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The Japanese Aggrenox (Extended-Release Dipyridamole plus Aspirin) Stroke Prevention versus Aspirin Programme (JASAP) Study: A Randomized, Double-Blind, Controlled Trial

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Key Words
Aspirin · Clinical trials · Ischemic stroke · Antiplatelet therapy · Secondary prevention

Abstract
Background: Despite improvements in treatment, stroke still carries a high death toll and disability in Asia. Extended-release dipyridamole (ER-DP) plus acetylsalicylic acid (ASA) has consistently been shown to be superior over conventional platelet inhibition by ASA. ER-DP plus ASA is well established in the secondary prevention of stroke in a lot of countries including the USA and Europe. DP has an established benefit in the treatment of heart disease in Japan; however, for the prevention of stroke, the fixed-dose combination of ER-DP plus ASA has only been investigated in a small number of patients in Japan. ***Methods:*** The aim of this double-blind, randomized clinical trial was to investigate the efficacy and safety of ER-DP plus ASA versus 81 mg ASA over 1 year. The primary end point of this study was the event rate of recurrent ischemic stroke (fatal or nonfatal) using the Kaplan-Meier method and Cox regression analysis. ***Results:*** Of the 1,294 enrolled patients, the primary end point was analyzed in 652 patients in the ER-DP plus ASA group and 639 in the ASA group. The incidence of ischemic stroke was 6.9% for ER-DP plus ASA and 5.0% for ASA with a hazard ratio of 1.47 (95% confidence interval 0.93–2.31) for the primary end point. The ASA treatment group was found to have a lower than expected yearly event rate, compared to other studies in Japanese stroke patients. Noninferiority of ER-DP plus ASA versus ASA could not be shown. The risks of major bleeding events and intracranial hemorrhage were found to be similar between the treatment arms. There were 4 deaths (0.6%) in the ER-DP plus ASA group and 10 (1.6%) in the ASA group. ***Conclusions:*** The results of the study are inconclusive. Noninferiority of ER-DP plus ASA versus ASA could not be established, a difference between treatments could not be shown for the primary end point. Possible reasons for this result include a small sample size, low event rates and too short a treatment duration (ClinicalTrials. gov number, NCT00311402).

The reported study was presented in part at the Japanese Stroke Society meeting on April 10, 2010, and the World Stroke Congress on October 14, 2010.

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Introduction

Asian countries generally have a higher stroke mortality than Western countries [1]. In Japan, stroke is a greater health problem than heart disease [2]. Recently, the lifetime risk of stroke has been given as high as nearly 20% for middle-aged and older adults, underlining the major public health burden in Japan [3]. Data from the Japan Multicenter Stroke Investigators' Collaboration study show that the death toll from cerebrovascular disease is higher than that of cardiovascular disease or even cancer after hospital discharge following ischemic stroke and transient ischemic attack (TIA) [4]. The incidence of stroke can differ within the same country; for example, a survey in China demonstrated that the percentage of patients with ischemic stroke in urban areas was more than 20% higher than that in rural areas [5], which is difficult to explain on the basis of genetic or ethnic variations, and makes conducting clinical trials challenging. Cerebrovascular disease is often seen as part of a common global atherothrombotic cardiovascular problem. However, large differences in the incidence of cerebrovascular disease in Western (USA and Europe) and Asian countries possibly suggest differences between the pathophysiology of these diseases in different parts of the world [1].

In 2009, data from the Japan Standard Stroke Registry showed that the relative frequencies of ischemic and hemorrhagic strokes in Japan were 75.4 and 24.6%, respectively [6]. The percentage of hemorrhagic strokes in East Asia is higher than in the Western world [1], even though the age-adjusted stroke mortality was reduced by about 63% in men and 43% in women between 1961 and 1986 [7]. Smoking and hypertension are more prevalent in Japan compared with other countries [8], which may lead to differences in the pathogenesis of vascular disease. Consequently, regulatory authorities have issued guidance on ethnic factors, such as differences in concomitant therapies, medical practices and food consumption, when evaluating foreign clinical data [9].

Dipyridamole (DP) was licensed in 1960 for clinical use in Japan for indications based on its coronary vasodilatory effect. DP in combination with warfarin received approval in 1982 for therapeutic use based on its antiplatelet effects, with the extended-release (ER) formulation receiving approval for these indications in 1994. ER-DP plus acetylsalicylic acid (ASA) has demonstrated a favorable efficacy and safety profile in all large, placebo-controlled, randomized stroke prevention trials, like the European Stroke Prevention Study 2 (ESPS-2) or the European/Australasian Stroke Prevention in Reversible Ischaemia Trial (ESPRIT), with bleeding rates similar or even lower compared with other antiplatelet therapies [10, 11].

Although international guidelines recommend a fixed-dose combination of ER-DP and ASA for secondary stroke prevention, the combination of ER-DP plus ASA (free or fixed dose) had not yet been approved for this indication in Japan [12–14]. Until now, there has been no formally conducted trial in Japan comparing ER-DP (200 mg twice daily) plus ASA (25 mg twice daily) with ASA 81 mg, one of the most commonly used dose in Japan.

The aim of the Japanese Aggrenox (ER-DP plus ASA) Stroke Prevention versus Aspirin Programme (JASAP) study (for investigators, see the Appendix) was to demonstrate the noninferiority of ER-DP plus ASA to ASA with regard to the event rate of recurrent ischemic stroke. Data from the ESPS-2 study were used to determine the clinically acceptable limit [10] as data were not available for secondary stroke prevention with ASA or ER-DP plus ASA in Japanese patients.

Methods

Study Design

This phase III, randomized, double-blind, parallel-group, active, controlled comparative study was conducted in 157 centers in Japan and sponsored by Boehringer Ingelheim. Patients provided written informed consent prior to participating in the trial.

The chosen noninferiority study design was the basis for the sample size calculation, which was approved by the Pharmaceuticals and Medical Devices Agency in Japan.

The study protocol was approved by the institutions' review committee on human research from all study centers. The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines and local laws and regulations.

Patient Numbers

The study was scheduled to have a 1-year treatment period and, according to the calculation based on the event rate after 1 year with a significance level of 5% (two-sided) and a power of 80% using the log-rank test. A total of 1,294 patients were randomly assigned (ER-DP plus ASA: 655, ASA: 639).

An interim review of the number of events and event rate was conducted under blinded conditions in week 26 (after 500 patients had been included). Following this interim analysis, the planned number of patients was increased from 1,000 to 1,250 in order to obtain at least 75 events.

Patient Selection

Patients aged 50 years or older with an ischemic stroke (excluding cardiogenic cerebral embolism) who met the National Institute of Neurological Disorders and Stroke ad hoc committee's diagnostic criteria of cerebrovascular disease III [15] were eligible for inclusion in the study. The onset of the ischemic stroke, including first and recurrent ischemic stroke, had to take place be-

tween 1 week and 6 months before enrollment in the study. Neurological signs and symptoms had to be considered stable by the investigator, and the responsible lesion had to be confirmed by computerized tomography (CT) or magnetic resonance imaging (MRI).

Patients had to have at least 2 of the following risk factors:

- diabetes [16];
- hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg), or being under treatment for hypertension;
- being a smoker at the time of onset of ischemic stroke;
- body mass index >25;
- previous vascular disease (stroke, myocardial infarction or peripheral arterial disease before the onset of the qualifying ischemic stroke);
- end organ damage (retinopathy, left ventricular hypertrophy or microalbuminuria);
- hyperlipidemia [17].

Patients who met the following criteria were excluded from the study: diagnosis of brain disorders with a bleeding risk; complications of cardiac disorders that may cause cerebral embolism; acute coronary syndromes <6 months before enrollment; history of peptic ulcer in the past 3 years; having undergone arterial reconstruction after developing ischemic stroke; severe disability (modified Rankin Scale 4 or 5); bleeding or bleeding tendencies; severe hypertension (systolic blood pressure ≥180 mm Hg or diastolic blood pressure ≥120 mm Hg); complications such as serious cardiac, renal or hepatic disorders; malignant tumor or having received cancer treatment in the past 5 years; pregnant or lactating women.

Outcomes

The primary end point of this study was recurrent ischemic stroke (fatal or nonfatal).

Secondary end points included: cerebral hemorrhage, subarachnoid hemorrhage, TIA, acute coronary syndromes (acute myocardial infarction, unstable angina, sudden cardiac death), other vascular events, ischemic vascular event composite end point (ischemic stroke, TIA, myocardial infarction, unstable angina or sudden death attributable to thromboembolism), stroke (composite end point of ischemic stroke, cerebral hemorrhage or subarachnoid hemorrhage).

For a post hoc analysis, the event rate of intracranial hemorrhage and the composite end point of stroke or major bleeding were evaluated for different subgroups of patients.

Safety was assessed using the end points, outcomes and definitions detailed in table 1.

All vascular events, sudden death or bleeding events were reviewed and adjudicated by a blinded Event Assessment Committee.

An independent, blinded Safety Monitoring Committee reviewed bleeding events and serious adverse events other than vascular events during the trial.

