31 (25.82)	13.11 (20.34)	1.22 (0.74, 1.99) 0.506	1.34 (0.82, 2.18) 0.327
Maximal Inhibition of Platelet Aggregation (%)		32	41	39	15 (8, 22) 0.002*	12 (5, 20) 0.007*

AUC_{0-2h}, AUC_{0-24h}, C_{max}, $t_{1/2}$, and metabolic ratio are presented as arithmetic mean (standard deviation). Periods 1, 2, and 3 denote baseline, two, and four weeks after daily oral administration of aspirin at 100 mg, respectively.

[1] Geometric mean ratio (90% confidence intervals) for AUC_{0-2h}, AUC_{0-24h}, C_{max}, $t_{1/2}$ and metabolic ratio. Mean (marginal mean) difference (90% confidence intervals) for maximal inhibition of platelet aggregation.

[2] Metabolic ratio: $AUC_{0-24h,\ H4}/AUC_{0-24h,\ clopidogrel}$

* P-value < 0.05

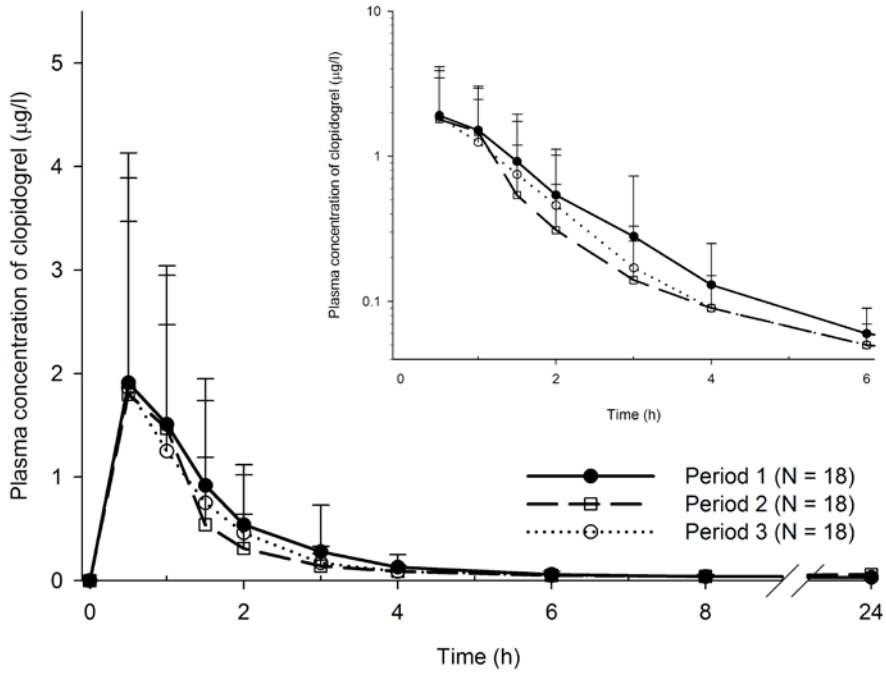

Figure 2. Mean plasma concentration-time profiles of clopidogrel by study period. The error bars represent the standard deviations. Period 1, 2, and 3 denote baseline, 2, and 4 weeks after daily oral administration of aspirin at 100 mg, respectively. Inserted figure is a semi-log scale mean plasma concentration-time profiles for first 6 hours of each period.

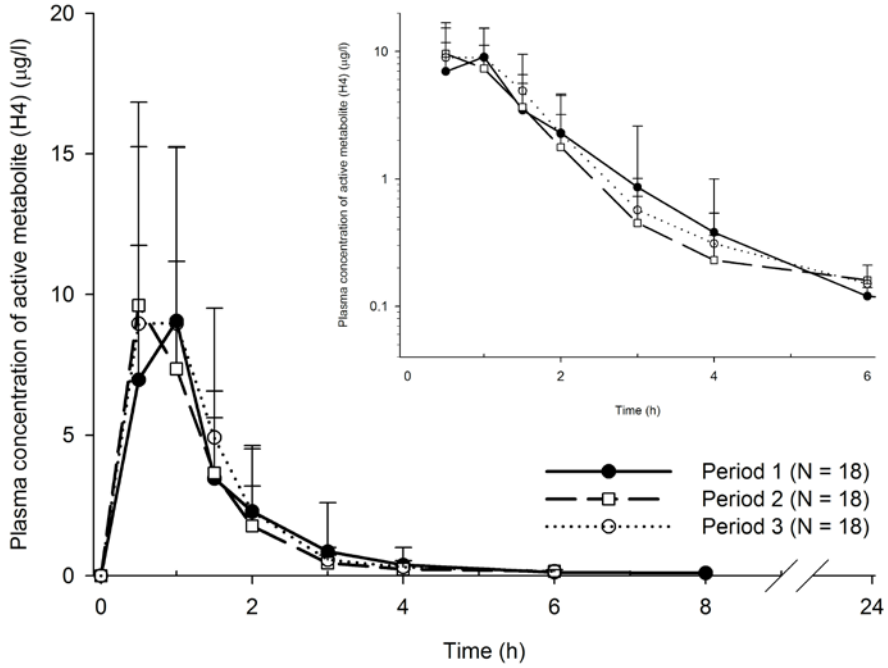

Figure 3. Mean plasma concentration-time profiles of H4, an active metabolite of clopidogrel by study period. The error bars represent the standard deviations. Period 1, 2, and 3 denote baseline, 2, and 4 weeks after daily oral administration of aspirin at 100 mg, respectively. Inserted figure is a semi-log scale mean plasma concentration-time profiles for first 6 hours of each period.

Effects of Aspirin on Clopidogrel Pharmacodynamics

The relative platelet inhibition (RPI, %) was increased after once-daily administration of aspirin at 100 mg and the increments were lasted for 24 hours (**Figure 4**). The maximal RPIs in periods 2 and 3 (RPI at 4 hours after clopidogrel administration) were increased by 15% and 12%, respectively, and the increment was statistically significant (p = 0.002 and p = 0.007, respectively) (**Table 1**, **Figure 4**). Baseline RPIs in each period (RPI before clopidogrel administrations) were significantly increased after aspirin daily administrations (both $p < 0.001$). The RPIs at 24 hours after clopidogrel administration were still higher in periods 2 and 3 than in period 1, but it was not statistically significant.

