Original Article

A Randomized Study of the Safety, Tolerability, Pharmacodynamics, and Pharmacokinetics of Clopidogrel in Three Different CYP2C19 Genotype Groups of Healthy Japanese Subjects

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Aim: We investigated the safety of 600/150 mg regimen of clopidogrel and the pharmacodynamics and pharmacokinetics of both 300/75 mg regimen and 600/150 mg regimen of clopidogrel in 72 Japanese subjects.

Methods: A randomized study was conducted in healthy Japanese male subjects. Eligible subjects were stratified by dose regimen (300 mg loading dose of clopidogrel on day 1 followed by a 75 mg maintenance dose from days 2 to 7 or a 600 mg loading dose of clopidogrel on day 1 followed by a 150 mg maintenance dose from days 2 to 7) and CYP2C19 metabolizer group [extensive metabolizers (EMs), intermediate metabolizers (IMs), and poor metabolizers (PMs)]. Platelet aggregation and platelet reactivity were evaluated by measuring the maximum platelet aggregation intensity (MAI) induced by 5 and 20 μ M ADP, phosphorylation of vasodilator-stimulated phosphoprotein (VASP), and P2Y₁₂ reaction units (PRU) using the VerifyNow system, respectively. We also measured the plasma concentrations of clopidogrel and its active metabolite H₄.

Results: No treatment emergent adverse events in the 300/75 mg and 600/150 mg regimen were observed in EMs, IMs, and PMs. All CYP metabolizer groups exhibited a lower MAI (%) induced by ADP in the 300/75 mg and 600/150 mg clopidogrel regimens, and MAI (%) in IM group was equipotent to EM irrespective of the clopidogrel dosage. The double dose regimen decreased MAI in the PM group as equipotent to the IM group receiving the standard dose regimen without the extension of bleeding time. No clear relationship of exposure to clopidogrel and CYP2C19 function was observed, whereas active metabolite H₄ exposure was likely to be related to CYP2C19 function.

Conclusion: Clopidogrel in the 600/150 mg regimen was well tolerated. All CYP metabolizer groups exhibited a lower MAI (%) induced by ADP and anti-platelet activities analyzed by VASP and VerifyNow test in the 300/75 mg and 600/150 mg regimens in healthy Japanese subjects.

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Key words: Clopidogrel, CYP2C19 genotype, Platelet aggregation, Active metabolite H₄

Introduction

Clopidogrel in a standard 300/75 mg regimen is used with aspirin to prevent thrombotic occlusion

during and after percutaneous coronary intervention (PCI), irrespective of stent implantation (PCI-stenting), because of its ability to inhibit platelet aggregation¹⁾.

Several studies indicated that 600 mg clopidogrel shows a faster onset of action as compared to 300 mg^{2,3)} and short-term treatment of 150 mg clopidogrel improves clinical outcomes in the acute/sub-acute phase in patients with coronary artery disease in whom early invasive treatment was applied⁴⁾. Since it has been well known

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that the risk of stent thrombosis during acute and sub-acute phases in coronary stent implanted patients is higher than that in the remote stage, a strong anti-platelet drug in those phases is required for the prevention of thromboembolism after PCI. Furthermore, the treatment of the higher dose regimen for this indication is considered. It is well established that a standard 300/75 mg regimen was well tolerated and has a potent inhibitory effect on platelet aggregation in patients^{1, 5)}. In addition, the safety and efficacy of clopidogrel with a double dose regimen (600 mg loading followed by 150 mg/day maintenance dose) is already shown in healthy subjects and patients with PCI outside Japan^{4, 6, 7)}. However, the 600/150 mg regimens have not been sufficiently evaluated in safety, pharmacodynamics, and pharmacokinetic studies in healthy Japanese subjects.

Clopidogrel is a pro-drug, metabolized by the hepatic enzymes, such as CYP2C19, CYP3A4, CYP1A2, CYP2B6, to its active metabolite H4, which inhibits ADP-induced platelet aggregation via its binding to P2Y₁₂ ADP receptors^{8, 9)}. *In vitro* investigations suggest that the oxidative metabolism of clopidogrel, linked to the active metabolite production, may be regulated by several cytochrome P450 (CYP) isozymes, including CYP2C19 (polymorphic enzyme). Moreover, associations have been reported between clopidogrel response and genetic polymorphisms in CYP2C19, specifically CYP2C19*2 and *3 a loss of function variant.

It is well known that the CYP2C19 genotype varies in frequency among different ethnic groups: the frequencies of EMs, IMs, and PMs were 35%, 46%, and 19%, respectively, in Asian population, 69%, 28%, 3% in Caucasians and 68%, 28%, and 4% in Africans¹⁰⁾. The inter-individual variability in clopidogrel metabolism and its effective inhibition of platelet aggregation between individuals with different CYP2C19 genotypes have been reported in previous studies^{11, 12)}.