Statistical Considerations

The primary objective of this study was the comparison of ER-DP plus ASA and ASA to show the noninferiority of ER-DP plus ASA with regard to the event rate of recurrent ischemic stroke. The clinically acceptable limit was based on results from the ESPS-2 study [10]. Additionally, the superiority test was planned

Table 1. Safety end points and definitions

Safety end points

- Incidence of bleeding events (including cerebral hemorrhage and subarachnoid hemorrhage)
  - Major bleeds included life-threatening or non-life-threatening bleeds
  - Minor bleeds included clinically important bleeds or other bleeds
- Incidence and severity of adverse events
- Discontinuation of treatment due to adverse events
- Incidence and severity of adverse events during run-in and maintenance periods, and discontinuation of treatments due to adverse events
- Onset of headache recorded in patient's diary

Safety-relevant outcomes[1]

- Frequency of:
  - Major bleed
  - Minor bleed
  - Number of headaches per day for run-in period
  - Intensity of headaches per day for run-in period
- Change of:
  - Daily dose of acetaminophen for headache during run-in period
  - Daily frequency of acetaminophen administration during run-in period

Major bleed

- Defined as at least 1 of the following: fatal hemorrhage; retroperitoneal hemorrhage, intracranial hemorrhage, intraocular hemorrhage (objective finding and subjective symptom leading to bleeding) or spinal/intraspinal hemorrhages (confirmed by objective findings); bleedings requiring surgery; clinically obvious bleeding requiring ≥4.5 units of blood transfusion or accompanied by a ≥2 g/dl decrease in hemoglobin level
  - Out of these criteria, those that met at least 1 of the following were considered to be a life-threatening bleed: fatal hemorrhage; required use of intravenous inotropic medication to maintain blood pressure; required surgical intervention, or required transfusion of ≥9 units of red blood cells or equivalent whole blood
- Bleeds not defined as major bleeds were classified as minor bleeds; those that met at least 1 of the following criteria were classified as clinically important bleeds:
  - Subcutaneous hematoma of 25 cm^2 or larger
  - Spontaneous epistaxis lasting for ≥5 min
  - Frank hematuria (spontaneous or lasting for ≥24 h after intervention)
  - Spontaneous rectal bleeding (severer than a blood stain on toilet paper)
  - Gingival bleeding lasting for ≥5 min
  - Bleeding requiring hospitalization
  - Bleeding requiring <4.5 units of blood transfusion
  - Others considered clinically relevant by the investigator
- The remaining bleeds were classified as other bleeds

[1] Analyzed according to the statistical principles of efficacy analysis.

Table 2. Study assessments

Assessments	Frequency
Adverse events, Bleeding events, Vascular events, and Vital signs	every 4 weeks
12-lead electrocardiogram, and Laboratory tests	weeks 4, 12, 28, 52 and every 4 weeks after week 56

to compare the 2 treatment groups if the noninferiority was shown.

In the ESPS-2 study, the event rates of recurrent ischemic stroke in the ER-DP plus ASA and ASA groups were 6.0 and 8.5% after 1 year, and 9.9 and 12.9% after 2 years, respectively [10].

The noninferiority limit was set to 2%, which equaled 1.37 with regard to the hazard ratio, for the level to suggest noninferiority of ER-DP plus ASA to ASA. Under these conditions, 500 patients per group were regarded to be sufficient to detect the noninferiority of ER-DP plus ASA to ASA with over 80% power.

The primary analysis population for efficacy was defined as the full analysis set, which excluded patients who did not meet the inclusion criteria, who had never taken the investigational products and who had no data after drug administration.

A similar analysis was conducted for the primary end point using the per-protocol set, to ensure universality of the results. The per-protocol set excluded patients who violated the exclusion criteria, had compliance rates <80% and deviated from the rules regarding concomitant treatment.

Safety was assessed in the treated set, which excluded patients who did not meet the inclusion criteria, had never taken the investigational products and had no data after drug administration. Patients who discontinued due to headache in the run-in period were included in the 'safety analysis in the run-in period'.

The event rates of recurrent ischemic stroke, and some of the secondary end points, were compared between ER-DP plus ASA and ASA when each treatment was given for 52 weeks or longer. The event rate was calculated using the Kaplan-Meier method, and the 2 treatment groups were compared using the Cox proportional hazard model including time from first stroke and age as covariates.

Exploratory analyses were used to assess those secondary end points based on frequencies or changes. The frequencies of major and minor bleeding in both treatment groups were compared using Fisher's exact test.

Treatment Schedule

After the screening phase (–1 to –2 weeks), clinic visits took place at weeks 0, 1 and then every 4 weeks to a maximum of 124 weeks. Visits also took place just before and 2 weeks (+14 days) after patients had terminated treatment.

CT/MRI was performed if onset of stroke or TIA was suspected to confirm the recurrence of disease. The study assessments are detailed in table 2; adverse events, bleeding and vascular events were assessed once a month.

The incidence, intensity and duration of headache, and the dose of acetaminophen were recorded in a patient's diary during the screening phase and for the first 4 weeks.

Randomization

Patients were randomized to a fixed-dose combination product containing ER-DP 200 mg and ASA 25 mg (ER-DP plus ASA) p.o. twice daily, or ASA 81 mg (ASA) p.o. once daily for a minimum of 52 weeks (including a 1-week run-in period), but not longer than 124 weeks, in blocks of 8, by an external enrollment center. Randomization was stratified by time from first stroke and age in order to minimize any confounding effect of these factors on the evaluation of efficacy of the tested treatment regimens.

During the 1-week run-in period to minimize potential headache with ER-DP, patients received ASA 81 mg once daily in the morning and ER-DP plus ASA once daily before bedtime.

Concomitant use of anticoagulant and antiplatelet therapies was prohibited from the run-in period until treatment discontinuation. Thrombolytic therapies, tissue plasminogen activator preparations and urokinase preparations were prohibited from week 0 until treatment discontinuation.

Results

A total of 1,294 patients were randomized and received either ER-DP plus ASA (n = 655) or ASA (n = 639). Of these 1,294 patients, 907 completed the study (n = 445 ER-DP plus ASA, n = 462 ASA). The reasons for withdrawal are included in figure 1.

There were no differences in baseline demographics or characteristics between the 2 treatment groups, given the formal inclusion criteria of 2 or more risk factors (table 3).

The majority of patients had at least 2–4 risk factors at baseline (88.4%; table 3).

The majority of patients were receiving concomitant ASA therapy (71.9%). Other concomitant antiplatelet therapies included DP (1.4%), cilostazol (18.8%), ticlopidine (total 4.9%; ER-DP plus ASA 4.7%, ASA 5.0%) and clopidogrel (total 12.9%; ER-DP plus ASA 11.8%, ASA 14.1%) for the last 12 weeks before screening.

There was an imbalance between the 2 groups for the proportion of patients receiving concomitant statin therapy (ER-DP plus ASA 32.4% vs. ASA 28.6%; p = 0.1479) before screening.

The mean treatment durations in the ER-DP plus ASA and ASA groups were 447 and 471 days, respectively.

Of the patients randomized to treatment, 3 patients in the ER-DP plus ASA group were not included in the analysis (2 took the wrong medication; 1 had no qualifying stroke). The efficacy results were therefore analyzed in a total of 1,291 patients (n = 652 ER-DP plus ASA, n = 639

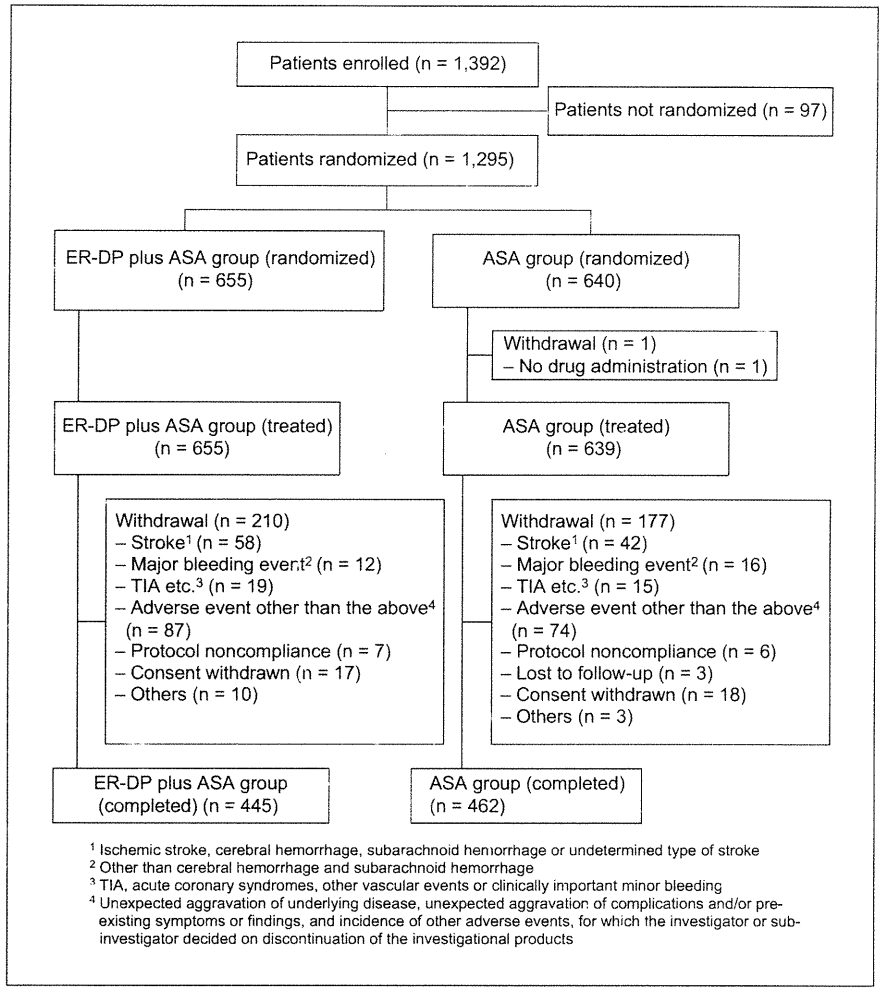

Fig. 1. Patient disposition.