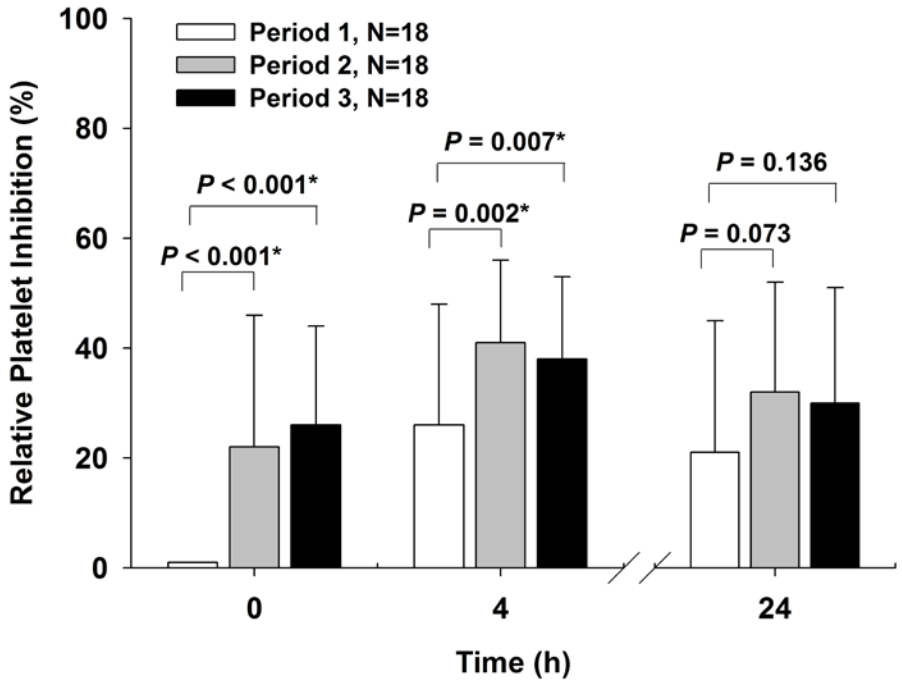

Figure 4. Relative inhibition of platelet aggregation (% change from predose baseline) with time after dose by period in each period. The maximal inhibition of platelet aggregation (i.e., 4h after clopidogrel administration) is shown. *P value <0.05.

Effects of Aspirin on P-gp Expression in the Blood

Aspirin significantly increased the level of plasma miR-27a, which peaked at 1 week after once-daily administration. For example, the mean fold increase in the plasma miR-27a was 1.89, 1.43, 1.53, and 1.14, respectively, after 1, 2, 3, and 4 weeks of aspirin administration (**Figure 5**, p = 0.004, linear mixed model). On the other hand, plasma miR-451 and mRNA were not increased after repeated administrations of aspirin.

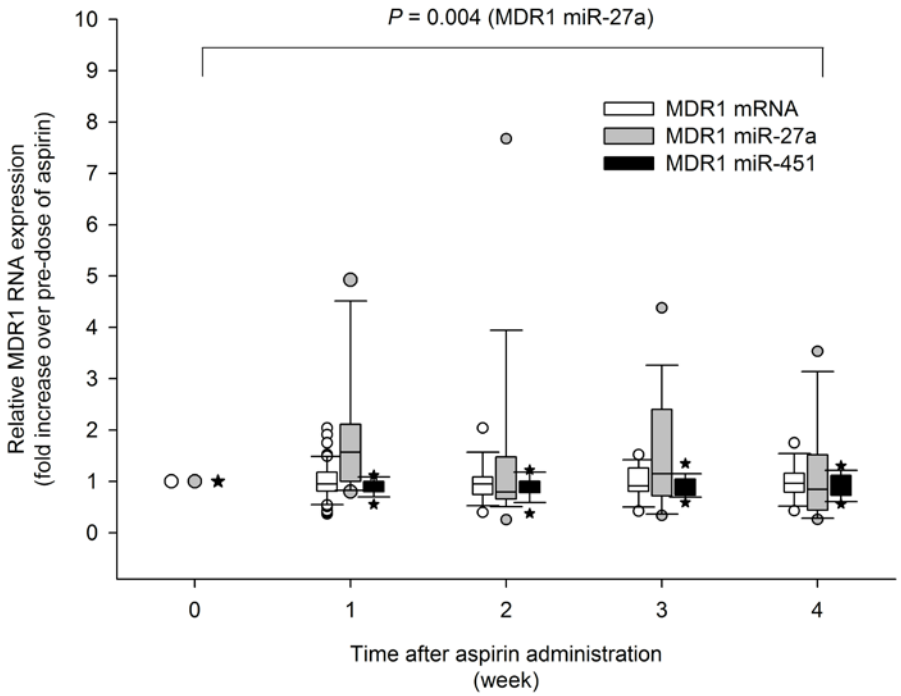

Figure 5. Relative MDR1 RNA expression after repeated daily oral administration of aspirin at 100mg.

Effects of Genotype on PK and PD

Whereas the exposure to clopidogrel was lower in CYP2C19 extensive metabolizers (EMs) than in poor metabolizers (PMs), the exposure to H4 and, thereby the metabolic ratio, were higher in EMs than PMs in all periods **(Table 2**, **Table 3**, and **Table 4**; **Figure 6** and **Figure 7)**. The GMRs for the metabolic ratio between EMs and PMs and their 90% confidence intervals (CI) were 3.41 [1.41 - 8.26], 4.56 [2.20 – 9.45] and 3.48 [1.68 - 7.23] in periods 1, 2 and 3, respectively. The maximal RPIs after clopidogrel administration were 22% higher (p = 0.008) in CYP2C19 EMs than in PMs without aspirin effect in period 1 **(Table 2**; **Figure 8)**. Although there was no statistically significant difference in the maximal RPIs between CYP2C19 genotypes after 2 weeks aspirin co-administration (**Table 3**; **Figure 9)**, after aspirin co-administrations for 4 weeks, the RPI was still 13% higher in CYP2C19 EMs than PMs (p = 0.042) **(Table 4**; **Figure 10)**.

Table 2. Comparison of pharmacokinetic and pharmacodynamic parameters of clopidogrel by genotype of CYP2C19 after a single administration of clopidogrel at 75 mg

		CYP2C19		GMR[1] (90% CI) *P*-value
		EM (N=9)	PM (N=9)	EM/PM
Clopidogrel	AUC_{0-24h} (μg*h/L)	2.49 (1.62)	3.73 (3.00)	0.65 (0.25, 1.67) 0.433
	C_{max} (μg*h/L)	1.77 (1.52)	3.09 (2.60)	0.59 (0.24, 1.47) 0.326
	$t_{1/2}$ (h)	5.28 (6.97)	4.26 (3.24)	0.81 (0.35, 1.9) 0.677
H4	AUC_{0-24h} (μg*h/L)	15.99 (4.88)	7.7 (3.22)	2.21 (1.52, 3.23) 0.002*
	C_{max} (μg*h/L)	14.68 (5.67)	7.17 (3.23)	2.12 (1.43, 3.13) 0.005*
	$t_{1/2}$ (h)	0.71 (0.19)	0.45 (0.11)	1.57 (1.24, 1.98) 0.004*
Metabolic Ratio[2]		22.13 (44.9)	4.32 (4.04)	3.41 (1.41, 8.26) 0.028*
Maximal Inhibition of Platelet Aggregation (%)		37 (22)	15 (18)	22 (10, 35) 0.008*

AUC_{0-24h}, C_{max}, $t_{1/2}$, and metabolic ratio are presented as arithmetic mean (SD). EM: extensive metabolizers, PM: poor metabolizers.