With this background, we investigated the safety, tolerability, pharmacodynamics, and pharmacokinetics of 7-day repeated oral doses of clopidogrel (300 mg loading dose followed by 75 mg/day and 600 mg loading dose followed by 150 mg/day) in healthy Japanese subjects with three different groups of CYP2C19 genotype. The primary objective of our study was to evaluate the safety of the 600/150 mg clopidogrel regimen, the major secondary objective was to assess the pharmacodynamics of clopidogrel, and an additional secondary objective was to assess the pharmacokinetics of clopidogrel and its active metabolite H4 after the administration of 300/75 mg and 600/150 mg clopidogrel regimens.

Methods

Subjects

This study was conducted in 72 healthy Japanese male subjects. All subjects were categorized according to their CYP2C19 genotypes as EMs: CYP2C19*1/*1, IMs: CYP2C19*1/*2 and CYP2C19*1/*3, and PMs: CYP2C19*2/*2, CYP2C19*2/*3, and CYP2C19*3/*3. All subjects were stratified by dosage regimens (300/75 mg and 600/150 mg regimens) and CYP2C19 genotyping groups. The major inclusion criteria for the participants were as follows: healthy male gender, age between 20 and 45 years, body weight between 50.0 and 90.0 kg, body mass index (BMI) between 18.0 and 28.0 kg/m² inclusive, and provision of written informed consent prior to any procedure related to the study. Subjects were excluded if they had any of the following characteristics: any contraindication to clopidogrel, such as hypersensitivity to the drug or any component of the product; active pathological bleeding, such as a peptic ulcer or intracranial hemorrhage; inability to abstain from the intake of any drug affecting hemostasis for 1 month before the administration of the study drug to the end of the study visit, especially the intake of clopidogrel, ticlopidine, salicylate derivatives, and steroidal and non-steroidal anti-inflammatory drugs; intake of any medication including St John's Wort within 14 days before the study inclusion or within 5 times the elimination of half-life or pharmacodynamic half-life of that drug, and abnormal hemostasis screen [platelet count <150 Giga/L; platelet aggregation induced by 5 μ M adenosine diphosphate (ADP) $\leq$ 50%, 1 mM arachidonic acid or 4 μ g/mL collagen $\leq$ 40%].

Ethics and Good Clinical Practice

This study was conducted in accordance with the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines¹³⁾. The investigators complied with all applicable regulatory and legal requirements, ICH GCP guidelines, and the Declaration of Helsinki¹⁴⁾ when obtaining and documenting the informed consent. No subject was involved in any trial-related activity without obtaining the appropriate written informed consent. The study protocol was reviewed by the ethics committee at the Sekino Clinical Pharmacology Clinic (Tokyo, Japan). Approval of the ethics committee was obtained before the study was initiated.

Study Design

A single-center, randomized, double-blind, placebo-controlled, repeated dose, two dose regimens,

Fig. 1. Flow charts of study design.

Each genotyping group by dosage regimen enrolled 12 subjects and they were randomly allocated to receive either Placebo ($n=3$) or clopidogrel ($n=9$). Because CYP2C19 genotyping cannot influence on PK and PD profile of placebo, subjects who received Placebo were accumulated as the Placebo group by dosage regimen strata.

safety, tolerability, pharmacodynamics, and pharmacokinetics study was conducted in the three different groups (EMs, IMs, and PMs) of CYP2C19 genotyped healthy Japanese subjects. The flow charts of study design are shown in **Fig. 1**. The study was divided into dose 1 (standard dose, 300/75 mg regimen) and dose 2 (double dose, 600/150 mg regimen). Subjects were screened within 2 to 21 days before randomization and eligible subjects were randomly allocated to receive either clopidogrel or the placebo under double-blinded fashion with the stratification of CYP2C19 metabolizer groups (EMs, IMs, and PMs) for each 300/75 mg and 600/150 mg dose regimen. An oral dose of 300 mg or 600 mg was used as a loading dose on day 1, and 75 mg or 150 mg was used as a maintenance dose (once daily, p.o.) from days 2 to 7 in EMs, IMs, or PMs groups (12 subjects/group: 9 subjects for clopidogrel and 3 subjects for the placebo) for each 300/75 mg and 600/150 mg regimen. The observation period was days 7 to 14 after the first clopidogrel dosing because the inhibition of platelet aggregation was

effective through the platelet lifespan.

All doses were orally administered with 240 mL of tap water after a fasting period of at least 10 h. On days 1 and 7, the subjects continued to fast for 4 h after clopidogrel dosing. On days 2 to 6, the subjects took a standard breakfast at 30 min after dosing.

Safety

Safety was assessed in the subjects after the administration of clopidogrel 300/75 mg and 600/150 mg regimens. Safety evaluations included adverse events, clinical laboratory tests (hematology, coagulation, biochemistry, and urinalysis), vital signs (heart rate and blood pressure), body temperature, 12-lead electrocardiography (ECG), and physical examination. Clinically significant abnormalities were monitored until the findings were resolved or until the subjects were clinically stable.