ASA). The efficacy results were also analyzed in the 1,280 patients in the per-protocol population (n = 646 ER-DP plus ASA, n = 634 ASA).

Efficacy

Recurrent ischemic stroke occurred in 45 (6.9%) of the 652 patients in the ER-DP plus ASA group, and in 32 (5.0%) of the 639 patients in the ASA group (fig. 2; table 4). Non-inferiority of ER-DP plus ASA compared with ASA was not shown (hazard ratio 1.47; 95% confidence interval 0.93–2.31; fig. 3). These results were consistent with those in the per-protocol population (table 4; fig. 4).

The event rate of stroke was significantly higher for ER-DP plus ASA compared with ASA (table 4; fig. 4). There was no statistically significant difference between treatment groups for the other secondary end points (table 4; fig. 4).

Post hoc Analysis

A multivariate analysis taking into account potential confounders for recurrence of ischemic stroke but only keeping covariates with a significant contribution in the model revealed a similar result for the comparison between treatments as the primary analysis. This analysis also revealed that higher modified Rankin Scale values and established end organ damage at baseline had a deleterious effect on the primary outcome, whereas the concomitant therapy with statins had a beneficial effect (table 5).

Table 3. Demographics, baseline characteristics and risk factors

	ER-DP plus ASA	ASA	Total	p value
Patients, n	655	639	1,294	
Sex, n				0.6666
Male	472 (72.1)	453 (70.9)	925 (71.5)	
Female	183 (27.9)	186 (29.1)	369 (28.5)	
Mean age ± SD, years	66.2 ± 8.1	66.0 ± 8.6	66.1 ± 8.4	0.1466
Mean BMI ± SD	24.08 ± 3.24	24.14 ± 3.08	24.11 ± 3.16	0.1851
Smoking history, n				
Never smoked	210 (32.1)	208 (32.6)	418 (32.3)	0.9447
Ex-smoker	323 (49.3)	309 (48.4)	632 (48.8)	
Current smoker	122 (18.6)	122 (19.1)	244 (18.9)	
Mean baseline systolic blood pressure ± SD, mm Hg	141.4 ± 18.5	140.4 ± 17.6	140.9 ± 18.1	0.2450
Mean baseline diastolic blood pressure ± SD, mm Hg	81.5 ± 12.0	81.4 ± 11.5	81.5 ± 11.8	0.3080
Timing of occurrence before enrollment, n				0.9802
≤1 month	285 (43.5)	274 (42.9)	559 (43.2)	
>1 month and ≤3 months	245 (37.4)	248 (38.8)	493 (38.1)	
>3 months and ≤6 months	125 (19.1)	117 (18.3)	242 (18.7)	
Clinical category of the last ischemic stroke, n				0.9627
Large-artery atherosclerosis	183 (27.9)	175 (27.4)	358 (27.7)	
Small-artery occlusion (lacune)	443 (67.6)	437 (68.4)	880 (68.0)	
Ischemic stroke of undetermined etiology	29 (4.4)	27 (4.2)	56 (4.3)	
Risk factors, n				0.8429
2	194 (29.6)	188 (29.4)	382 (29.5)	
3	225 (34.4)	238 (37.2)	463 (35.8)	
4	167 (25.5)	132 (20.7)	299 (23.1)	
5	57 (8.7)	60 (9.4)	117 (9.0)	
6	10 (1.5)	20 (3.1)	30 (2.3)	
7	2 (0.3)	1 (0.2)	3 (0.2)	
Hypertension, n	581 (88.7)	562 (87.9)	1,143 (88.3)	0.7292
Diabetes, n	275 (42.0)	248 (38.8)	523 (40.4)	0.2573
Hyperlipidemia, n	448 (68.4)	408 (63.8)	856 (66.2)	0.0885
Obesity, n	244 (37.3)	257 (40.2)	501 (38.7)	0.2787
End organ damage, n	106 (16.2)	126 (19.7)	232 (17.9)	0.1107
History of vascular disease, n	145 (22.1)	154 (24.1)	299 (23.1)	0.4288
Smoking history – time of previous ischemic stroke, n	291 (44.4)	290 (45.4)	581 (44.9)	0.7376
Concomitant therapy with angiotensin receptor blocker, n	299 (45.6)	304 (47.6)	603 (46.6)	0.5038

SD = Standard deviation; figures in parentheses indicate percentages.

Visit-to-visit variability of systolic blood pressure was found to be surprisingly large (up to ±32 mm Hg).

Safety

ER-DP plus ASA and ASA were well tolerated. The total number of adverse events was greater in the ER-DP plus ASA group compared with the ASA group (640 vs. 611, p = 0.04; table 6).

Major bleeding events and clinically relevant minor bleeding events were comparable in the ER-DP plus ASA and ASA groups (table 7).

The difference in drug-related adverse events was mainly due to headache in the early stages of treatment with ER-DP plus ASA (table 6). More patients in the ER-DP plus ASA group discontinued treatment due to headache (table 6).

No relevant changes in laboratory parameters, vital signs and electrocardiography were noted in either treatment group.

There were 4 deaths (0.6%) in the ER-DP plus ASA group and 10 (1.6%) in the ASA group.

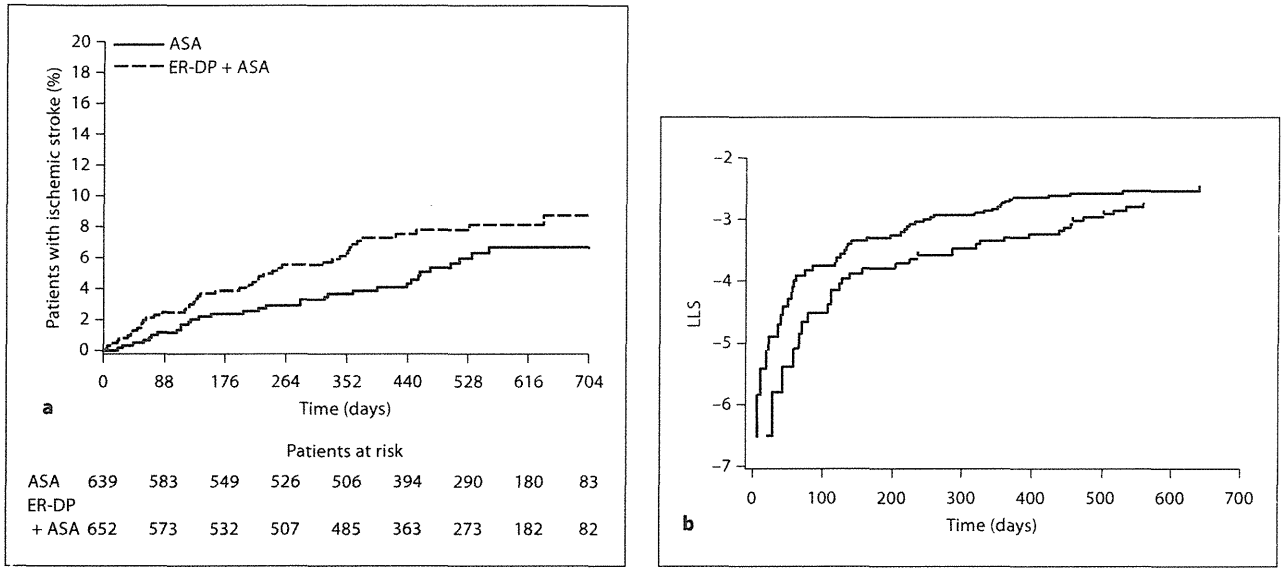

Fig. 2. Kaplan-Meier curve (**a**) and log-log survival (LLS) plot (**b**), with -log(nonevent), for recurrence of ischemic stroke. The estimated hazard ratios are based on a Cox proportional hazards model with the covariates of age and time from qualifying event to entry.

