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A comparison of the antiplatelet effects of prasugrel and high-dose clopidogrel as assessed by VASP-phosphorylation and light transmission aggregometry

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Summary

Platelet inhibition as measured by vasodilator-stimulated phosphoprotein (VASP) and light transmission aggregometry (LTA) have shown concordance following dosing of clopidogrel. No reports have directly compared the VASP assay and LTA at the levels of $P2Y_{12}$ blockade after loading doses (LDs) of prasugrel or high dose clopidogrel (600 and 900 mg). The aim was to compare the VASP assay and LTA during the loading dose phase of a comparative study of prasugrel and clopidogrel. Prasugrel 60 mg LD/10 mg maintenance dose (MD) and clopidogrel 300 mg/75 mg and 600 mg/75 mg LD/MD regimens were compared in a 3-way crossover study in 41 healthy, aspirin-free subjects. Each LD was followed by seven daily MDs and a 14-day washout period. $P2Y_{12}$ receptor blockade was estimated using the VASP assay, expressed as platelet reactivity index (VASP-PRI). Platelet aggregation was assessed by light transmission aggregometry (20 and 5 μM ADP). Twenty-four hours after prasgurel 60 mg or clopidogrel 300 mg and 600 mg, respectively, VASP-PRI decreased from ~80% to 8.9%, 54.7%, and 39.0 %, and maximal platelet aggregation (MPA) decreased from ~79% to 10.8%, 42.7%, and 31.2%, with an overall VASP:MPA correlation of 0.88 ($p<0.01$). VASP assay responses after the clopidogrel LDs showed a wider range of values (300 mg: 0–93%; 600 mg: 0–80%) than prasugrel (0–13%); MPA responses followed a similar trend. Pearson's correlation suggested a strong agreement between VASP and LTA (20 μM ADP) for MPA ($r=0.86$, $p<0.0001$). VASP and LTA demonstrated concordance across the response range of $P2Y_{12}$ receptor blockade following thienopyridine LDs.

Keywords

Clopidogrel, prasugrel, $P2Y_{12}$, thienopyridine, VASP

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Introduction

The pharmacodynamic (PD) response of platelets to ADP and other agonists is commonly measured to assess the inhibitory effects of antiplatelet agents such as thienopyridines. The most common method employed to determine a platelet PD response is light transmission aggregometry (LTA) (1, 2). This method has been widely applied to the characterization and clinical development of antiplatelet agents (3–7). In more recent years it has also been employed to assess the variability in response to antiplatelet agents such as clopidogrel (8–16). However, LTA has several limitations, including the need for a specialized laboratory and trained staff, and a lack of standardization in reagents, protocols, and equipment (1, 2) Accordingly, LTA measurement is not widely available in clinical laboratories and its routine use to guide antithrombotic therapy is unproven (1, 2).

The pharmacological target of clopidogrel and other thienopyridines is the $P2Y_{12}$ class of ADP receptor that when activated by ADP results in a sustained platelet aggregation response (17–20). Vasodilator stimulated phosphoprotein (VASP) is a relatively new assay that measures $P2Y_{12}$ function more directly and is less dependent than ADP-induced LTA on other pathways to yield the final assay endpoint (21–25). The flow cytometric measurement of VASP has been described (21, 22) and used to

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evaluate $P2Y_{12}$ blockade and the antiplatelet effects of clopidogrel (26, 27). An additional advantage of the VASP assay, compared to LTA methods, is the stability of blood for at least 48 hours prior to assay, thus allowing shipment and central laboratory evaluation (28, 29).

Previous studies have shown good concordance between levels of platelet inhibition measured by VASP phosphorylation and LTA following administration of clopidogrel doses of 75–300 mg (25, 27). In recent years the use of higher LDs of clopidogrel (600 and 900 mg) has become common, (10–11, 30–34) and, in addition newer agents have been developed, such as prasugrel, that provide substantially higher levels of $P2Y_{12}$ blockade and platelet inhibition (7, 15, 35–37). Prasugrel's higher levels of inhibition in large part reflect increased generation of, and exposure to, its active metabolite, compared to clopidogrel (35, 38). However few data are available on the relative performance of the VASP and LTA assays at the higher levels of $P2Y_{12}$ blockade associated with these regimens. Accordingly, in the present study, we compared the flow cytometric assessment of VASP phosphorylation and LTA in an evaluation of the platelet response to standard and high LDs of clopidogrel (300 and 600 mg, respectively) and a 60 mg LD of prasugrel (7, 16)