Pharmacodynamics

The primary pharmacodynamics variable was the

maximum platelet aggregation intensity (MAI) induced by 5 μ M ADP. The main secondary pharmacodynamics variables were MAI induced by 20 μ M ADP, PRI-VASP (platelet reactivity index, %), VerifyNow [P2Y₁₂ reaction unit (PRU), inhibition rate of PRU relative to baseline (%)], and bleeding time (prolongation factor).

1) ADP-Induced MAI (%)

To measure the platelet aggregation, the MAI (%) induced by ADP (5 and 20 μ M) in platelet-rich plasma was measured using an optical aggregometer (MCM Hematracer 313M SSR Engineering Co. Ltd, Tokyo, Japan)¹⁵. The blood samples were collected at 0 (predose), 1, 2, 4, and 6 h after clopidogrel dosing on day 1 and at 0 h (predose) on day 2. To assess the platelet response under steady-state conditions, the blood samples were collected at 2, 4, and 6 h after dosing on day 7 and at 72 h after dosing on day 10. The blood samples were also collected in duplicate at each time point. Blood collection, sample management, and testing procedures were standardized to avoid systematic errors.

2) Vasodilator-Stimulated Phosphoprotein Test

Platelet reactivity was evaluated by measuring the phosphorylation of VASP^{16, 17}. Blood samples were collected at 0 h (predose), 1, 2, 4, and 6 h after dosing on day 1; at 0 h (predose) on day 2; and at 2, 4, and 6 h after dosing on day 7. VASP phosphorylation was compared in the presence or absence of ADP by measuring the optical density of each sample at 405 nm and calculating the platelet reactivity index (PRI-VASP).

3) VerifyNow P2Y₁₂ Assay

The VerifyNow-P2Y₁₂ Assay (Accumetrics, Inc., San Diego, CA, USA) was used as described previously¹⁸. This assay system is designed to measure platelet P2Y₁₂ receptor blockade in subjects and patients already receiving clopidogrel. The blood samples were collected at 0 (predose), 1, 2, 4, and 6 h on day 1; at 0 h (predose) on day 2; and at 2, 4, and 6 h after dosing on day 7. The extent of platelet aggregation was evaluated in P2Y₁₂ reaction units (PRUs), and the inhibition rate of PRUs (%) was calculated relative to the baseline. PRU was determined based on the rate and extent of platelet aggregation. The inhibition rate of PRU (%) represented the percent change from baseline aggregation, and it was calculated from the PRU values at each time point and the PRU value at baseline.

4) Bleeding Time

The prolongation factor of bleeding time was

measured as the ratio of bleeding time between 4 and 6 h on day 7 to the bleeding time at baseline. Bleeding time was measured using the Ivy method on the skin of the volar surface of the subject's forearm¹⁹. A blood pressure manchette was placed on the upper arm and inflated to 40 mmHg. A lancet was used to make a stab wound on the underside of the forearm. The bleeding time was measured from when the incision was made until the bleeding stopped. Filter paper was used to draw the blood every 30 s. The test was finished when bleeding had ceased (defined as blood on filter paper <1 mm). The absolute bleeding time was expressed in seconds.

Pharmacokinetics

For pharmacokinetic assessment, the plasma concentrations of unchanged clopidogrel and its active metabolite H4 were measured, and PK parameters such as C_{max}, AUC_{0–24}, t_{max}, and t_{1/2} were calculated.

To determine the plasma concentrations of clopidogrel, the blood samples were collected at 0 (predose), 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h after clopidogrel dosing on days 1 and 7, and at 0 h (predose) on day 5. For the determination of H4 plasma concentrations, blood samples were also collected at 0 (predose), 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, and 8 h after dosing on days 1 and 7. Plasma concentrations of clopidogrel were measured using a validated liquid chromatography/tandem mass spectrometry (LC-MS/MS) method with a limit of quantification (LOQ) of 5 pg/mL at the Drug Disposition Department, Sanofi-Aventis, Malvern, Great Valley (USA). The plasma concentration of H4 was measured using a validated LC-MS/MS method with an LOQ of 0.5 ng/mL at the Drug Disposition Department, Sanofi-Aventis (Montpellier, France). Pharmacokinetic parameters including C_{max}, t_{max}, AUC_{0–24}, and t_{1/2} were calculated using WinNonlin software (PKDMS Version 2.1 incorporating WinNonlin Professional, Version 5.2.1, Pharsight, Mountain View, CA, USA) using the plasma concentrations of clopidogrel and H4.