Table 4. Primary and secondary efficacy end points and post hoc analysis

End point	Event rate and HR, n		
	ER-DP plus ASA	ASA	HR of ER-DP plus ASA
Primary end point			
Number (full analysis set)	652	639	
Ischemic stroke	45 (6.9)	32 (5.0)	1.47 [0.93–2.31]
Secondary end points			
Cerebral hemorrhage	12 (1.8)	7 (1.1)	1.79 [0.70–4.54]
Subarachnoid hemorrhage	0	1 (0.2)	0 [0.00, n.a.]
TIA	3 (0.5)	3 (0.5)	1.02 [0.21–5.07]
Acute coronary syndromes	9 (1.4)	16 (2.5)	0.58 [0.26–1.31]
Other vascular events	11 (1.7)	6 (0.9)	1.88 [0.69–5.07]
Ischemic vascular composite end point	57 (8.7)	51 (8.0)	1.16 [0.79–1.69]
Stroke	57 (8.7)	39 (6.1)	1.52 [1.01–2.29]
Post hoc analysis			
Intracranial hemorrhage	13 (2.0)	13 (2.0)	1.04 [0.48–2.25]
Composite end point of stroke or major bleeding	71 (10.9)	55 (8.6)	1.34 [0.94–1.91]

Figures in parentheses indicate percentages, those in square brackets 95% confidence intervals.

n.a. = Not assessed; HR = hazard ratio, calculated using Cox regression analysis; acute coronary syndromes = acute myocardial infarction, unstable angina and sudden cardiac death; other vascular events = pulmonary embolism, retinal vascular disorder, deep vein thrombosis, peripheral artery obstruction and vascular intervention like percutaneous coronary intervention; ischemic vascular composite end point = ischemic stroke, TIA, myocardial infarction, unstable angina or sudden death attributable to thromboembolism; stroke = composite end point of ischemic stroke, cerebral hemorrhage or subarachnoid hemorrhage.

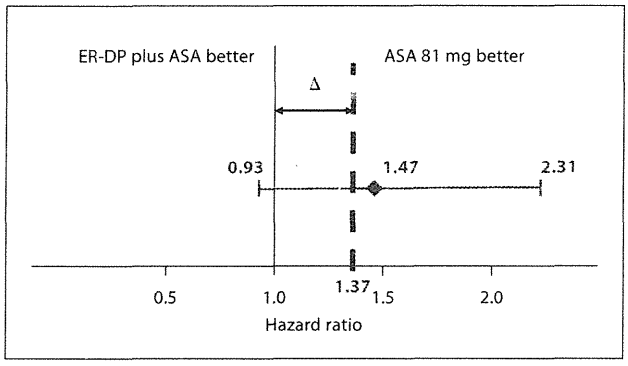

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Fig. 3. Confidence interval for recurrence of ischemic stroke (full analysis set).

Table 5. Post hoc multivariate analysis of hazard ratios for the primary end point of recurrent ischemic stroke, including baseline characteristics with significant contribution to prediction of risk ($p < 0.05$)

Baseline characteristics	Hazard ratio
Treatment (p = 0.078)	
ER-DP plus ASA vs. ASA	1.50 (0.96–2.37)
Modified Rankin Scale (p = 0.003)	
1 vs. 0	0.97 (0.52–1.81)
2 vs. 0	1.43 (0.71–2.90)
3 vs. 0	2.80 (1.41–5.56)
End organ damage (p = 0.012)	
Yes vs. no	1.88 (1.15–3.09)
Concomitant therapy with statin (p = 0.0007)	
Yes vs. no	0.32 (0.16–0.61)

Figures in parentheses indicate 95% confidence intervals.

Sex, age, timing of stroke occurrence before enrollment, clinical category of the qualifying ischemic stroke, number of risk factors, previous stroke (prior to qualifying ischemic stroke), hypertension, diabetes, hyperlipidemia, obesity, history of vascular disease, smoking history (at time of previous ischemic stroke), concomitant therapy with angiotension receptor blocker and concomitant therapy with angiotensin-converting enzyme inhibitor have also been entered into the initial model. Forward selection was used to identify significant contributors.

Discussion

In the JASAP trial, ER-DP plus ASA failed to demonstrate noninferiority over ASA for the event rate of recurrent ischemic stroke, which was the primary objective of this study. Consequently, the study could not prove superiority of one treatment over the other. The observed yearly event rates for ischemic stroke in the JASAP were smaller than expected for the sample size calculation (expected/observed event rate 8.5/3.9% in the ASA group vs. 6.0/5.4% in the ER-DP plus ASA group). The ASA arm showed a far lower event rate than in the ASA arms of other studies conducted about the same time; for example, the Sarpogrelate-Aspirin Comparative Clinical Study for Efficacy and Safety in Secondary Prevention of Cerebral Infarction [18] reported in patients with less pronounced risk profiles than in the JASAP an event rate for ASA of 4.9%/year (table 8), while in the JASAP we expected an event rate for ASA of 8.5%/year but we observed 3.9%/year. In contrast, for the ER-DP plus ASA arm, the expected event rate was 6.0%/year and was in the range of the observed event rate 5.6%/year. A retrospective power calculation revealed that, based on the actually observed lower-than-expected yearly event rates, the JASAP trial had only a 53% power to achieve its primary statistical objective that was to demonstrate noninferiority. Despite the fact that the number of enrolled patients had been increased by 125 patients in each arm and the enrollment period extended by 4 months during the conduct of the trial, the ASA incidence rates from previous trials could not be replicated. Similar underestimations have been observed in another trial conducted at the same time (CSPS 2 [19]).

A very early separation of the 2 Kaplan-Meier plots (within the first few days of treatment initiation) was observed in the JASAP, in the ER-DP plus ASA treatment arm (fig. 2a). This has never been observed in any other stroke prevention trials or trials with ER-DP plus ASA. These findings in the JASAP are unexplained and unexpected. Nevertheless, separation of the Kaplan-Meier curves has been observed after 6 months' treatment in ESPS-2 [10]. Similarly, the slope of the log-log plot is consistently different in the second part of the treatment period potentially crossing at a later time (fig. 2b).

Study design and patient population differed substantially from previous and other trials as well; the JASAP enrolled patients as late as 180 days after the qualifying event, more than 50% of the patients had been recruited more than 1 month after the index stroke, approximately 19% of the patients were recruited more than 3 months after the event, compared with 3% of the patients in PRoFESS and 1.2% in ESPS-2 [10, 20]. Early initiation of treatment has shown a clear benefit, as demonstrated in the recently published EARLY trial, which compared early (within 24 h after the event) initiation of treatment with ER-DP plus ASA (control group received ASA) with initiation after 7 days' standard ASA treatment [21]. The ben-

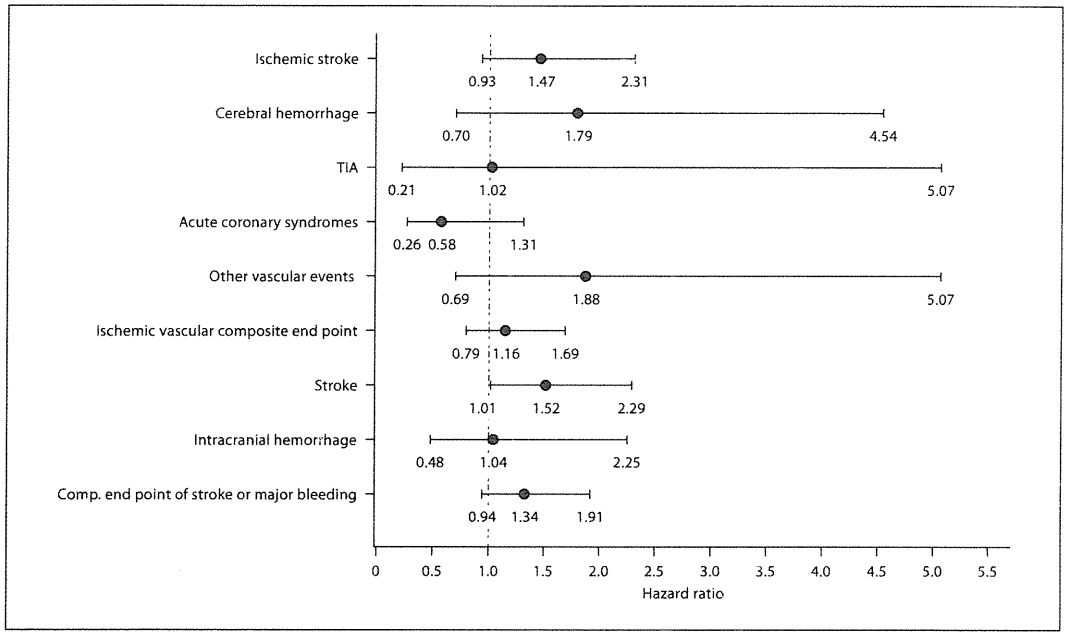

Fig. 4. Forest plot for primary and secondary efficacy end points and post hoc analysis.

Table 6. Adverse events by system organ class and preferred term with a frequency of ≥10% of the patients in either treatment group, other adverse events and mortality data

Adverse event ≥10%	ER-DP plus ASA, n	ASA, n	p value
Patients	655	639	
Total number with adverse events	640 (97.7)	611 (95.6)	0.0431
Serious adverse events	178 (27.2)	167 (26.1)	0.7061
Deaths	4 (0.6)	10 (1.6)	0.1125
Infection and infestations	348 (53.1)	339 (53.1)	1.0000
Nasopharyngitis	281 (42.9)	261 (40.8)	0.4642
Metabolism and nutrition disorders	69 (10.5)	84 (13.1)	0.1682
Nervous system disorders	415 (63.4)	301 (47.1)	<0.0001
Headache	293 (44.7)	187 (29.3)	<0.0001
Eye disorders	104 (15.9)	105 (16.4)	0.8208
Vascular disorders	62 (9.5)	80 (12.5)	0.0908
Respiratory, thoracic and mediastinal disorders	103 (15.7)	96 (15.0)	0.7581
Gastrointestinal disorders	316 (48.2)	320 (50.1)	0.5408
Diarrhea	78 (11.9)	43 (6.7)	0.0016
Skin and subcutaneous tissue disorders	156 (23.8)	152 (23.8)	1.0000
Musculoskeletal and connective tissue disorders	233 (35.6)	216 (33.8)	0.5208
Injury, poisoning and procedural complications	184 (28.1)	197 (30.8)	0.2998
Fall	58 (8.9)	69 (10.8)	0.1069
Other adverse events			
Drug-related adverse events	265 (40.5)	171 (26.8)	<0.0001
Headache <7 days of starting treatment	215 (32.8)	79 (12.4)	<0.0001
Ischemic stroke	45 (6.9)	34 (5.3)	0.2485
Discontinuation of treatment due to adverse events	172 (26.3)	142 (22.2)	0.0922
Discontinued treatment due to headache	17 (2.6)	7 (1.1)	0.0620

Figures in parentheses indicate percentages.

efit of the treatment with DP plus ASA was correlated with a significant reduction in innate inflammation [22], which was shown the highest immediately after stroke [23].