Materials and methods

Subject population

As described previously, healthy subjects, 25 males and 16 females, with a median age of 39 (20 to 63 years); median body mass index of 27.2 kg/m^2 (19.1 to 31.3 kg/m^2), and a maximal platelet aggregation (MPA) response ≥ 70% with 20 μM ADP and 1.5 mM arachidonic acid at baseline, were enrolled in this study (16). There were 32 subjects of Caucasian descent and nine subjects of African descent. Thirty-three subjects completed the study as planned; eight subjects were withdrawn from the study; one was withdrawn due to adverse events not related to the study drug, and seven subjects withdrew themselves from the study due to personal reasons. The institutional review board approved the protocol, and the study was conducted in accordance with regulatory standards and good clinical practice guidelines stipulated in the Declaration of Helsinki. All subjects provided written, informed consent.

Study design

This study was part of a larger open-label, randomized, three treatment, three sequence crossover safety study described in detail elsewhere (16). Subjects were admitted on Day 1 to the research unit for baseline physical and laboratory testing. On Day 1, each subject received a single loading dose of prasugrel 60 mg, clopidogrel 300 mg, or clopidogrel 600 mg. The original study included a seven-day maintenance dose (MD) phase, however the VASP assay was only used during the LD phase. Hence, details of the MD phase, including the LTA results, are not given here but can be found elsewhere (16).

Study drugs

Prasugrel 10 mg tablets, as the HCl salt, were supplied by Eli Lilly and Company, Indianapolis, IN, USA. Clopidogrel was furnished as commercially available clopidogrel bisulfate (Plavix™) 75 mg tablets, Bristol-Myers Squibb, New York, NY, USA, and Sanofi-Aventis, LLC, Bridgewater, NJ, USA).

VASP

Blood samples for measurement of VASP were collected predose and at 2, 4, 6 and 24 hours after the clopidogrel or prasugrel LDs on Day 1. While LTA was additionally measured at 0.25, 0.5 and 1 hours, for logistic reasons, VASP was not assessed at these time points. The blood samples were anticoagulated with 3.8% sodium citrate and shipped within eight hours of collection, at room temperature, by overnight courier to Esoterix Clinical Trial Services, Brentwood, TN, USA, for analysis. VASP was measured by quantitative flow cytometry using a platelet VASP/$P2Y_{12}$ kit (Platelet VASP®; Biocytex, Marseille, France). For this assay, as described in full in the package insert, citrated whole blood was incubated with PGE_1 with or without ADP (10 μM) (28). After fixation with paraformaldehyde, the cells were permeabilized and incubated with a primary mouse monoclonal antibody specific for phosphorylated VASP, followed by a secondary fluorescein isothiocyanate (FITC)-conjugated polyclonal goat-antimouse antibody. Samples were then analyzed by flow cytometry to measure the level of phosphorylated VASP. Results were expressed as the platelet reactivity index (PRI), which is calculated from the corrected mean fluorescence intensity (cMFI) of the PGE_1 and PGE_1 + ADP samples as follows:

$$PRI = \left[\frac{cMFI_{PGE1} - cMFI_{PGE1+ADP}}{cMFI_{PGE1}}\right] \text{X } 100\%$$

The manufacturer of the VASP kit and others (28, 29) report that whole blood samples for VASP analysis are stable for up to 48 hours at room temperature. During pre-study assay qualification with the central laboratory, we determined whole blood samples to actually be stable at room temperature for up to 72 hours after sampling (data on file, Eli Lilly and Company). Accordingly, blood samples exceeding the 72 hour limit (16, representing 3% of total VASP samples) were excluded from data analysis.