[1] Geometric mean ratio (90% confidence intervals) for $AUC_{0-2h,}$ AUC_{0-24h}, C_{max}, $t_{1/2}$ and metabolic ratio. Mean (marginal mean) difference (90% confidence intervals) for maximal inhibition of platelet aggregation.

[2] Metabolic ratio: $AUC_{0-24h,\ H4}/AUC_{0-24h,\ clopidogrel}$

* *P*-value < 0.05

Table 3. Comparison of pharmacokinetic and pharmacodynamic parameters of clopidogrel by genotype of CYP2C19 after aspirin 100 mg co-administration for 2 weeks

		CYP2C19		GMR[1] (90% CI) *P*-value
		EM (N=9)	PM (N=9)	EM/PM
Clopidogrel	AUC_{0-24h} (μg*h/L)	1.67 (1.35)	3.37 (2.35)	0.41 (0.19, 0.92) 0.073
	C_{max} (μg*h/L)	1.58 (1.66)	2.58 (1.97)	0.51 (0.20, 1.31) 0.227
	$t_{1/2}$ (h)	5.01 (3.25)	4.03 (2.66)	1.21 (0.71, 2.06) 0.531
H4	AUC_{0-24h} (μg*h/L)	14.9 (4.70)	8.13 (3.53)	1.89 (1.36, 2.63) 0.004*
	C_{max} (μg*h/L)	15.3 (6.45)	7.77 (2.86)	1.95 (1.42, 2.67) 0.002*
	$t_{1/2}$ (h)	0.58 (0.23)	0.49 (0.10)	1.15 (0.93, 1.42) 0.263
Metabolic Ratio[2]		23.05 (34.58)	3.57 (2.70)	4.56 (2.20, 9.45) 0.003*
Maximal Inhibition of Platelet Aggregation (%)		44 (16)	37 (13)	7 (-3, 16) 0.239

AUC_{0-24h}, C_{max}, $t_{1/2}$, and metabolic ratio are presented as arithmetic mean (SD). EM: extensive metabolizers, PM: poor metabolizers.

[1] Geometric mean ratio (90% confidence intervals) for $AUC_{0-2h,}$ AUC_{0-24h}, C_{max}, $t_{1/2}$ and metabolic ratio. Mean (marginal mean) difference (90% confidence intervals) for maximal inhibition of platelet aggregation.

[2] Metabolic ratio: $AUC_{0-24h,\ H4}/AUC_{0-24h,\ clopidogrel}$

* *P*-value < 0.05

Table 4. Comparison of pharmacokinetic and pharmacodynamic parameters of clopidogrel by genotype of CYP2C19 after aspirin 100 mg co-administration for 4 weeks

		CYP2C19		GMR[1] (90% CI) *P*-value
		EM (N=9)	PM (N=9)	EM/PM
Clopidogrel	AUC_{0-24h} (μg*h/L)	2.13 (2.41)	3.06 (2.29)	0.58 (0.25, 1.33) 0.263
	C_{max} (μg*h/L)	1.96 (2.63)	2.26 (1.43)	0.55 (0.21, 1.40) 0.28
	$t_{1/2}$ (h)	3.00 (2.05)	3.49 (1.26)	0.7 (0.38, 1.27) 0.305
H4	AUC_{0-24h} (μg*h/L)	17.59 (9.52)	8.83 (4.32)	2.01 (1.32, 3.06) 0.012*
	C_{max} (μg*h/L)	15.19 (7.25)	7.16 (3.00)	2.05 (1.31, 3.22) 0.014*
	$t_{1/2}$ (h)	0.74 (0.24)	0.55 (0.13)	1.3 (1.01, 1.67) 0.085
Metabolic Ratio[2]		21.64 (26.52)	4.58 (3.44)	3.48 (1.68, 7.23) 0.010*
Maximal Inhibition of Platelet Aggregation (%)		45 (12)	32 (16)	13 (3, 23) 0.042*

AUC_{0-24h}, C_{max}, $t_{1/2}$, and metabolic ratio are presented as arithmetic mean (SD). EM: extensive metabolizers, PM: poor metabolizers.

[1] Geometric mean ratio (90% confidence intervals) for $AUC_{0-2h,}$ AUC_{0-24h}, C_{max}, $t_{1/2}$ and metabolic ratio. Mean (marginal mean) difference (90% confidence intervals) for maximal inhibition of platelet aggregation.

[2] Metabolic ratio: $AUC_{0-24h,\ H4}/AUC_{0-24h,\ clopidogrel}$

* *P*-value < 0.05

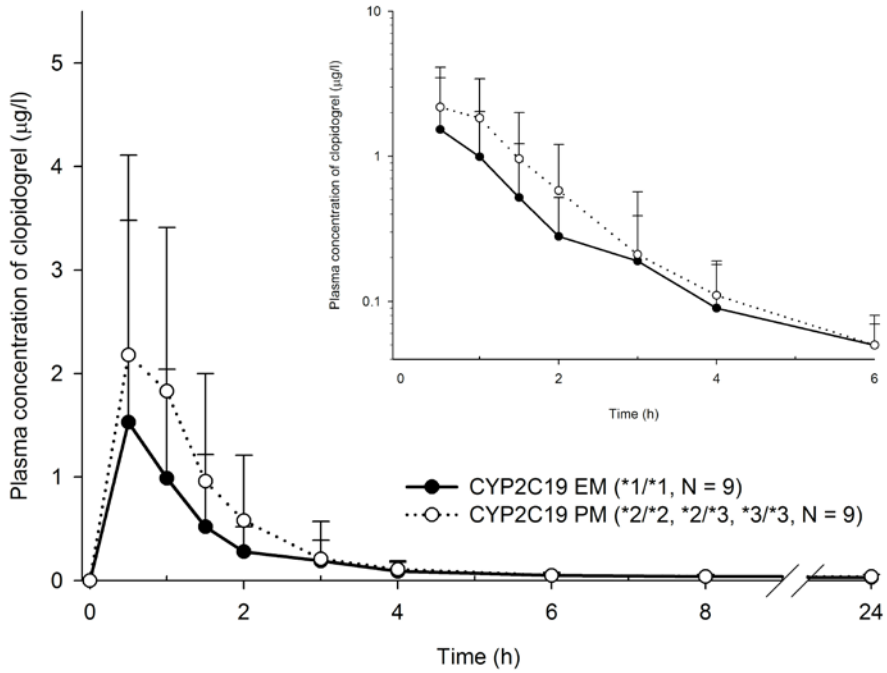

Figure 6. Mean plasma concentration-time profiles of clopidogrel by genotype of CYP2C19. The error bars represent the standard deviations. Data were pooled to visualize the effect of genotypes on the pharmacokinetics of clopidogrel. Inserted figure is a semi-log scale mean plasma concentration-time profiles for first 6 hours of each period.