Compared to previous large stroke studies, this trial had the shortest patient exposure to medication (1.3 years vs. 1.4 years in ESPS-2, vs. 2.5 years in PRoFESS and vs. 3.5 years in ESPRIT) [10, 11, 20]. Trials investigating the prevention of a second stroke required several years of observation and follow-up in order to arrive at stable and conclusive data [11, 24], strengthening the argument that in the JASAP trial, patients may have not been treated sufficiently long enough.

Concomitant treatment with irreversible platelet inhibitors (thienopyridines) was observed more frequently in the ASA arm (122 vs. 108 patients). This may have led to a confounding, irreversible inhibition of platelet function (table 3).

More patients with stroke had diabetes in the ER-DP plus ASA group (42.0 vs. 38.8%). Similar differences were observed for patients with concomitant hyperlipidemia (68.4 vs. 63.8%) and those receiving angiotensin receptor blockers (45.6 vs. 47.6%; table 3).

Even though patients with severe hypertension were excluded and concomitant antihypertensive treatment was in line with the Japanese Society of Hypertension guidelines [14], a high rate of hypertension and high visit-to-visit blood pressure differences were observed in both treatment arms. Neither excessive high blood pressure nor high instability has been reported for previous stroke prevention trials with any of the tested study medications.

A post hoc analysis of the visit-to-visit blood pressure measurements revealed a standard deviation of up to ±32 mm Hg of the mean blood pressure for individual patients. Recent publications argued that blood pressure fluctuations are even more predictive of vascular and thromboembolic complications than stable elevated blood pressure alone [25–27].

While direct platelet inhibitors such as ASA or thiopyridines show immediate benefit for controlling highly thrombogenic states such as after angioplasty or stenting, other preventive treatments have a protective or enhancing effect on the wall of blood vessels, such as DP, cilostazol or statins. Statins were found to not only reduce blood lipids, but through their pleiotrophic effects also positively influence antithrombotic/anti-inflammatory properties of the blood vessel walls [28, 29]. However, the

Table 7. Adjudicated bleeding events (numbers, with percentages in parentheses)

	ER-DP plus ASA	ASA	p value
Patients	652	639	
Cerebral hemorrhage	12 (1.8)	7 (1.1)	
Subarachnoid hemorrhage	0	1 (0.2)	
Intracranial hemorrhage	13 (2.0)	13 (2.0)	
Patients	655	639	
Major bleeding event	26 (4.0)	24 (3.8)	0.8859
Life-threatening	0	2 (0.3)	0.2437
Minor bleeding event	166 (25.3)	163 (25.5)	0.9492
Clinically important	37 (5.6)	38 (5.9)	0.9054

Table 8. Comparison of sample size, exposure to drug, event rate for recurrent stroke events and prevalence of risk factors in the JASAP study, the PRoFESS study, the S-ACCESS study, ESPS-2 and ESPRIT

	JASAP (ASA vs. ER-DP plus ASA)	PRoFESS (clopidogrel vs. ER-DP plus ASA)	S-ACCESS (ASA vs. sarpogrelate)	ESPS-2 (ASA vs. ER-DP plus ASA)	ESPRIT (ASA vs. DP plus ASA)
Number of patients	1,294	20,332	1,499	6,602 (4 arms)	2,739
Mean exposure, years	1.3	2.0	1.6	1.4	3.5
Yearly event rate for ischemic strokes[1], %	3.9 (ASA) vs. 5.6 (ER-DP plus ASA)	3.8 (clopidogrel) vs. 4.0 (ER-DP plus ASA)	4.9 (ASA) vs. 6.1 (sarpogrelate)	8.9 (ASA) vs. 6.8 (ER-DP plus ASA)	2.4 (ASA) vs. 2.0 (DP plus ASA)
Risk factor at baseline, n					
Previous cerebral infarction (before qualifying event)	251 (19.4)	3,706 (18.2)	200 (13.3)	1,394 (21.1)	314 (11.5)
Diabetes mellitus	523 (40.4)	5,743 (28.2)	419 (28.0)	1,011 (15.3)	512 (18.7)
Hypertension	1,143 (88.3)	15,048 (74.0)	1,037 (69.2)	3,997 (60.5)	1,631 (59.5)
Hyperlipidemia	856 (66.2)	9,493 (46.7)	593 (39.6)	1,509 (22.9)	1,272 (46.4)

PRoFESS = Prevention Regimen for Effectively Avoiding Second Strokes; S-ACCESS = Sarpogrelate-Aspirin Comparative Clinical Study for Efficacy and Safety in Secondary Prevention of Cerebral Infarction. Figures in parentheses indicate percentages.

[1] In the ESPS-2, reported for all stroke only.

benefit for these approaches has only been shown after a longer period of time, typically 2.5 years and more. Reduction of inflammation has recently also been observed for DP experimentally [30–32] as well as clinically [22], setting this compound apart from conventional inhibitors of platelet function.

Given the high rate of diabetics in this study, it is tempting to compare these data with the diabetic subgroup of SPARCL [24]. A treatment benefit there was only shown in the later part of another trial, such as for nonfatal stroke. Both SPARCL and ESPRIT [11] showed benefit after significantly longer periods of observation. SPARCL also showed a numerical disadvantage for the active treatment of nonfatal stroke in diabetic patients in the early phase of the observation period. This is similar to the JASAP data. The JASAP then showed a convergence of the log-log plots of events, towards the end of the (short) observation period (fig. 2). One could argue that a longer duration of the study would have shown clearer results, as suggested by the extrapolation of the incidence seen in figure 2.

This study was originally designed for registration of ER-DP plus ASA in Japan, and was not of a sufficiently long duration. Modern treatment approaches, directed to restore and enhance antithrombotic functions of the blood vessel, not only require early initiation but also a longer period of treatment to produce the full benefit. This has been demonstrated with ER-DP plus ASA in EARLY and ESPRIT, and with statins in SPARCL.

The results from the JASAP are inconclusive. Neither noninferiority nor superiority of one treatment over the other was demonstrated. However, because of the frequent visits and thus good longitudinal observations in each patient, the JASAP trial highlights the importance of aggressive risk control, particularly blood pressure management, the importance of control of concomitant medication as well as the importance of very early initiation of treatment which is also capable of controlling acute inflammatory processes as well as sufficient length of the trial/treatment period to allow repair/recovery.

Conclusions

The JASAP study did not meet its primary study objective to demonstrate noninferiority of ER-DP plus ASA versus ASA 81 mg. Taken together, the favorable assessment of the combination treatment with ER-DP plus ASA over conventional platelet inhibition by ASA remains unchanged.

Appendix

The JASAP study investigators are as follows.

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Statistical advisor: Hideki Origasa.

Event Assessment Committee: Masayasu Matsumoto (chair), Makoto Takagi, Yoshihiko Seino, Shinya Goto.

Safety Monitoring Committee: Masahiro Yasaka (chair), Takehiko Nagao, Haruko Yamamoto.

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REVIEW

Cardiovascular Therapeutics

Clopidogrel Resistance: Identifying and Overcoming a Barrier to Effective Antiplatelet Treatment

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Keywords
Clopidogrel; Clopidogrel resistance; Cytochrome P450; Drug interaction; Platelets; Polymorphism.

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SUMMARY

Clopidogrel is an inhibitor of the ADP receptor P2Y12 and platelet aggregation. It is widely used for the management of atherothrombotic disease in patients who have experienced severe vascular events such as stroke or myocardial infarction or with peripheral artery disease. However, some patients show "resistance" to clopidogrel, and show impaired inhibition of platelet aggregation. In this review, I discuss the clinical evidence of the extent of the problem, potential implications for future cardiovascular events and clinical tests to assess platelet aggregation. I also discuss the mechanisms that appear responsible for clopidogrel resistance. Clopidogrel is administered as a prodrug and the active metabolite is generated by the cytochrome P450 system. Therefore, inadequate responses to clopidogrel may be caused by polymorphisms in one or more of the cytochrome P450 enzymes and interaction/competition with other drugs metabolized by the cytochrome P450 system (e.g., statins and proton pump inhibitors). Finally, I discuss the therapeutic options available for patients with known or suspected clopidogrel resistance, including the use of drugs with alternative molecular targets (e.g., cilostazol), metabolized via different pathways (e.g., prasugrel) or administered in an active form (e.g., ticagrelor). Clopidogrel resistance is a clinically significant problem with potentially severe consequences if it is not identified or managed appropriately. The availability of point-of-care assays and novel treatments provide clinicians with an extensive array of tools that should aid in the management of atherothrombotic diseases/events, and reduce the risk of future severe events in these patients.