Light transmission aggregometry (LTA)

Blood samples for measurement of platelet aggregation were collected predose and at 0.25, 0.5, 1, 2, 4, 6 and 24 hours after the prasugrel and clopidogrel LDs. Platelet-rich (PRP) and platelet-poor plasma (PPP) were prepared by differential centrifugation at room temperature as previously described (39). Platelet counts of the PRP were adjusted to approximately 250 x 10^9/l using autologous PPP. Platelet aggregation was measured by light transmission aggregometry (LTA) using a Chrono-Log™ 4-channel optical aggregometer (Chrono-Log Corporation, Havertown, PA, USA). Following addition of ADP (20 μM or 5 μM final concentrations) the platelet aggregation response was recorded for 8 min. Two parameters were derived from each aggregation tracing:

- Maximal Platelet Aggregation: (MPA, defined as the maximum extent of aggregation achieved at any time during the 8 min); and,
- Residual Platelet Aggregation (RPA, defined as the level of aggregation present at 6 min after addition of ADP).

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Table 1: Published definitions of poor pharmacodynamic response after treatment with a thienopyridine

Method	Definition	Citation
LTA	MPA (5 μM ADP) > 75th percentile of baseline at any time after a thienopyridine dose	Gurbel (10)
LTA	RPA (5μM ADP) ≥ 15% at any time after a thienopyridine dose	Hochholzer (32)
LTA	ΔMPA (20 μM ADP) <15% at 4 hours or 24 hours after a thienopyridine LD	Weerakkody (41)
VASP	VASP (PRI, %) value greater than the value determined by subtracting 2-fold the standard deviation from mean baseline VASP-PRI applied 24 hours after a LD	Aleil (27)
VASP	PRI >50% after a thienopyridine dose	Barragan (26)

Statistical methods

A crossover design permitted within-subject comparison of the treatment effect with either the VASP or LTA methodologies across the prasugrel and clopidogrel LD regimens. Negative VASP-PRI values (n=47/514; 9.1%) were imputed as 0 for the VASP analysis. Values less than –90% (n=2; 0.4%) were treated as missing, as these values suggested an analytical problem. The Day 1 predose MPA (20 and 5 μM ADP) and VASP-PRI from all three LD regimens were used to test for a period effect using a mixed-effect model; no detectable period effect was observed. A linear mixed effect model was used to assess MPA for each treatment at different scheduled time points (40). The primary comparisons were prasugrel 60 mg LD to clopidogrel 300 and 600 mg LD, and clopidogrel 600 to 300 mg LD. The pharmacodynamic parameter change in MPA, or delta MPA (ΔMPA) was calculated using the following equation:

$$\Delta MPA \text{ at time } t = \Delta MPA_t = MPA_0 - MPA_t$$

where MPA_0 is the MPA at baseline (predose) and MPA_t is the MPA at time t.

The inhibition of platelet aggregation, maximum (IPAmax) for each post dose sample was calculated with the following formula:

$$IPAmax = ([MPA_0 - MPA_t]/MPA_0) \times 100\%$$

where MPA_0 is the MPA at baseline and MPA_t is the MPA at time t. RPA, defined above as the level of aggregation present at 6 min after addition of agonist, was substituted for MPA in the same formula and used to calculate the inhibition of residual platelet aggregation (IPAresid) in a similar fashion.

Scatter plots of VASP-PRI versus MPA, RPA, IPAmax, IPAresid and ΔMPA for 20 and 5 μM ADP were generated across all time points with concurrent data (predose, 2, 4, 6 and 24 hours post-LD) and Pearson correlation coefficients were calculated.

Exploratory analyses of the entire data set were performed by five published criteria (Table 1) (10, 26–27, 32, 41) in order to assess the incidence of pharmacodynamic (PD) poor responses to thienopyridine LDs by the VASP and LTA methods.