Statistical Analysis

Pharmacodynamics

MAI (%) and PRI-VASP (%) were analyzed using a linear mixed effects model to compare the three CYP2C19 metabolizer groups (IMs minus EMs, PMs minus EMs, and PMs minus IMs) for each dose regimen separately. MAI (%) and PRI-VASP (%) were analyzed to compare the three metabolizer groups (PMs receiving 600/150 mg minus EMs receiving 300/75 mg, and PMs receiving 600/150 mg minus

IMs receiving 300/75 mg) on day 7. The results are presented as the mean difference, with 2-sided 90% confidence intervals (CIs). Equipotency was concluded if the 90% CIs for the differences in MAI (%) and PRI-VASP (%) between the CYP2C19 groups were entirely within -15%; +15%. With a total of 72 subjects (9 subjects under clopidogrel and three subjects under the placebo per metabolizer group for each dose regimen), if the true total variability in MAI(%) was less than or equal to 15% (total SD was 11.6% in the previous Japanese study, PDY10393), the difference in means between the three metabolizer groups was estimated in an acceptable range of -15%; +15%, for any true difference in the means of MAI (%). The MAI (%) test, PRI-VASP (%) test, VerifyNow P2Y₁₂ assay, and bleeding time analysis were summarized according to each treatment group and CYP2C19 metabolizer group for each dose regimen using descriptive statistics.

Pharmacokinetics

For unchanged clopidogrel and H4, the effects of CYP2C19 genotypes in the three metabolizer groups (IMs vs. EMs, PMs vs. EMs, and PMs vs. IMs) were assessed on log-transformed parameters with a linear fixed effects model for each dose regimen. The dose proportionality on day 1 (loading dose of 300 and 600 mg) and day 7 (maintenance doses of 75 and 150 mg) for PK parameters (based on days 1 and 7) was also assessed in each CYP2C19 metabolizer group for each dose regimen separately.

No sample size calculation was performed, because the sample size for this study was based upon empirical considerations.

Results

Subjects Characteristics

The study was conducted at the Sekino Clinical Pharmacology Clinic between August 2010 and January 2011. A total of 72 healthy Japanese male subjects were enrolled with an age range from 20 to 35 years and weight range from 50.4 to 87.4 kg. Thirty-six subjects were randomized to each dosage regimen (standard dose 300/75 mg regimen or double dose 600/150 mg regimen) and classified into three CYP2C19 metabolizer groups according to their CYP2C19 genotypes. All subjects receiving the 300/75 mg regimen strata completed the study, and these subjects were included in the safety, pharmacodynamics, and pharmacokinetic populations. Each subject in the placebo group and the IM group of the 600/150 mg regimen strata withdrew on days 2 and 7, respectively, owing to per-

Fig. 2. The effect of 300/75 mg clopidogrel regimen on the MAI (%) induced by 5 μ M ADP in EMs, IMs, and PMs.

Mean $\pm$ SE,

EMs: extensive metabolizers, IMs: intermediate metabolizers, PMs: poor metabolizers; MAI: maximum platelet aggregation intensity

sonal reasons. Demographics and subjects characteristics were comparable among the treatment groups.

Safety Profile

No treatment emergent adverse events (TEAEs) were observed in any of the 72 subjects in the 300/75 mg and 600/150 mg regimens. No clinical changes in vital signs (heart rate and blood pressure), ECG, and laboratory test parameters were observed. Seventeen subjects had on-treatment potentially clinically significant abnormality (PCSAs) for biochemistry parameters in uric acid (3 subjects in the placebo group, 9 subjects in the 300/75 mg regimen group, and 5 subjects in the 600/150 mg regimens group). Nevertheless, no particular distribution in the CYP2C19 groups was observed and these findings were not clinically significant.

Pharmacodynamics

MAI Induced by 5 and 20 μ M ADP

The MAI (%) induced by 5 μ M ADP after treatment with clopidogrel 300/75 mg regimen is shown for each metabolizer group in **Fig. 2**. All CYP2C19 metabolizer groups exhibited a lower mean MAI (%) value on day 7 than the placebo group; 71.72% for the placebo, 32.87% for EMs, 39.41% for IMs, and 47.48% for PMs. Mean differences of MAI (%) induced by 5 μ M ADP on day 7 between CYP2C19 metabolizer groups after treatment with the 300/75 mg dose regimen are given in **Table 1** with their 2-sided CIs. The 90% CIs for the difference in MAI

Table 1. Estimates of CYP2C19 group difference in MAI (%) induced by 5 μ M ADP with 90% confidence interval at day 7 for the 300/75 mg regimen

Parameter	Comparison at day 7	Estimate	90% CI
MAI (%) - ADP 5 μ M	IMs minus EMs	5.85	(0.68 to 11.02)
	PMs minus EMs	14.05	(10.17 to 17.93)
	PMs minus IMs	8.20	(3.50 to 12.90)

(%) induced by 5 μ M ADP on day 7 between the IM and EM groups as well as between the PM and IM groups were entirely within the protocol-specified boundaries (-15% ; $+15\%$), thus demonstrating equipotency between these groups.