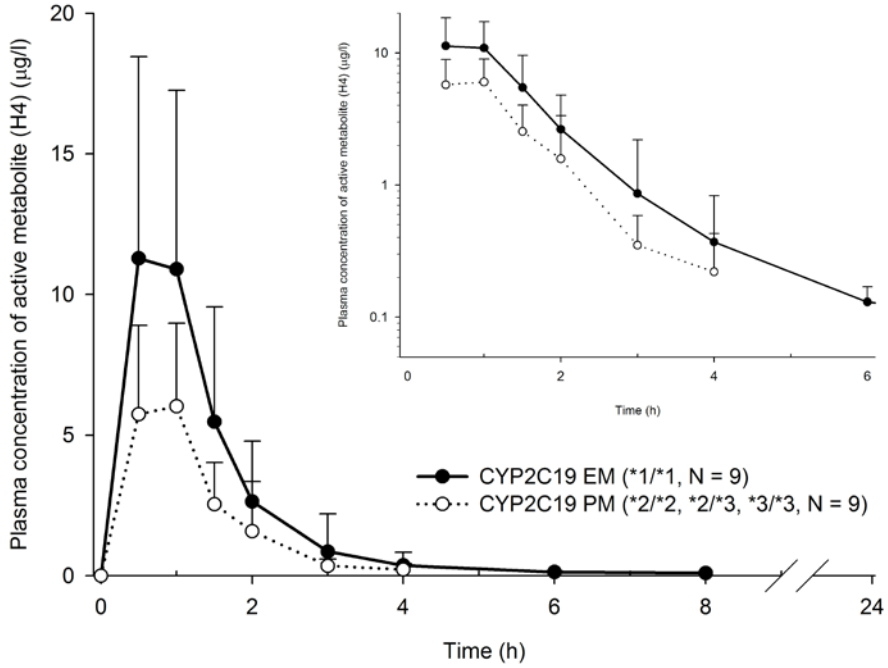

Figure 7. Mean plasma concentration-time profiles of H4, an active metabolite of clopidogrel by genotype of CYP2C19. The error bars represent the standard deviations. Data were pooled to visualize the effect of genotypes on the pharmacokinetics of H4. Inserted figure is a semi-log scale mean plasma concentration-time profiles for first 6 hours of each period.

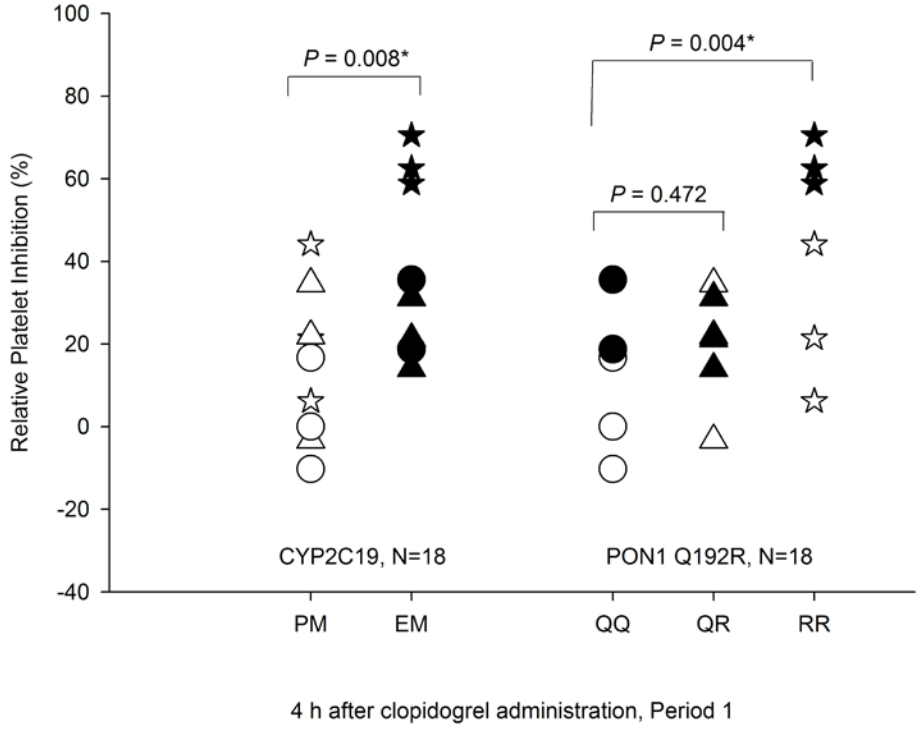

Figure 8. Relative inhibition of platelet aggregation (% change from predose baseline) at 4 hours after clopidogrel administration in period 1 by genotypes. *CYP2C19/PON1* EM/QQ (filled circle), EM/QR (filled triangle), EM/RR (filled star), PM/QQ (open circle), PM/QR (open triangle), and PM/RR (open start); EM, extensive metabolizer; PM, poor metabolizer; **P* value <0.05.

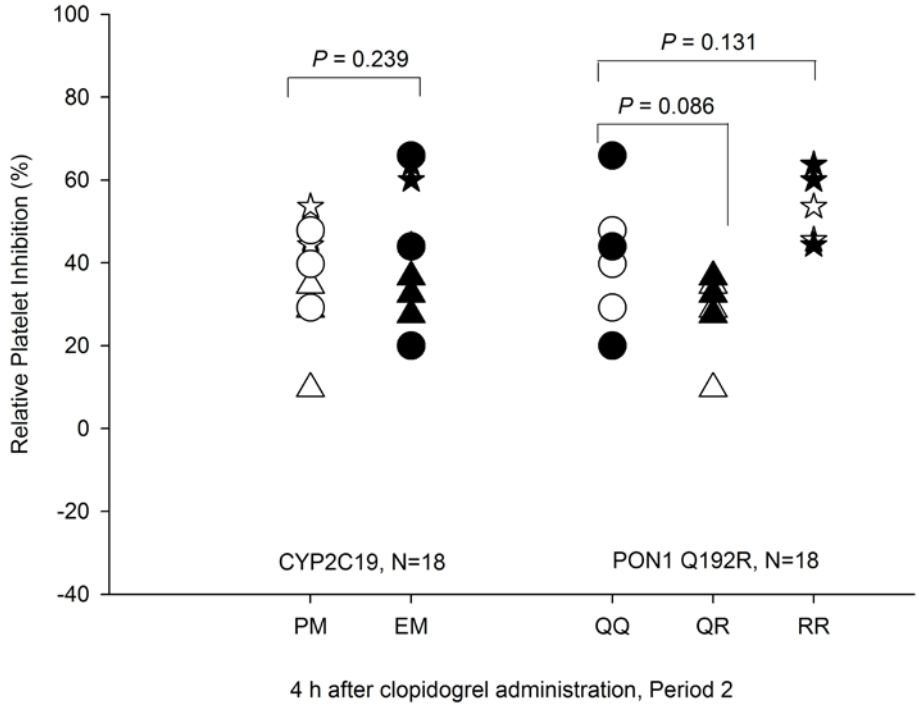

Figure 9. Relative inhibition of platelet aggregation (% change from predose baseline) at 4 hours after clopidogrel administration in period 2 by genotypes. *CYP2C19/PON1* EM/QQ (filled circle), EM/QR (filled triangle), EM/RR (filled star), PM/QQ (open circle), PM/QR (open triangle), and PM/RR (open start); EM, extensive metabolizer; PM, poor metabolizer; **P* value <0.05.