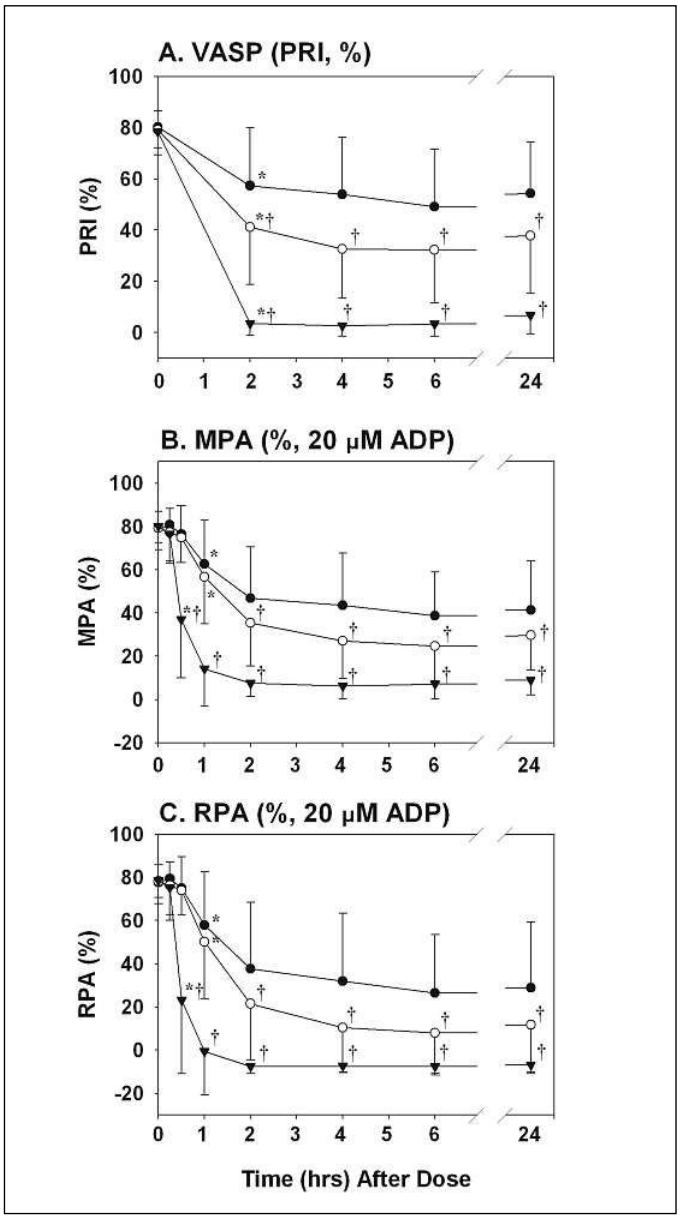

Figure 1: Responses to thienopyridine loading doses. A) Vasodilator-stimulated phosphoprotein (VASP, Platelet Reactivity Index, PRI, %); B) Maximal platelet aggregation (MPA, %; 20 μM ADP); and, C) Residual platelet aggregation (RPA, %; 20 μM ADP). Symbols represent clopidogrel 300 mg (●), clopidogrel 600 mg (○), and prasugrel 60 mg (▼) doses, respectively. *earliest time point that was statistically significantly different (p<0.0001) *vs.* baseline; †p<0.001 *vs.* clopidogrel 300 mg dose.

Results

Comparison of PD data by alternative methods

Figures 1A-C present a composite of the mean (± SD) VASP-PRI responses and the MPA and RPA responses to 20 μM ADP, from baseline to 24 hours after each of the thienopyridine LDs. The majority of LTA results shown reflect data obtained with 20 μM ADP; data obtained with 5 μM ADP followed similar patterns but are not shown.