Introduction

Resistance, or poor responsiveness, to antiplatelet agents [1,2] is a significant clinical phenomenon characterized by the occurrence of cardiovascular events, despite adequate therapy with glycoprotein IIb/IIIa (GpIIb/IIIa) inhibitors, the P2Y12-subtype adenosine diphosphate (ADP) receptor (P2Y12) inhibitor clopidogrel, or aspirin, for example. Several studies have shown that an inadequate response to clopidogrel is associated with future cardiovascular events [3–10]. However, despite the awareness of its importance, clopidogrel resistance remains poorly understood—there is no clear definition of clopidogrel resistance in terms of the drug's pharmacologic effect, such as targeting the P2Y12 receptor or lack thereof, or in terms of treatment failure: recurrent vascular atherothrombotic events despite drug adherence. Furthermore, there are no well-defined standardized methods by which to determine the presence of clopidogrel resistance in individuals. There is also no consensus on what alternative treatment strategies may improve outcomes in patients showing clopidogrel resistance. This review aims to provide a summary of the clinical impact of clopidogrel resistance, the potential mechanisms involved, and how clopidogrel resistance can be identified in a clinical setting. It also presents alternative therapeutic options for patients who are either known to exhibit or who may exhibit clopidogrel resistance based on clinical findings.

Platelets are activated by a number of physiologic agonists including thromboxane (Tx) A_2, ADP, thrombin, serotonin, and collagen. Shear stress within blood vessels also plays an important role. Clopidogrel is a thienopyridine derivative prodrug that upon hepatic biotransformation to its active metabolite *in vivo* inhibits platelet activity through irreversible blockade of the platelet surface receptor P2Y12. The clinical efficacy of clopidogrel was first demonstrated in the Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) study [11], which was conducted in nearly 20,000 patients with recent myocardial infarction (MI), recent stroke, or established peripheral artery disease (PAD). The CAPRIE study showed that clopidogrel is at least as effective as aspirin for preventing clinical thrombotic events such as MI, ischemic stroke, and vascular death, with no major difference between these agents in terms of safety. Based on these studies, and on the findings of the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) [12] and the Clopidogrel for the Reduction of Events During Observation (CREDO) [13] studies, for example, clopidogrel is now widely used for the prevention of

vascular events in patients with acute coronary syndrome (ACS) or atherothrombotic diseases, and in patients undergoing percutaneous coronary intervention (PCI).

Based on these findings and the potential risk of future events, it is important to distinguish between treatment failure (which can be attributed to platelet aggregation induced by other stimuli, distinct from the effects of clopidogrel) and the inability of clopidogrel to inhibit its target receptor. In this review, clopidogrel resistance is defined as inability of clopidogrel to exert an effective antiplatelet response via the P2Y12 receptor.

What is Clopidogrel Resistance?

Patients' responses to clopidogrel, as assessed by an array of available tests including aggregometry, follow a normal distribution [14] and the mean response to ADP (i.e., the decrease in platelet aggregation) was 41.9% relative to that of normal plasma. In that study, hypo- and hyper-responsiveness to clopidogrel were considered to be two standard deviations less than and greater than the mean, respectively, with prevalences of 4.2% and 4.8%, respectively. In a systematic review [15], the prevalence of clopidogrel resistance was reported to range from as low as 5% to as high as 44% (Table 1).

Based on these studies of clopidogrel resistance, it appears that there are two distinct processes at work; however, there is some potential for these to combine and increase the risk of future events. Within a clinical setting, clopidogrel resistance is characterized by the occurrence of thrombotic events despite clopidogrel administration. On the one hand, this may be due to decreased activity of clopidogrel induced by competition with other drugs or endogenous molecules, for example, those belonging to the cytochrome P450 system, which is essential to generate the active metabolite of clopidogrel. On the other hand, some patients may show an impaired ability to absorb the drug or to generate the active metabolite because of altered functioning of the cytochrome P450 system, possibly due to polymorphisms of genes encoding these isozymes, and alterations to the molecular structure or expression level of the P2Y12 receptor.

Factors Involved in Clopidogrel Resistance

Clopidogrel Metabolism

Clopidogrel metabolism occurs via two major pathways. In the first pathway, clopidogrel undergoes esterase-mediated hydrolysis, which results in the production of the M1 metabolite (SR26334) and other inactive by-products (Figure 1) [16]. The second pathway is involved in the formation of the active metabolite, R-130964, in two stages. First, the cytochrome P450 isozymes CYP1A2, CYP2C19, and CYP2B6 induce the formation of

Table 1 Prevalence of clopidogrel resistance [15]

Study	n	Patients	Dose (mg, load/qd)	Method and definition of clopidogrel resistance	Time	Prevalence
Gurbel et al. [1]	92	PCI	300/75	5 and 20 μM ADP-induced aggregation: <10% absolute change	24 h	31–35%
Jaremo et al. [81]	18	PCI	300/75	ADP-induced fibrinogen binding <40% of baseline	24 h	28%
Müller et al. [2]	119	PCI	600/75	5 and 50 μM ADP-induced aggregation: <10% relative change	4 h	5–11%
Mobely et al. [82]	50	PCI	300/75	1 μM ADP-induced aggregation, TEG and Ichor PW: <10% absolute inhibition	Pre and post	30%
Lepantalo et al. [83]	50	PCI	300/75	2 or 5 μM AD-induced aggregation and PFA-100: <10% inhibition	2.5 h	40%
Angiolillo et al. [84]	48	PCI	300/75	6 μM ADP-induced aggregation: <40% inhibition	10 min, 4 and 24 h	44%
Matetzky et al. [10]	60	STEMI	300/75	5 μM ADP-induced aggregation and CPA: <10% inhibition	Daily for 5 days	25%
Dziewierz et al. [85]	31	CAD	300	20 μM ADP-induced aggregation: <10% absolute change	5 days 24 h	23%
Lev et al. [86]	150	PCI	300	5 μM ADP-induced aggregation: <10% absolute change	20–24 h	24%
Angiolillo et al. [87]	52	Diabetics and nondiabetics	300	<10% relative inhibition	24 h	38% diabetic; 8% nondiabetic
Gurbel et al. [88]	190	PCI	300 or 600/75	5 and 20 μM ADP-induced aggregation <10% absolute inhibition	24 h	28–32% (300 mg) 8% 600 mg

PCI, percutaneous coronary interventions; ADP, adenosine diphosphate; CAD, coronary artery disease; TEG, thromboelastography; Ichor PW, Ichor Plateletworks; PFA-100, platelet function analyzer-100; CPA, cone and platelet analyzer; STEMI, ST-segment elevation myocardial infarction.

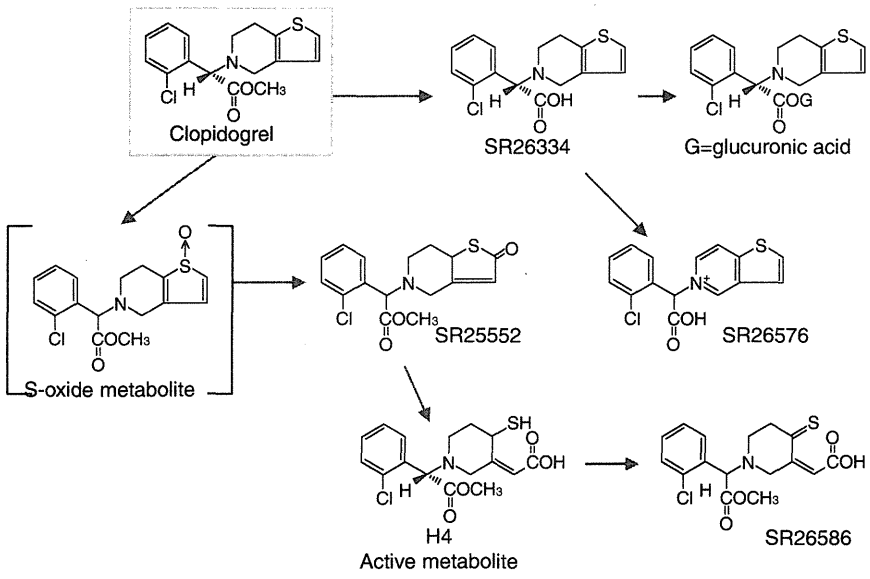

Figure 1 Metabolism of clopidogrel to its inactive and active metabolites.

2-oxo-clopidogrel; then, activity of CYP3A, CYP2C9, CYP2C19, or CYP286 leads to the formation of R-130964 (Figure 1).

Based on these metabolic pathways, a number of mechanisms could underlie the phenomenon of clopidogrel resistance. One such mechanism is the potential for interactions with other drugs that rely on the cytochrome P450 system; another is altered expression and activity of the cytochrome p450 system.

Pharmacology and Drug Interactions

A large number of pharmaceutical products and natural compounds are metabolized via the cytochrome P450 system. Of particular interest are the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitors (statins) and proton pump inhibitors (PPIs), both of which are commonly used in the patient populations that are most likely to use clopidogrel and related antithrombotic agents.