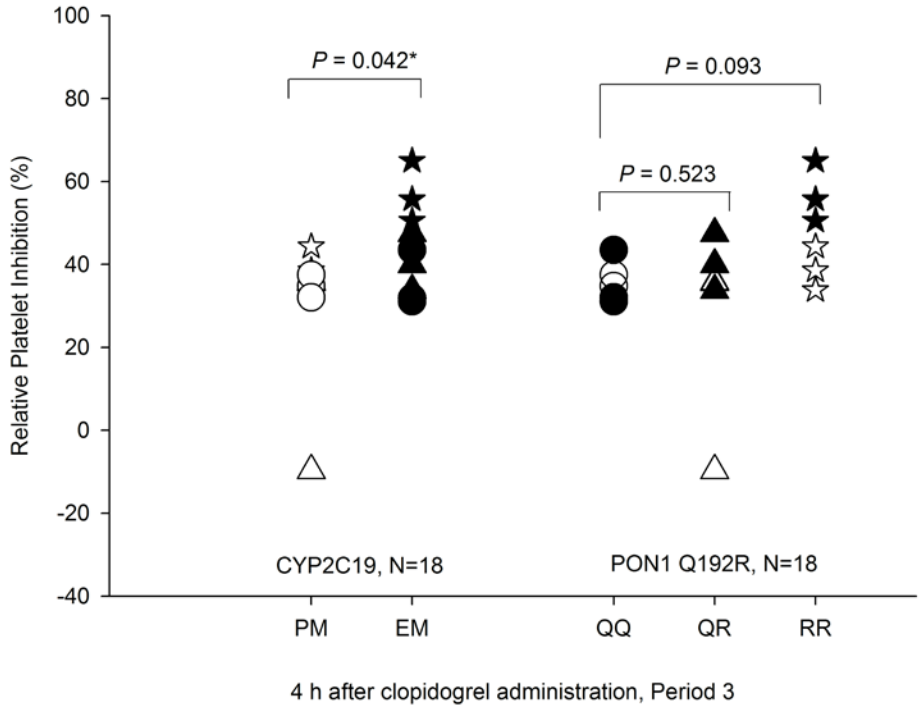

Figure 10. Relative inhibition of platelet aggregation (% change from predose baseline) at 4 hours after clopidogrel administration in period 3 by genotypes. *CYP2C19/PON1* EM/QQ (filled circle), EM/QR (filled triangle), EM/RR (filled star), PM/QQ (open circle), PM/QR (open triangle), and PM/RR (open start); EM, extensive metabolizer; PM, poor metabolizer; **P* value <0.05.

Although the exposure to clopidogrel was higher in PON1 Q192R RR subjects than in QQ subjects, the exposure to H4 and, thereby the metabolic ratio, were lower in PON1 Q192R RR subjects than in QQ subjects in all the periods **(Table 5**, **Table 6**, and **Table 7**; **Figure 11** and **Figure 12)**. The GMRs (ratio of RR/QQ and [90% CI]) for the metabolic ratio were 0.40 [0.14 – 1.20], 0.48 [0.20 – 1.17] and 0.31 [0.13 – 0.75] in periods 1, 2 and 3, respectively. However, the maximal RPIs after clopidogrel administration were 30% higher in RR subjects than in QQ subjects (p = 0004) without aspirin effect in period 1 **(Table 5**; **Figure 8).** After aspirin co-administration for 2 and 4 weeks, the difference in RPIs between RR and QQ subjects were not statistically significant (p = 0.131 and p = 0.093, respectively) **(Table 6** and **Table 7**; **Figure 9** and **Figure 10)**. The exposure to clopidogrel and H4, the metabolic ratios, and the maximal RPIs after clopidogrel administration were not statistically different between PON1 Q192R QR and QQ subjects in all the periods (**Table 2**, **Table 3**, **Table 4**, **Table 5**, **Table 6**, and **Table 7)**.

Table 5. Comparison of pharmacokinetic and pharmacodynamic parameters of clopidogrel by genotype of PON1 Q192R after a single administration of clopidogrel at 75 mg

		PON1 Q192R			GMR[1] (90% CI) *P*-value	
		QQ (N=6)	QR (N=6)	RR (N=6)	QR/QQ	RR/QQ
Clopidogrel	AUC_{0-24h} (μg*h/L)	2.94 (3.15)	2.93 (2.85)	3.47 (1.32)	0.76 (0.24, 2.40) 0.678	1.66 (0.52, 5.28) 0.452
	C_{max} (μg*h/L)	1.74 (1.68)	1.86 (1.65)	3.69 (2.77)	0.82 (0.27, 2.51) 0.760	2.01 (0.66, 6.15) 0.291
	$t_{1/2}$ (h)	5.01 (3.92)	3.03 (1.71)	6.27 (8.42)	0.63 (0.22, 1.77) 0.441	0.97 (0.34, 2.74) 0.960
H4	AUC_{0-24h} (μg*h/L)	13.01 (5.92)	12.55 (6.21)	9.97 (6.04)	0.91 (0.58, 1.45) 0.738	0.67 (0.42, 1.07) 0.153
	C_{max} (μg*h/L)	9.38 (2.79)	13.67 (7.49)	9.72 (6.44)	1.27 (0.79, 2.05) 0.390	0.88 (0.55, 1.43) 0.656
	$t_{1/2}$ (h)	0.53 (0.14)	0.58 (0.16)	0.63 (0.29)	1.08 (0.81, 1.44) 0.629	1.1 (0.82, 1.46) 0.578
Metabolic Ratio[2]		8.21 (4.50)	27.71 (55.82)	3.75 (3.53)	1.21 (0.4, 3.57) 0.763	0.4 (0.14, 1.2) 0.164
Maximal Inhibition of Platelet Aggregation (%)		14 (16)	20 (14)	44 (25)	7 (-9, 22) 0.472	30 (15, 46) 0.004*

AUC_{0-24h}, C_{max}, $t_{1/2}$, and metabolic ratio are presented as arithmetic mean (SD).

[1] Geometric mean ratio (90% confidence intervals) for $AUC_{0-2h,}$ AUC_{0-24h}, C_{max}, $t_{1/2}$ and metabolic ratio. Mean (marginal mean) difference (90% confidence intervals) for maximal inhibition of platelet aggregation.