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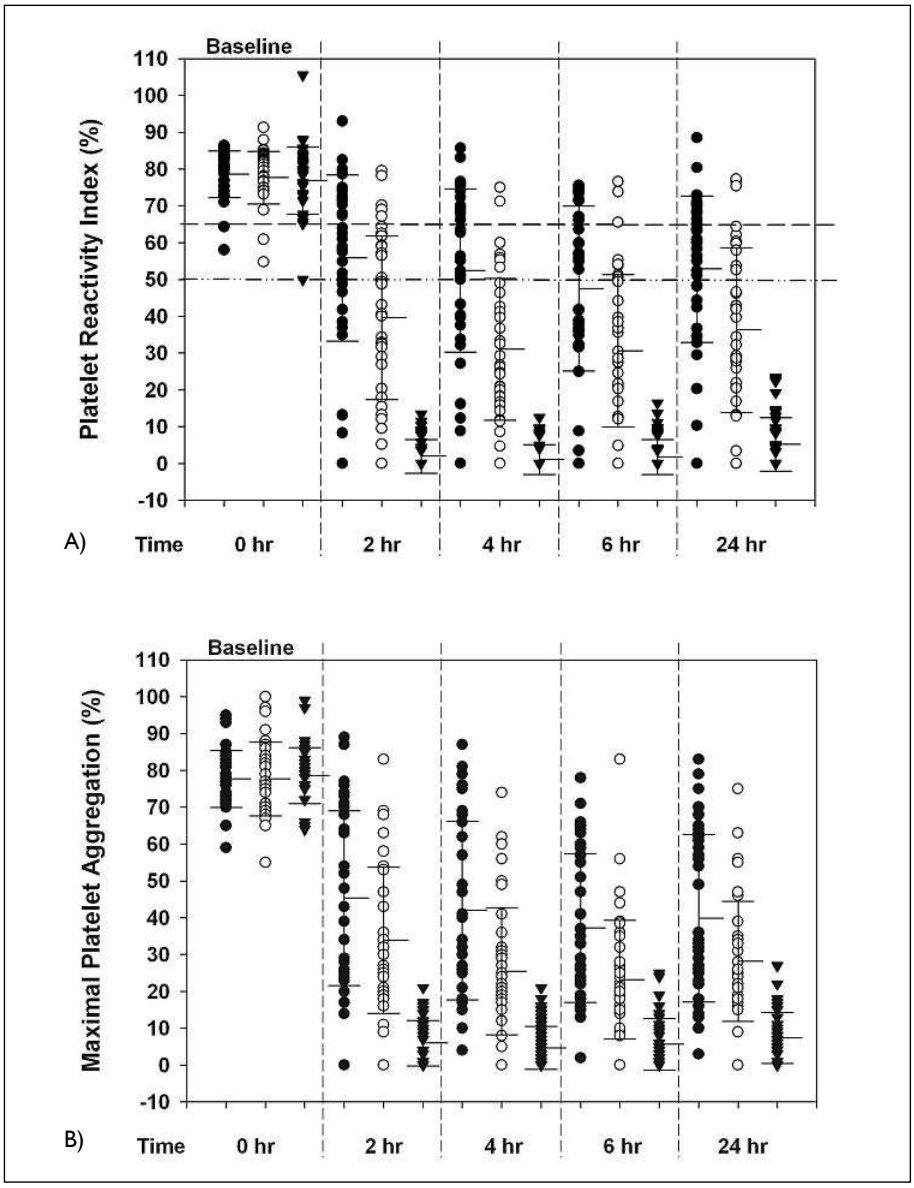

Figure 2: Individual VASP and LTA data during clopidogrel and prasugrel LDs. A) Individual VASP (platelet reactivity index, PRI; %) responses. Symbols represent clopidogrel 300 mg (●), clopidogrel 600 mg (○), and prasugrel 60 mg (▼) doses; horizontal lines represent mean ± SD. Broken lines represent the poor responder cutoffs defined by Aleil et al. (27) (- – – – , mean PRI at baseline – 2 SD; PRI = 64.1% in this study) and Barragan, et al, (- – on treatment PRI >50%) (26). B) Individual MPA (maximal platelet aggregation, MPA; %, 20 μM ADP) responses. Symbols represent clopidogrel 300 mg (●), clopidogrel 600 mg (○), and prasugrel 60 mg (▼) doses; horizontal lines represent mean ± SD.

VASP

With the VASP assay, the three treatments achieved a statistically significant difference ($p<0.0001$) from baseline at the first post-LD assay point of 2 hours (Fig. 1A). The prasugrel 60 mg LD and clopidogrel 600 mg LD were statistically significantly different ($p<0.001$) at 2 hours compared to the clopidogrel 300 mg LD. The prasugrel 60 mg LD achieved significantly lower VASP-PRI ($p<0.001$) than the clopidogrel 600 mg LD at 2 hours and through all subsequent time points.