The MAI (%) induced by 5 μ M ADP after treatment with the 600/150 mg dose regimen is shown for each metabolizer group in **Fig. 3**. All CYP2C19 metabolizer groups exhibited a lower mean MAI (%) value on day 7 than the placebo group; 75.19% for the placebo, 30.02% for EMs, 31.13% for IMs, and 44.06% for PMs. The mean differences of MAI (%) induced by 5 μ M ADP on day 7 between CYP2C19 metabolizer groups after treatment with the 600/150 mg dose regimen are given in **Table 2A** with their 2-sided CIs. The 90% CI for the difference in MAI (%) induced by 5 μ M ADP between the IM and EM CYP2C19 metabolizer groups on day 7 was entirely within the protocol-specified boundaries (-15% ; $+15\%$), thus demonstrating equipotency between these two groups.

The mean differences of MAI (%) induced by 5 μ M ADP on day 7 between the PM group after the 600/150 mg dose regimen and the EM and IM groups after the 300/75 mg dose regimen are shown in **Table 2B**. Equipotency at the double dose for PMs compared to the standard dose for IMs was demonstrated.

The inhibitory response of clopidogrel on 20 μ M ADP-induced platelet aggregation in the 300/75 mg and 600/150 mg regimens was almost similar to that observed against platelet aggregation induced by 5 μ M ADP (data not shown).

PRI-VASP

The mean values of PRI-VASP (%) up to 7 days after treatment with clopidogrel are shown for each metabolizer group in **Fig. 4** and **5** for the 300/75 mg dose regimen and for the 600/150 mg dose regimen, respectively. For the 300/75 mg dose regimen on day 7, EM, IM, and PM groups had PRI-VASP (%) of 31.26%, 48.90%, and 68.55%, respectively, whereas the placebo group had PRI-VASP (%) of 94.60%. For the 600/150 mg dose regimen on day 7, EM, IM, and PM groups had PRI-VASP (%) of 21.06%, 19.75%,

Fig. 3. The effect of 600/150 mg clopidogrel regimen on the MAI (%) induced by 5 μ M ADP in EMs, IMs, and PMs.

Mean $\pm$ SE,

EMs: extensive metabolizers, IMs: intermediate metabolizers, PMs: poor metabolizers; MAI: maximum platelet aggregation intensity

and 54.14%, respectively, whereas the placebo group had PRI-VASP (%) of 91.93%.

The mean differences of PRI-VASP (%) on day 7 between CYP2C19 metabolizer groups after treatment with clopidogrel are given with their 2-sided CIs in **Table 3** for the 300/75 mg dose regimen and in **Table 4A** for the 600/150 mg dose regimen.

The 90% CIs for the difference in PRI-VASP (%) between the IM and EM CYP2C19 metabolizer groups for the 600/150 mg dose regimen on day 7 were entirely within the protocol-specified boundaries (-15% ; $+15\%$), thus demonstrating equipotency between these two groups.

The mean differences of PRI-VASP (%) on day 7 between the PM group after the 600/150 mg dose regimen and the EM and IM groups after the 300/75 mg dose regimen are shown in **Table 4B**. Equipotency at the higher dose for PMs compared to the lower dose for EMs or IMs was not demonstrated.

VerifyNow PRU

For the 300/75 mg dose regimen on day 7, the

Table 2A. Estimates of CYP2C19 group difference in MAI (%) induced by ADP 5 μ M with 90% confidence interval at day 7 for the 600/150 mg regimen

Parameter	Comparison at day 7	Estimate	90% CI
MAI (%) - ADP 5 μ M	IMs minus EMs	1.02	(-5.38 to 7.42)
	PMs minus EMs	12.93	(7.10 to 18.75)
	PMs minus IMs	11.91	(4.84 to 18.97)

Table 2B. Estimates of CYP2C19 group difference in MAI (%) induced by ADP 5 μ M with 90% confidence interval at day 7 for the dose regimens clopidogrel 600/150 mg (PMs) minus 300/75 mg (EMs and IMs) - pharmacodynamic population

Parameter	Comparison	Estimate	90% CI
MAI (%) - ADP 5 μ M	PMs (600/150 mg) minus EMs (300/75 mg) at day 7	11.12	(5.21 to 17.04)
	PMs (600/150 mg) minus IMs (300/75 mg) at day 7	4.58	(-1.83 to 10.99)

Fig. 4. The effects of 300/75 mg clopidogrel on PRI-VASP in EMs, IMs, and PMs.Mean $\pm$ SE,

EMs: extensive metabolizers, IMs: intermediate metabolizers, PMs: poor metabolizers; PRI-VASP: platelet reactivity index vasodilator-stimulated phosphoprotein

EM, IM, and PM groups had an inhibition rate of PRU relative to the baseline (%) of 78.64%, 59.85%, and 34.55%, respectively. For the 600/150 mg dose regimen on day 7, the EM, IM, and PM groups had an inhibition rate of PRU relative to baseline (%) of 89.57%, 83.19%, and 44.04% respectively. The inhibitory rate of PRU was very similar to the results of MAI (%) induced by 5 μ M ADP.