[2] Metabolic ratio: $AUC_{0-24h,\ H4}/AUC_{0-24h,\ clopidogrel}$

* *P*-value < 0.05

Table 6. Comparison of pharmacokinetic and pharmacodynamic parameters of clopidogrel by genotype of PON1 Q192R after aspirin 100 mg co-administration for 2 weeks

		PON1 Q192R			GMR[1] (90% CI) *P*-value	
		QQ (N=6)	QR (N=6)	RR (N=6)	QR/QQ	RR/QQ
Clopidogrel	AUC_{0-24h} (μg*h/L)	1.82 (1.73)	2.86 (2.82)	2.87 (1.60)	1.18 (0.44, 3.14) 0.769	1.81 (0.68, 4.82) 0.304
	C_{max} (μg*h/L)	1.16 (1.24)	2.18 (1.94)	2.9 (2.08)	1.67 (0.52, 5.29) 0.45	2.38 (0.75, 7.56) 0.207
	$t_{1/2}$ (h)	5.21 (3.21)	5.70 (2.87)	2.77 (2.07)	1.23 (0.63, 2.37) 0.594	0.54 (0.29, 1.01) 0.106
H4	AUC_{0-24h} (μg*h/L)	11.99 (5.32)	12.16 (6.81)	10.39 (4.42)	0.96 (0.64, 1.43) 0.852	0.87 (0.58, 1.30) 0.546
	C_{max} (μg*h/L)	11.59 (4.40)	12.59 (9.40)	10.43 (4.48)	0.94 (0.64, 1.38) 0.784	0.87 (0.59, 1.29) 0.549
	$t_{1/2}$ (h)	0.64 (0.25)	0.43 (0.08)	0.54 (0.09)	0.71 (0.55, 0.92) 0.034*	0.89 (0.69, 1.15) 0.427
Metabolic Ratio[2]		12.06 (11.58)	22.53 (44.02)	5.35 (3.78)	0.81 (0.33, 1.98) 0.685	0.48 (0.20, 1.17) 0.169
Maximal Inhibition of Platelet Aggregation (%)		41 (16)	29 (10)	52 (9)	-13 (-24, -1) 0.086	11 (-1, 23) 0.131

AUC_{0-24h}, C_{max}, $t_{1/2}$, and metabolic ratio are presented as arithmetic mean (SD).

[1] Geometric mean ratio (90% confidence intervals) for $AUC_{0-2h,}$ AUC_{0-24h}, C_{max}, $t_{1/2}$ and metabolic ratio. Mean (marginal mean) difference (90% confidence intervals) for maximal inhibition of platelet aggregation.

[2] Metabolic ratio: $AUC_{0-24h,\ H4}/AUC_{0-24h,\ clopidogrel}$

* *P*-value < 0.05

Table 7. Comparison of pharmacokinetic and pharmacodynamic parameters of clopidogrel by genotype of PON1 Q192R after aspirin 100 mg co-administration for 4 weeks

		PON1 Q192R			GMR[1] (90% CI) *P*-value	
		QQ (N=6)	QR (N=6)	RR (N=6)	QR/QQ	RR/QQ
Clopidogrel	AUC_{0-24h} (μg*h/L)	1.49 (1.36)	3.19 (3.41)	3.11 (1.65)	1.37 (0.5, 3.81) 0.591	2.28 (0.82, 6.32) 0.177
	C_{max} (μg*h/L)	1.01 (0.90)	2.09 (2.16)	3.23 (2.45)	1.5 (0.48, 4.74) 0.544	3.04 (0.96, 9.62) 0.11
	$t_{1/2}$ (h)	3.89 (2.30)	3.02 (1.54)	2.81 (1.04)	0.73 (0.35, 1.52) 0.464	0.82 (0.40, 1.72) 0.651
H4	AUC_{0-24h} (μg*h/L)	16.43 (12.37)	12.49 (6.77)	10.72 (4.95)	0.77 (0.46, 1.29) 0.391	0.7 (0.42, 1.18) 0.248
	C_{max} (μg*h/L)	12.81 (8.15)	11.95 (7.70)	8.77 (4.45)	0.87 (0.5, 1.5) 0.65	0.71 (0.41, 1.23) 0.29
	$t_{1/2}$ (h)	0.66 (0.24)	0.59 (0.09)	0.69 (0.27)	0.94 (0.70, 1.28) 0.748	1.05 (0.77, 1.43) 0.778
Metabolic Ratio[2]		16.26 (16.00)	18.17 (31.79)	4.89 (3.92)	0.56 (0.23, 1.37) 0.275	0.31 (0.13, 0.75) 0.036*
Maximal Inhibition of Platelet Aggregation (%)		35 (5)	31 (21)	48 (11)	-5 (-17, 8) 0.523	13 (0, 25) 0.093

AUC_{0-24h}, C_{max}, $t_{1/2}$, and metabolic ratio are presented as arithmetic mean (SD).

[1] Geometric mean ratio (90% confidence intervals) for $AUC_{0-2h,}$ AUC_{0-24h}, C_{max}, $t_{1/2}$ and metabolic ratio. Mean (marginal mean) difference (90% confidence intervals) for maximal inhibition of platelet aggregation.

[2] Metabolic ratio: $AUC_{0-24h,\ H4}/AUC_{0-24h,\ clopidogrel}$

* *P*-value < 0.05

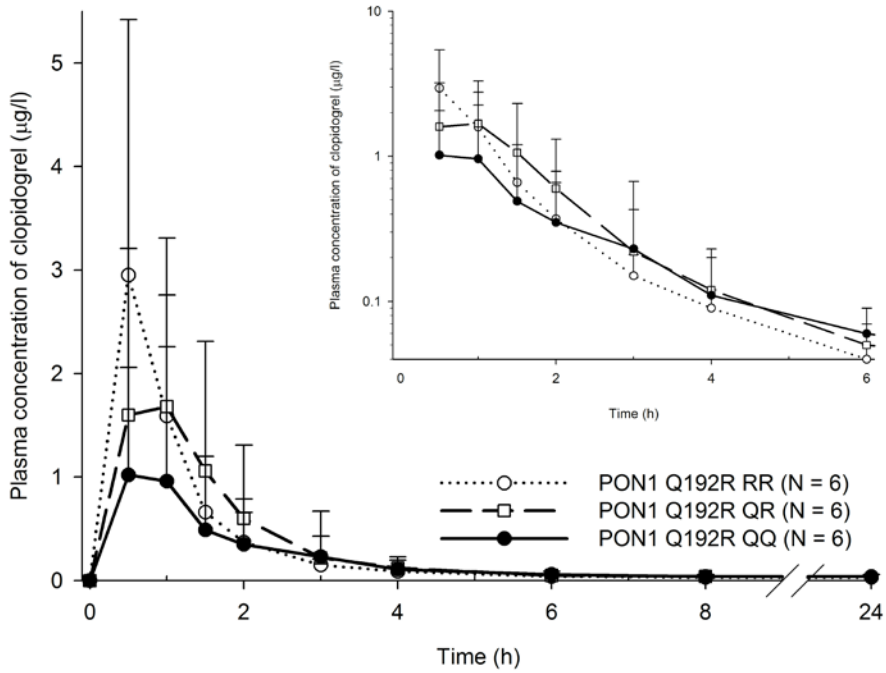

Figure 11. Mean plasma concentration-time profiles of clopidogrel by genotype of PON1 Q192R. The error bars represent the standard deviations. Data were pooled to visualize the effect of genotypes on the pharmacokinetics of clopidogrel. Inserted figure is a semi-log scale mean plasma concentration-time profiles for first 6 hours of each period.