LTA

For MPA and RPA after 20 μM ADP, the prasugrel 60 mg LD achieved a statistically significant difference from baseline at 30 minutes (Fig. 1B, C); the clopidogrel 300 mg and 600 mg LDs achieved a statistically significant difference ($p<0.001$) from baseline at 1 hour. Compared to the clopidogrel 300 mg LD, the prasugrel 60 mg LD and the clopidogrel 600 mg LD were statistically significantly different ($p<0.001$) at 30 minutes and 2 hours, respectively. The prasugrel 60 mg LD achieved significantly lower MPA and RPA ($p<0.001$ for each measure) than the clopidogrel 600 mg LD at 30 minutes through all subsequent time points. In response to 5 μM ADP, the prasugrel 60 mg LD achieved a statistically significant difference from baseline at 15 minutes, whereas both clopidogrel doses were different from baseline at 30 minutes ($p<0.05$; data not shown).

The three curves demonstrated a similar pattern of increasing platelet inhibition (decreasing platelet response) over the 24 hour period and suggested a concordance between the VASP measurement of $P2Y_{12}$ inhibition and LTA evaluation of ex-vivo platelet responses.

As depicted in Figures 2A and B, reductions in individual VASP-PRI and MPA (20 μM ADP) values were observed from 2 to 24 hours following administration of a single LD of prasugrel or clopidogrel. The range of values at baseline for VASP-PRI (prasugrel 60 mg: 50–105%; clopidogrel 300 mg: 58–86%; clopidogrel 600 mg: 55–91%) and MPA (prasugrel 60 mg:

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64–99%; clopidogrel 300 mg: 59–95%; clopidogrel 600 mg: 55–100%) were similar across all three treatment groups pre-dose. At 2 hours after a thienopyridine LD, the range of values for each assay were similar for all three treatments both for VASP-PRI (prasugrel 60 mg: 0–13%; clopidogrel 300 mg: 0–93%; clopidogrel 600 mg: 0–78%) and for MPA (prasugrel 60 mg:0–21%; clopidogrel 300 mg: 0–89%; clopidogrel 600 mg: 0–83%). In this crossover study, the pattern of change in VASP-PRI and MPA values after a thienopyridine LD (Fig. 2A, B; Table 2) was similar (correlation coefficient 0.88; p<0.001) from 2 hours after the LD through all time points, with both clopidogrel LD treatments demonstrating a much wider range, and a greater number of higher VASP-PRI and MPA values (lower platelet inhibition) than the prasugrel LD.

Using the definition of Aleil et al., (Table 1: baseline mean – 2 standard deviations) the lower limit of normal for the VASP assay in the absence of thienopyridine therapy was a PRI of 64.1%, based on a pre-dose VASP-PRI mean of 79.3% and standard deviation of 7.6% (27). By this criterion, 346/512 (67.6%) of all VASP measurements from 2 to 24 hours after the LD were identified as a positive response to either thienopyridine. All subjects (34/34) receiving a prasugrel 60 mg LD had a VASP-PRI below 64.1% at 24 hours post dose, compared to only 21/33 (64.6%) and 29/33 (88%) of subjects following clopidogrel 300 mg and 600mg LDs, respectively. By the definition of Barragan et al. (Table 1) 284/512 (55.47%) of VASP-PRI measurements were <50%, and, accordingly, identified as a positive response to a thienopyridine (26). Again, all subjects (34/34) receiving a prasugrel 60 mg LD had a VASP-PRI below 50% at 24 hours post dose, compared to only 11/33 (33%) and 22/33 (66.7%) subjects following clopidogrel 300 mg and 600 mg LDs, respectively.

Table 2: Pearson correlations of VASP with measures of platelet aggregation by light transmission aggregometry.

Measure correlated with VASP-PRI	20 μM ADP	5 μM ADP
Inhibition of platelet aggregation (IPAmax, %)	–0.87, p<.0001	–0.79, p<.0001
Inhibition of platelet aggregation, Residual (IPAresid, %)	–0.85, p<.0001	–0.72, p<.0001
Maximal platelet aggregation (MPA, %)	0.86, p<.0001	0.78, p< 0001
Residual platelet aggregation (RPA, %)	0.85, p<.0001	0.72, p<.0001
Change in maximal platelet aggregation (ΔMPA, %)	–0.86, p<.0001	–0.73, p<.0001

Correlation of data by alternative methods

Figure 3 A and B presents a comparison of individual subjects' data obtained by the VASP method, with MPA and RPA to 20 μM ADP. The baseline values for each sequence are represented as one symbol (open squares) and cluster in the upper right hand corner of each panel. The Pearson correlations reported in Table 2 suggest a strong agreement between VASP-PRI and IPAmax, IPAresid, MPA, RPA, and ΔMPA as measures of $P2Y_{12}$ receptor blockade. The responses for these healthy subjects were distributed throughout the dose response range (Fig. 3A, B). The responses to prasugrel clustered in the lower left hand corner suggesting near maximal platelet $P2Y_{12}$ inhibition. Correlation coefficients for these scatter plots can be found in Table 2.