Statins

Evidence for clinical effects of an interaction between clopidogrel and statins has been reported in several studies. First, Lau et al. [17] compared platelet aggregation in 44 patients undergoing coronary artery stent implantation who were treated with clopidogrel alone or in combination with pravastatin or atorvastatin. They found that patients treated with clopidogrel in combination with atorvastatin showed significantly higher residual platelet aggregation compared with clopidogrel alone, indicating reduced clopidogrel activity, with a dose–response relationship. It must be noted that the authors of that study used a PlateletWorks assay that has not been extensively validated for measuring platelet aggregation, and which may be less reliable than other methods for assessing platelet defects in cardiac surgical patients [18,19]. In a separate study in healthy volunteers, however, although simvastatin and fluvastatin affected the antiaggregation effect of clopidogrel, atorvastatin, pravastatin, and rosuvastatin did not [20], suggesting that the drug interaction is not a class effect of statins.

Numerous studies have subsequently revealed that coadministration of a statin with clopidogrel does not affect the efficacy of clopidogrel [21–25]. Indeed, one study indicated that the effect may be the result of a prior history of coronary stent thrombosis, rather than the use of a statin [21]. In a large-scale study of 1395 patients [26] scheduled for elective coronary stent placement, clopidogrel 600 mg was given ≥2 h before PCI and 75 mg daily thereafter. Statin medication on admission was continued unaltered until discharge (atorvastatin, n = 255; simvastatin, n = 355, fluvastatin, n = 42; pravastatin, n = 81). The use of clopidogrel after stratification for concomitant use of statins had no effect on antiplatelet activity or clinical outcomes after PCI.

Proton Pump Inhibitors

PPIs are commonly administered for the treatment of gastroesophageal reflux disease and gastric ulcers. However, concomitant administration of antithrombotic agents is known to increase the risk of gastrointestinal adverse events, particularly upper gastrointestinal bleeding, and this risk is more pronounced in patients with gastric ulcers [27–29]. However, it has also been noted that clopidogrel activity is commonly impaired when used in combination with PPIs.

Gilard et al. [30] evaluated vasodilator-stimulated phosphoprotein (VASP) phosphorylation as an index for platelet reactivity in patients taking clopidogrel in combination with either placebo or omeprazole. Using this approach, the mean percentage platelet reactivity is inversely correlated with clopidogrel treatment efficacy. Of note, the platelet reactivity index measured at the start and after 7 days of clopidogrel treatment was significantly augmented by omeprazole compared with placebo (from 83.9% and

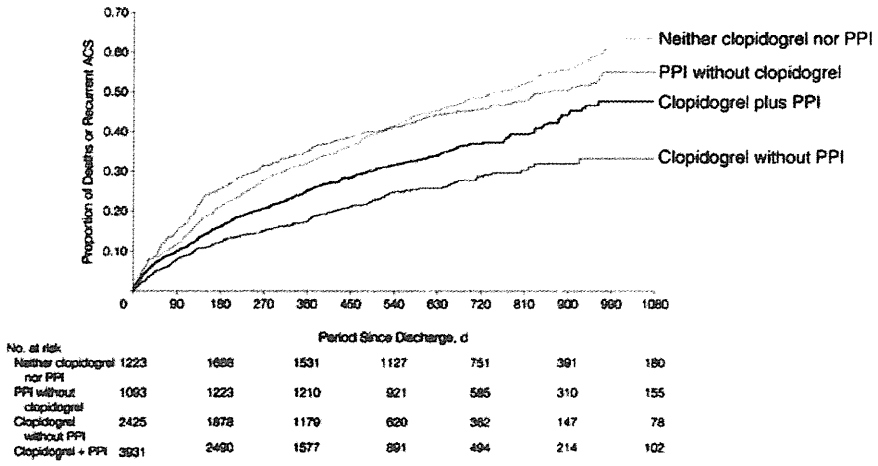

Figure 2 Cumulative risk of all-cause mortality and recurrent acute coronary syndrome in patients using clopidogrel and/or a PPI after discharge for acute coronary syndrome (n = 5244) [35]. ACS, acute coronary syndrome; PPI, proton pump inhibitor; d, days.

83.2% to 39.8% and 51.4%, respectively [−32.6 vs. −43.3%], respectively; $P < 0.0001$), indicating impaired clopidogrel activity in patients using omeprazole. Similar findings have been shown in other studies [31–34], including randomized controlled trials and population-based studies. Moreover, it appears that patients using PPIs in combination with clopidogrel have a higher risk for mortality or rehospitalization for cardiac adverse events than those not treated with PPIs [35]. Indeed, Ho et al. reported that the cumulative risk of all-cause mortality and recurrent acute coronary syndrome was significantly lower in patients using clopidogrel alone than in those using clopidogrel in combination with a PPI (Figure 2) [35].

The results of two randomized trials investigating the efficacies of clopidogrel and prasugrel with or without a proton-pump inhibitor (at the physician's discretion) were recently reanalyzed [36]. In the larger of the two studies, TRITON-TIMI 38, patients (n = 13,608) were randomly assigned to prasugrel (n = 6813) or clopidogrel (n = 6795) and 33% (n = 4295) of patients were using PPIs at randomization. This analysis revealed no association between PPI use and risk of the primary endpoint (composite of cardiovascular death, MI or stroke) in patients treated with clopidogrel (hazard ratio [HR] = 0.94; 95%confidence interval [95%CI] = 0.80–1.11) or prasugrel (HR = 1.00; 95%CI = 0.84–1.20).

Drug–drug interactions involving PPIs and clopidogrel may occur as a result of their metabolism by cytochrome P450 enzymes including CYP2C19 in the liver. However, based on the above findings, the effect of PPI administration on clinical outcomes following clopidogrel treatment seems to be inconsistent. For example, Juurlink et al. [33] reported a retrospective population-based nested case-control study of 13,636 patients prescribed clopidogrel after acute MI, including 734 cases readmitted with MI and 2057 controls. As in the above studies, current use of PPIs was associated with an increased risk of reinfarction (adjusted odds ratio [OR] = 1.27; 95%CI = 1.03–1.57). However, in a stratified analysis, pantoprazole, which does not inhibit CYP2C19, was not associated with readmission (adjusted OR: 1.02; 95%CI = 0.70–1.47); the adjusted OR for other PPIs was 1.40 (95%CI = 1.10–1.77). Other studies have revealed that esomeprazole [37] and omeprazole [31] are associated with adverse outcomes of clopidogrel therapy, likely because of their potent inhibition of CYP2C19 [38]. Interestingly, in the COGENT randomized controlled study [38] of patients with ACS or undergoing placement of a coronary stent, there was no difference in the effects of a fixed-dose combination of clopidogrel (75 mg) with omeprazole (20 mg) or clopidogrel alone (both groups also used aspirin at 75–325 mg/day) on the cardiovascular endpoint (a composite of cardiovascular death, nonfatal MI, CABG or PCI or ischemic stroke; HR = 1.02, 95%CI = 0.70–1.51). As would be expected, the rate of gastrointestinal outcomes was lower in the combination therapy group than in the placebo group (HR = 0.55; 95%CI = 0.36–0.85); however, the study did not address whether clopidogrel reduced the efficacy of omeprazole. Although this study could indicate a real absence of an effect of the PPI omeprazole on clopidogrel activity, several limitations of the study should be considered. First, the median follow-up was only 133 days (maximum 362 days). Second, the study did not directly measure platelet activity, which may indicate subclinical clopidogrel resistance, indicative of a risk of future events. Third, the study used a novel formulation of clopidogrel/omeprazole with altered release kinetics that differs from the commercially available formulations.

Overall, it seems that the ability of PPIs to inhibit CYP2C19 may influence clopidogrel activity. Although clopidogrel can be used concomitantly with PPIs, physicians should be aware of the potential for an inadequate response; regular assessment of platelet aggregation should be a priority in such cases to ensure that platelet aggregation is inhibited appropriately to reduce the risk of future adverse events.

Potential Genetic Factors Involved in Clopidogrel Resistance

Because the response to clopidogrel shows wide variation among individuals, many studies have been conducted to investigate possible biological factors that may mediate these differences. As a