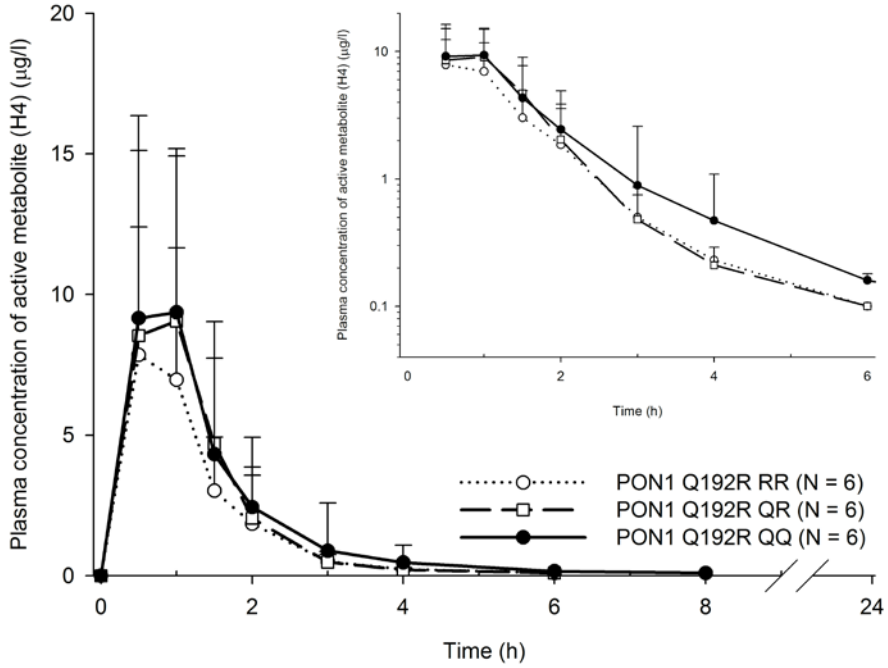

Figure 12. Mean plasma concentration-time profiles of H4, an active metabolite of clopidogrel by genotype of PON1 Q192R. The error bars represent the standard deviations. Data were pooled to visualize the effect of genotypes on the pharmacokinetics of H4. Inserted figure is a semi-log scale mean plasma concentration-time profiles for first 6 hours of each period.

DISCUSSION

In this study, the exposure to clopidogrel ($AUC_{0\text{-}2h}$ and C_{max}) was decreased after oral administration of aspirin at 100 mg for 2 and 4 weeks, but the decrements were not statistically significant. The absorption of clopidogrel is affected by intestinal P-gp expression and if aspirin induced intestinal P-gp, the plasma level of clopidogrel is expected to be lower than without aspirin. Although the exposure to clopidogrel was lowered, the level of H4 was not changed after aspirin co-administration (**Table 1**). Furthermore, the anti-thrombotic effects were increased due to aspirin's additive effect on the inhibition of platelet aggregation, and the increased PD effects were lasted for 24 hours (**Figure 4**). RPIs at 4 hours after clopidogrel administration were thought to reflect the maximal PD effect of clopidogrel (24) and aspirin significantly increased clopidogrel's maximal PD effect. Clopidogrel is rapidly absorbed and transformed after oral administration, but most of absorbed clopidogrel is hydrolyzed by carboxylesterases into inactive carboxylic acid and only a small proportion of it is converted to H4 (26, 27). Therefore only small amount of clopidogrel is observed in plasma (28). The decrement in clopidogrel exposure in this study might not be sufficient to show the difference in H4 exposure. The unchanged exposure to H4 by co-administered aspirin can be also explained by the metabolism of effluxed clopidogrel in the intestine. The intestinal CYP3A4, together with other CYP enzymes in the liver, plays an important role in the bioactivation of

clopidogrel (13). Therefore, clopidogrel that was transported back into the intestine by P-gp could have been metabolized to H4 or inactive carboxylic acid by the intestinal CYP3A4 or carboxylesterases, respectively. Depending on the relative contribution of these metabolic pathways in the intestine, the reabsorption of H4 might have resulted in no difference in its systemic level between before and after aspirin coadministration.

In the study performed by Jung *et al.* (5), aspirin induced P-gp proteins by increasing its mRNA expressions. P-gp mRNA expressions and the drug efflux proteins were increased in Caco-2 cells after exposure to aspirin for 72 hours. Also a significant delay in clopidogrel permeabilization was observed in the cells exposed to aspirin. In the rat intestine, the P-gp gene expressions and the P-gp protein expressions were increased after aspirin administration for 4 weeks. And oral absorption of clopidogrel was decreased due to P-gp expression caused by aspirin exposure. Similar tendency of clopidogrel's PK characteristic was observed in this study but the insufficient dose of aspirin might lead to failure to show its statistical significance. The doses of aspirin as acetylsalicylic acid (ASA) used in the rats were 15, 60, and 150 mg/kg, which were equivalent to human doses of 2.4, 9.6 and 24 mg/kg based on body surface area. Therefore, a 70 kg man should take more than 168 mg up to 1680 mg of daily aspirin as ASA to maintain the same environment used in the rats. The aspirin doses as ASA used in *in vitro* study were 0.5, 1 and 2 mmol/L for 72 hours, which were equivalent to 90, 180, and 360 μg/L for 72 hours. In the study performed by Bae *et al.* (29), mean plasma aspirin exposure as ASA was 279 μg/L for 6 hours (46.5 μg/L/h) after single oral

administration of 100 mg of aspirin in 10 healthy Korean males. Based on these data, the exposure to aspirin in subjects from this study are thought to be lower than that of the rats and cells used in the study performed by Jung *et al.* although plasma aspirin concentrations were not determined in this study. And the aspirin exposure in this study was likely to result in insufficient P-gp expressions in the human intestine, which was not enough to make significant differences in the oral absorption of clopidogrel.