Bleeding Time

After the administration of clopidogrel, the bleeding time prolongation ratio increased from 1.05 to 1.58-fold with the 300/75 mg regimen and from 1.24

Fig. 5. The effect of 600/150 mg clopidogrel on PRI-VASP in EMs, IMs, and PMs.Mean $\pm$ SE,

EMs: extensive metabolizers, IMs: intermediate metabolizers, PMs: poor metabolizers; PRI-VASP: platelet reactivity index vasodilator-stimulated phosphoprotein

to 1.55-fold with the 600/150 mg regimen on day 7 with no clear differences among EMs, IMs, and PMs.

Pharmacokinetic Profile

PK Parameters of Clopidogrel After the Administration of the 300/75 mg and 600/150 mg Regimens

PK parameters such as $t_{\max}$, $t_{1/2}$, $C_{\max}$, and AUC_{0-24} of clopidogrel were investigated after the administration of the 300/75 mg and 600/150 mg clopidogrel regimens in EMs, IMs, and PMs. In the 300/75 mg and 600/150 mg regimen groups, the $t_{\max}$ of clopidogrel was generally 0.75–1.5 h on days 1 and 7, and $t_{1/2}$ was approximately 8 h and 7–11 h on days 1 and

Table 3. Estimates of CYP2C19 group difference in PRI-VASP (%) with 90% confidence interval at day 7 for the 300/75 mg regimen

Parameter	Comparison	Estimate	90% CI
PRI-VASP (%)	IMs minus EMs at day 7	14.95	(3.20 to 26.70)
	PMs minus EMs at day 7	38.70	(26.52 to 50.88)
	PMs minus IMs at day 7	23.75	(11.85 to 35.65)

Table 4A. Estimates of CYP2C19 group differences in PRI-VASP (%) with 90% confidence interval at day 7 for the 600/150 mg regimen

Parameter	Comparison	Estimate	90% CI
PRI-VASP (%)	IMs minus EMs at day 7	-3.53	(-14.45 to 7.38)
	PMs minus EMs at day 7	32.99	(23.07 to 42.91)
	PMs minus IMs at day 7	36.52	(25.92 to 47.13)

Table 4B. Estimates of CYP2C19 group difference in PRI-VASP (%) with 90% confidence interval at day 7 for the dose regimens clopidogrel 600/150 mg (PMs) minus 300/75 mg (EMs and IMs) -pharmacodynamic population

Parameter	Comparison	Estimate	90% CI
PRI-VASP (%)	PMs (600/150 mg) minus EMs (300/75 mg) at day 7	25.42	(14.37 to 36.46)
	PMs (600/150 mg) minus IMs (300/75 mg) at day 7	9.56	(-0.80 to 19.91)

7. For the 300/75 mg dose regimen, on both day 1 and day 7, a clopidogrel IM/EM ratio ranging from 3.35 to 3.99 was observed for $C_{\max}$ and AUC_{0-24} . These unexpected values were likely due to two IM subjects with much higher $C_{\max}$ and AUC_{0-24} values as compared with those of the other subjects of the IM subpopulation group. For the 600/150 mg dose regimen group, the ratios are estimated as 0.82 to 1.24 on day 1 and 0.54 to 1.12 on day 7; there were no significant differences in clopidogrel exposures among the three CYP2C19 metabolizer groups.

The Effect of CYP2C19 on the PK Parameters of Active Metabolite H4 After the Administration of the 300/75 mg and 600/150 mg Regimens

The plasma concentration-time profile and PK parameters of H4 after the administration of clopidogrel at 300/75 mg and 600/150 mg in EMs, IMs, and PMs are shown in **Fig. 6** and **Table 5**, respectively. $t_{\max}$ and $t_{1/2}$ in both 300/75 mg and 600/150 mg regimens were generally 0.75–1.0 h and 0.4–0.7 h, respectively, on days 1 and 7 after the administration of clopidogrel in EMs, IMs, and PMs. For both 300/75 mg and 600/150 mg regimens on day 7, a 59% and 62% decrease in active H4 metabolite exposure (AUC_{0-24})

was reported in PMs compared to EMs, while a slight decrease (34% and 12%) was observed in IMs compared to EMs. For each dosing regimen separately (300/75 mg or 600/150 mg), there was a slight difference between day 1 and day 7. After a two-fold increase of dose, (from 75 to 150 mg or 300 to 600 mg), active H4 metabolite exposure (AUC_{0-24}) increased close to the dose proportionally in IMs (1.88- and 1.84-fold increase of AUC_{0-24} on day 1 and day 7, respectively) and less than dose proportionally in both EMs (1.56- and 1.38-fold increase of AUC_{0-24} on day 1 and day 7, respectively) and PMs (1.44- and 1.26-fold increase of AUC_{0-24} on day 1 and day 7, respectively).