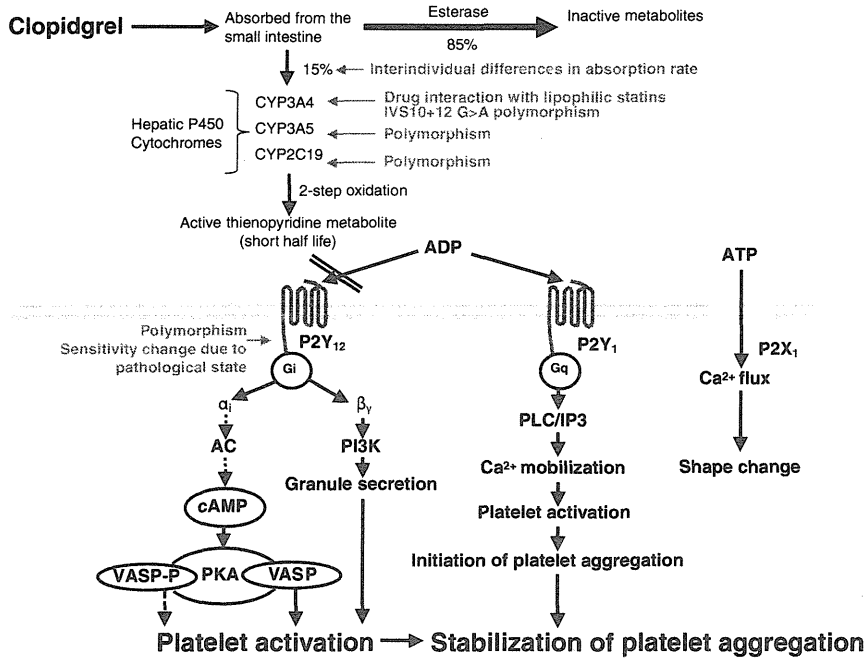

Figure 3 Pathways involved in platelet aggregation and possible mechanisms involved in response to clopidogrel. Modified from Gurbel et al. Thromb Res 2007 [15] and Angiolillo et al. Eur Heart J 2008 [89]. AC, adenylate cyclase; ADP, adenosine diphosphate; AMP, adenosine monophosphates; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; CYP, cytochrome P450; Gi, inhibitory G protein; Gq, stimulatory G protein; IP3, inositol triphosphate; PDE, phosphodiesterase; PKA, protein kinase A; VASP, vasodilator-stimulated phosphoprotein; PI3K, phosphatidyl inositol 3-kinase; PLC, phospholipase C.

result, a number of sites of interest have been identified, as summarized in Figure 3. Although there is some variation in the rate of clopidogrel absorption from the small intestine, it appears that the main causes of variation are polymorphisms in hepatic cytochrome P450 isozymes (for example, CYP3A4, CYP3A5, and CYP2C19) and in the P2Y12 receptor itself.

In terms of polymorphisms in CYP2C19, loss-of-function polymorphisms were first shown to significantly reduce the response to clopidogrel in platelets from healthy subjects, as demonstrated by increased platelet aggregation measured using light transmission aggregometry [39]. In that study, the baseline platelet activity was not influenced by the CYP2C19 genotype. In the presence of ADP, platelet aggregation decreased gradually during treatment with clopidogrel in individuals with the wild-type *1/*1 homozygote (reaching 48.9% at day 7; $P < 0.001$ vs. baseline). By contrast, platelet aggregation did not change significantly in individuals with the *1/*2 heterozygote (71.8% at day 7; $P = 0.22$ vs. baseline). Thus, the CYP2C19*2 loss-of-function allele was associated with a marked decrease in platelet responsiveness (i.e., greater platelet aggregation) to clopidogrel.

Similar findings have been reported in a number of other studies [39–43], and CYP2C19 polymorphisms have been detected in many individuals worldwide. Indeed, the prevalence of the CYP2C19*2 mutant allele has been reported to be as high as 70% in some populations [44]. Elsewhere, the prevalence of either the *2 and *3 polymorphisms seems to be <40% (Figure 4) [45]. Of note, however, the prevalence of poor metabolizers or intermediate metabolizers of clopidogrel seems to be higher in Asian (18–23%) than in Caucasian (approximately 3%) populations, and this may be related to the common gene variants of CYP2C19 in these populations [46].

What is the Clinical Impact of Clopidogrel Resistance?

Because of the widespread use of clopidogrel to protect patients undergoing critical cardiovascular interventions, resistance to this agent presents a significant clinical problem in patients with atherothrombotic diseases.

Hochholzer et al. [47] investigated the relationship between ADP-induced platelet aggregation, measured using light transmission aggregometry, and major adverse cardiac events in 802 consecutive patients within a 30-day period after elective coronary stent placement. All patients received a loading dose of clopidogrel 600 mg followed by a maintenance dose of 75 mg daily, and were classified according to the quartiles of platelet aggregation (quartile 1, <4%; quartile 2, 4–14%; quartile 3, 15–32%; quartile 4, >32%). Although the incidence of subsequent coronary events was low (<1%) in patients in the first and second quartiles of platelet aggregation, the incidence was significantly higher in patients in the third and fourth quartiles (>3%; $P = 0.034$).

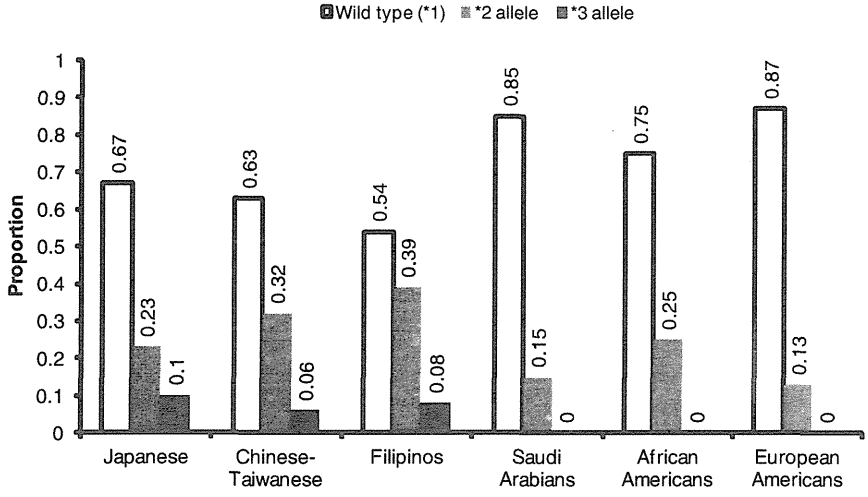

Figure 4 Estimated prevalence of polymorphisms in CYP2C19 [35].

In terms of the implications of genetic polymorphisms, the results of a number of studies have been reanalyzed to investigate whether these polymorphisms might be at least partly responsible for differences in treatment efficacy and long-term outcomes. Trenk et al. [43] reported that patients carrying at least one CYP2C19*2 allele were more prone to show impaired platelet reactivity (measured by light transmission aggregometry) upon clopidogrel treatment, which was associated with significantly worse clinical outcomes after coronary stent placement. In that study, 552 patients were homozygous for the wild-type CYP2C19 allele and 245 carried at least one *2 allele. Compared with patients homozygous for the wild-type allele, those with the *2 allele showed significantly higher levels of residual platelet aggregation when on clopidogrel (23.0% vs. 11.0%; $P < 0.0001$). In addition, patients with residual platelet aggregation >14% versus ≤14% (41.3% vs. 22.5%, respectively) showed a 3-fold greater increase (95%CI = 1.4–6.8; $P = 0.004$) in the 1-year incidence of death and MI.

In a study involving 1477 patients with acute coronary syndromes [16], the rates of the primary efficacy outcome such as death from cardiovascular causes, nonfatal MI, and nonfatal stroke were significantly higher in patients carrying loss-of-function polymorphisms of CYP2C19 (12.1% vs. 8.0%; $P = 0.01$) than in those with wild-type CYP2C19. Similar results were found for the rates of definite or probable stent thrombosis (2.6% vs. 0.8%; $P = 0.02$) over 450 days after the start of clopidogrel treatment.

In another study of 259 patients who used clopidogrel for ≥1 month after a first MI, 5-year event-free survival was significantly lower in patients with a polymorphism in CYP2C19 (HR = 3.69; 95%CI = 1.69–8.5; $P = 0.0005$) [48].

A study of 2208 consecutively enrolled patients presenting with acute MI who received clopidogrel [49] assessed the association between allelic variants of several genes related to clopidogrel (*ABCB1*, *CYP3A5*, *CYP2C19*, *P2RY12*, and *ITGB3*) and the risk of death from any cause, nonfatal stroke, or MI during 1 year of follow-up. A total of 225 patients died and nonfatal MI or stroke occurred in 94 patients during the follow-up period. Of note, the rate of cardiovascular events at 1 year was higher in patients carrying any two *CYP2C19* loss-of-function alleles than in patients with none (21.5% vs. 13.3%; adjusted HR = 1.98; 95%CI = 1.10–3.58). Similarly, the rate of cardiovascular events was higher among patients with a variant allele of *ABCB1*, a gene involved in clopidogrel absorption, than in patients with the wild-type *ABCB1* genotype (15.5% vs. 10.7%; adjusted HR = 1.72; 95%CI = 1.20–2.47). Interestingly, these rates were even higher among the patients who underwent PCI during hospitalization, as the rate of cardiovascular events among patients with two *CYP2C19* loss-of-function alleles was 3.58 times (95%CI = 1.71–7.51) that of patients with no loss-of-function alleles. By contrast, the *ABCB1* alleles had no significant, independent effect in these patients. Taken together, the findings of this study indicate that polymorphisms in a range of genes may affect the outcomes of clopidogrel therapy; however, the effect is most marked for *CYP2C19*. This may be attributed to differences in the effects of these polymorphisms on the function of the protein product, because polymorphisms of *CYP2C19* cause loss of function, whereas those of the *ABCB1* gene did not.

"Measuring" Clopidogrel Resistance

Platelet function, and hence clopidogrel activity, can be measured using a number of different approaches. The most commonly used approaches include ADP-induced platelet aggregation, VASP phosphorylation/flow cytometry, thromboelastography, the platelet drop-count method, and light transmission elastography.

It was initially considered that ADP-induced platelet aggregation could be used as a marker for clopidogrel activity. However, clopidogrel specifically inhibits the P2Y12 receptor rather than the P2Y1 receptor, which is responsible for the initial wave of ADP-induced platelet aggregation. Furthermore, because the