The plasma mir-27a was significantly increased after aspirin administrations (**Figure 5**). However, no change in plasma MDR1 mRNA and mir-451 were observed. Expression of miR-27a and miR-451 is known to upregulate the expression of P-gp protein by inhibiting some transcriptional factors suppressing P-gp expressions (30). Although we did not observed the gene and protein expression in the human intestine, the increment observed in plasma mir-27a and its weak negative correlation with clopidogrel exposure suggests that aspirin can upregulate P-gp protein expression in human.

PON1 Q192R RR subjects showed less exposure to H4 than QR or QQ groups in all the periods, but the corresponding PD effects in RR subjects were highest among the genotype groups. *PON1* Q192R RR variation is known to be associated with higher PON1 enzyme activity (4). Therefore if PON1 involved in the metabolism of clopidogrel, higher metabolite activity could have been observed in RR subjects (4, 18, 31). The lower exposure to H4 and lower metabolic ratios in RR subjects is thought to be caused by accelerated metabolism of clopidogrel to the metabolite which is not H4. In period 1, only clopidogrel's effect on platelet aggregation was evaluated and

the maximal platelet aggregation after clopidogrel administration was highest in RR subjects among the *PON1* Q192R genotype groups (**Figure 8**). However, this tendency in PD was observed in only 3 subjects, whose genotypes were *CYP2C19* EM and *PON1* Q192R RR (filled star in **Figure 8**). When the effect of *PON1* Q192R genotypes on clopidogrel's PD were analyzed in *CYP2C19* PMs, no difference between *PON1* genotype groups was observed. And the PD difference among the *PON1* Q192R genotypes was not consistent after once-daily aspirin 100 mg was co-administered for 2 and 4 weeks (**Table 5**, **Table 6**, and **Tabl4 7**; **Figure 8**, **Figure 9**, and **Figure 10**). The PD difference observed in RR subjects in period 1 might not be caused by the variation in the PON1 enzyme activity but by the variation in the CYP2C19 enzyme activity in *CYP2C19* EMs itself. Due to the small sample size in each genotype groups (N=3), PD result of *CYP2C19*/*PON1* Q129R EM/RR subjects may affect the result of statistical analysis. The PD difference observed in RR subjects in Period 1 is not thought to be repeated in the general population. Aspirin is known to induce PON1 enzyme *in vitro* and *in vivo* (32, 33). In this study, however, neither the exposure to H4 nor the metabolic activity was changed after daily co-administration of aspirin at 100 mg (**Table 1**; **Figure 3** and **Figure 12**). Daily aspirin 100 mg might not be sufficient to induce PON1 enzyme.

In this study, the *CYP2C19**2 or *3 genotypes (PMs) were associated with the lower metabolic activity of the CYP2C19 enzyme and the lower exposure to H4, thereby leading to the lower inhibition of platelet aggregation. The metabolic activity was 3-fold greater and the exposure to H4 was 2-fold

greater in CYP2C19 EMs than in PMs (**Table 2**). The maximal PD effect after clopidogrel administration was also greater in *CYP2C19* EM subjects in period 1 and a similar tendency was observed after once-daily aspirin 100 mg co-administrations for 2 and 4 weeks (**Table 2**; **Figure 8**, **Figure 9**, and **Figure 10**). These data support earlier study about the effect of the *CYP2C19* genetic polymorphisms on clopidogrel's PK and PD (4, 6, 8, 10). However, other drug metabolizing enzymes such as CYP3A4 are also known to contribute to the bioactivation of clopidogrel to H4, and there is still a possibility that variations in the expression of those enzymes could have confounded the study results.

The present study had several limitations. First, the number of subjects was not sufficiently large enough to detect the observed decrement in clopidogrel exposure between before and after aspirin administrations. A post hoc power analysis showed that the actual statistical power was only 70% and 24 %, respectively, for $AUC_{0\text{-}2h}$ and $AUC_{0\text{-}24h}$, and > 15% and 42% decrements in these PK parameters, respectively, should have been observed to attain the same power (i.e., 80%) with 18 subjects at the same significance level of 5%. Therefore, the number of subjects in each genotype group, although balanced, was not large enough to detect a possible difference between genotypes. Second, the P-gp expression in the guts was not directly measured in the present study; it was rather indirectly assessed using clopidogrel's PK parameter and mRNA and microRNA expression in whole blood. The hypothesis can be tested in future studies by comparing the change of clopidogrel exposure with change of known P-gp substrate exposure such as

digoxin, after the aspirin administrations. Third, the concentrations of aspirin were not determined because the primary focus was clopidogrel. However, the pharmacokinetic profile of aspirin could have helped to interpret the results of the present study in a more mechanistic way.

In summary co-administered once-daily aspirin decreased the systemic exposure to clopidogrel, but its antithrombotic effect was not affected. Variations of *CYP2C19* genotypes, but not of the *PON1* genotype appeared to play a significant role in the antithrombotic effect of clopidogrel. Combination of low dose aspirin can be still recommended in patients receiving treatment with clopidogrel.

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국문 초록

서론: 아스피린 복용에 의한 장상피세포에서 P-당단백질의 발현의 증가로 클로피도그렐의 경구 흡수율이 감소됨이 동물모형에서 알려져 있다. 본 연구의 목적은 사람에서 아스피린 병용투여에 의한 클로피도그렐의 약동학 및 약력학의 변화를 규명하기 위한 것이다.

방법: 클로피도그렐의 대사효소의 유전 변이에 의한 차이를 고려하여 CYP2C19 과 PON1 의 유전형에 따라서 등록된 18 명의 건강한 남성 자원자를 대상으로 시험을 진행하였다. 아스피린과 병용투약 전, 매일 100 mg 아스피린 경구투약을 2 주 시행한 후, 4 주 시행한 후, 클로피도그렐 75mg 을 각각의 시기에 단회 경구 투여하였다. 이후 클로피도그렐과 그 활성대사체인 H4 의 혈장 약물농도와 혈소판 응집 억제 정도(relative platelet inhibition, RPI)를 측정하여 약동학적, 약력학적 특성변화를 관찰하였다. 또한 아스피린 병용투약에 따른 말초혈액에서의 P-당단백질 발현의 영향을 평가하기 위하여 MDR1 mRNA 와 miR-27a, miR-16 의 발현을 측정하였다.

결과: 아스피린 병용투여 이후 P-당단백질의 마이크로 RNA 중 mir-27a 는 7.67 배 증가하였고 (P=0.004), 클로피도그렐의 혈중 노출량 (area under the concentration-time curve, AUC)은 14%

감소하였다 (P>0.05). 활성대사체인 H4 의 혈중노출량은 변화가 없었으며, 혈소판 응집 억제 정도는 15%까지 상승하였다 (*P*=0.002).

결론: 결론적으로 저용량의 아스피린을 병용 투여하였을 경우 클로피도그렐의 체내흡수를 감소시킬 가능성이 있으나, 그로 인한 치료효과의 감소 가능성은 적은 것으로 판단된다.

주요어 : 아스피린, 클로피도그렐, 약물상호작용, 약동학, P-당단백질

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