No clear relationship of the exposure of clopidogrel and CYP2C19 function was observed, whereas active metabolite H4 exposure was likely to be related to CYP2C19 function.

Discussion

This study suggested that the double dose regimen (600/150 mg) of clopidogrel was tolerated in all CYP2C19 metabolizer groups. No TEAEs including bleeding were observed in any genotyping group. Vital signs, ECG, and laboratory parameters did not show

Fig. 6. Plasma concentration profiles of H4 after the administration of 300/75 mg and 600/150 mg clopidogrel in EMs, IMs, and PMs.

Mean $\pm$ SD,

EMs: extensive metabolizers, IMs: intermediate metabolizers, PMs: poor metabolizers

any clinically relevant findings.

With respect to pharmacodynamics, the equipotency in MAI (%) induced by 5 μ M ADP was demonstrated between the IM and the EM groups as well as between the PM and the IM groups after the administration of the standard dose regimen (300/75 mg) and between the IM and the EM groups after the administration of the double dose regimen (600/150 mg) on day 7. When subjects in the PM group took the double dose regimen, MAI on day 7 in double dose regimen was equipotent with that in the IM group receiving the standard dose regimen. The relationship among the genotype groups (EM, IM, and PM) in anti-platelet activities analyzed by the VASP and VerifyNow test were well consistent with the

results of MAI (%) induced by ADP 5 μ M in both dose regimens. Although several bibliographic reports suggested the relationship between the variability of platelet aggregation after receiving clopidogrel and the loss/gain of CYP2C19 function^{11, 12)}, there had still been unknown facts on the magnitude of CYP2C19 functions on the anti-platelet activity of clopidogrel as compared to the placebo, equipotency of anti-platelet activities between CYP2C19 functions, and dose escalation effect of clopidogrel on MAI (%). Our results suggested that 1) MAI (%) after clopidogrel administration was definitely lower than that of the placebo irrespective of CYP2C19 genotyping group, 2) MAI (%) in the IM group was equipotent to that in the EM group regardless of clopidogrel dosage regimen,

Table 5. PK parameters of the active metabolite, H4, in healthy Japanese male subjects

Mean $\pm$ SD (Geometric Mean) [CV%]	300 mg loading dose (day 1)			After 75 mg/day maintenance dose (day 7)			Ratio estimates with 90% CIs on H4 pharmacokinetic parameters on day 7 300/75 mg regimen		
	EM (<i>n</i> =9)	IM (<i>n</i> =9)	PM (<i>n</i> =9)	EM (<i>n</i> =9)	IM (<i>n</i> =9)	PM (<i>n</i> =9)	Comparison *	Estimate	90% CI
C_{max} (ng/mL)	29.8 $\pm$ 9.88 (28.2) [33]	19.6 $\pm$ 4.73 (19.0) [24]	11.4 $\pm$ 4.25 (10.6) [38]	11.1 $\pm$ 4.67 (10.2) [42]	7.00 $\pm$ 3.81 (5.97) [55]	3.90 $\pm$ 1.36 (3.64) [35]	C_{max} IM/EM	0.58	(0.39 to 0.88)
t_{max} ^a (h)	0.75 (0.50–1.50)	1.00 (0.75–1.50)	1.00 (0.50–1.50)	0.75 (0.50–1.50)	0.75 (0.50–1.50)	0.75 (0.50–1.50)	PM/EM PM/IM	0.36 0.61	(0.24 to 0.53) (0.41 to 0.91)
$t_{1/2\alpha}$ (h)	0.524 $\pm$ 0.163 (0.504) [31]	0.415 $\pm$ 0.0478 (0.413) [12]	0.452 $\pm$ 0.101 (0.443) [22]	0.461 $\pm$ 0.155 (0.439) [34]	0.584 $\pm$ 0.392 (0.507) [67]	0.736 $\pm$ 0.636 (0.557) [86]	AUC _{0–24} IM/EM	0.66	(0.51 to 0.85)
AUC _{0–24} (ng•h/mL)	39.9 $\pm$ 16.8 (36.7) [42]	25.7 $\pm$ 6.06 (25.0) [24]	15.9 $\pm$ 4.73 (15.1) [30]	11.1 $\pm$ 3.79 (10.6) [34]	7.20 $\pm$ 1.93 (6.97) [27]	4.58 $\pm$ 1.61 (4.37) [35]	PM/EM PM/IM	0.41 0.63	(0.32 to 0.53) (0.49 to 0.80)
Mean $\pm$ SD (Geometric Mean) [CV%]	600 mg loading dose (day 1)			After 150 mg/day maintenance dose (day 7)			Ratio estimates with 90% CIs on H4 pharmacokinetic parameters on day 7 600/150 mg regimen		
	EM (<i>n</i> =9)	IM (<i>n</i> =9)	PM (<i>n</i> =9)	EM (<i>n</i> =9)	IM (<i>n</i> =9)	PM (<i>n</i> =9)	Comparison *	Estimate	90% CI
C_{max} (ng/mL)	33.3 $\pm$ 20.8 (29.2) [62]	32.1 $\pm$ 18.3 (28.0) [57]	12.0 $\pm$ 4.28 (11.4) [36]	12.3 $\pm$ 6.34 (11.3) [52]	11.4 $\pm$ 6.11 (9.88) [54]	4.42 $\pm$ 1.66 (4.17) [38]	C_{max} IM/EM	0.88	(0.61 to 1.27)
t_{max} ^a (h)	1.00 (0.75–3.00)	1.00 (0.75–1.50)	1.00 (0.50–2.00)	0.77 (0.50–1.00)	0.75 (0.50–1.00)	0.75 (0.50–1.50)	PM/EM PM/IM	0.37 0.42	(0.26 to 0.53) (0.29 to 0.61)
$t_{1/2\alpha}$ (h)	0.701 $\pm$ 0.562 (0.595) [80]	0.572 $\pm$ 0.218 (0.542) [38]	0.680 $\pm$ 0.300 (0.632) [44]	0.533 $\pm$ 0.263 (0.489) [49] ^b	0.659 $\pm$ 0.508 (0.553) [77]	0.702 $\pm$ 0.339 (0.630) [48] ^c	AUC _{0–24} IM/EM	0.88	(0.69 to 1.12)
AUC _{0–24} (ng•h/mL)	60.7 $\pm$ 23.4 (57.2) [39]	50.5 $\pm$ 21.1 (46.9) [42]	22.6 $\pm$ 6.95 (21.8) [31]	15.1 $\pm$ 4.84 (14.6) [32] ^b	13.4 $\pm$ 4.18 (12.8) [31]	5.63 $\pm$ 1.28 (5.51) [23] ^c	PM/EM PM/IM	0.38 0.43	(0.29 to 0.49) (0.33 to 0.55)

* Ratio estimates with 90% CIs on H4 pharmacokinetic parameters between CYP2C19 metabolizer groups

^a: median (Min-Max), ^b: *n*=8, ^c: *n*=7

and 3) the double dose regimen decreased MAI in the PM group as equipotent to that in the IM group receiving the standard dose regimen without the extension of bleeding time.

Regarding pharmacokinetics, a decrease in the active metabolite H4 exposure (AUC_{0–24}) was reported in PMs compared to EMs and a slight decrease was observed in IMs compared to EMs, while we could not find a conclusive effect of CYP2C19 function on the exposure of clopidogrel.

Our study has some limitations. Although approximately 80% of clopidogrel can be biotransformed to an inactive metabolite through hydrolysis by esterase, the effect of this hydrolysis on inter-individual variability was not adjusted in the present study, and therefore, we do not know how the differences of esterase activity among individuals affect the clopidogrel content and its metabolism by CYP enzymes. The effects of other CYP enzymes that oxidize clopidogrel, excluding CYP2C19, were not considered in the present study. Therefore, it is unclear whether the degree

of contribution rates of metabolism by those CYP enzymes may be excluded or should be considered. Similarly, the present study does not take account of the effects of transporters that metabolize clopidogrel. The effects of transporters on some PK/PD parameters of clopidogrel have been reported, but in the present study, the distribution and effects of several transporters were not investigated. This study was a non-crossover design. Therefore, it is unknown exactly how the dose increases would affect the equipotency between the phenotypes. The study was also conducted in only young healthy adult males.

Conclusion

No TEAEs in the 300/75 mg and 600/150 mg regimen were observed. Clopidogrel in the 600/150 mg regimen was well tolerated. All CYP metabolizer groups exhibited a lower MAI (%) induced by ADP and MAI (%) in IMs that was equipotent to that in EMs irrespective of the clopidogrel dosage. MAI (%)

in PMs receiving a double dose of clopidogrel was equipotent to IMs receiving the standard dose clopidogrel in healthy Japanese subjects.

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Authors' Contributions

M. Kobayashi and M. Kajiwarra are employees of Sanofi K.K., Tokyo, Japan. Dr. Hasegawa reviewed and revised the draft manuscript. Sanofi K.K. was responsible for the study design, the statistical analysis plan, and the drafting. All authors had reviewed and approved the final version of this manuscript.

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Conflict of Interests

M. Kobayashi and M. Kajiwarra are employees of Sanofi K.K., Tokyo, Japan. Dr. Hasegawa has no conflicts of interest to disclose.

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