Figure 4A-D presents a comparison of the individual subjects' data obtained by the VASP method, with MPA or RPA to 20 μM or 5 μM ADP at 24 hours after the thienopyridine LD. The MPA and RPA values obtained using 20 μM ADP appear to more

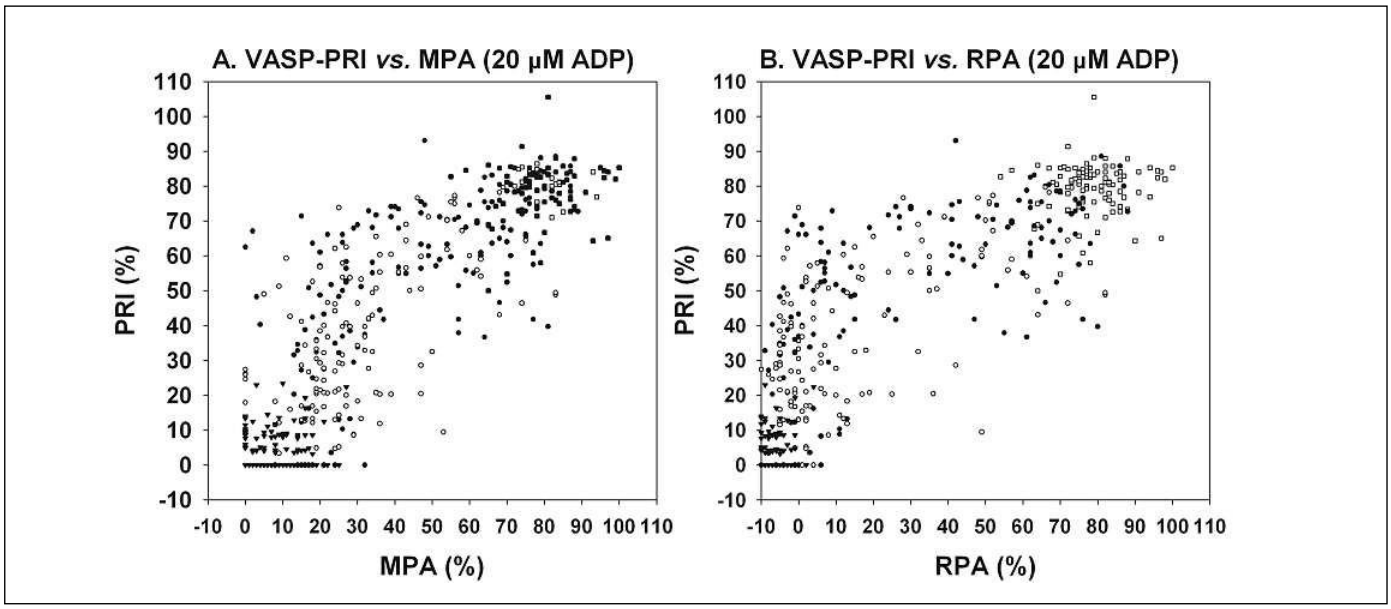

Figure 3: Plots of Individual VASP (Platelet reactivity index, PRI, %) vs. maximal platelet aggregation (MPA, %, 20 μM ADP) (A) or residual platelet aggregation (RPA, %, 20 μM ADP) (B) at all timepoints. Responses at time zero, 2, 4, 6 and 24 hours after a thienopyridine loading dose. Symbols represent baseline (❑, combined time-zero values for all 3 treatment sequences), clopidogrel 300 mg (●), clopidogrel 600 mg (❍), and prasugrel 60 mg (▼) doses, respectively.

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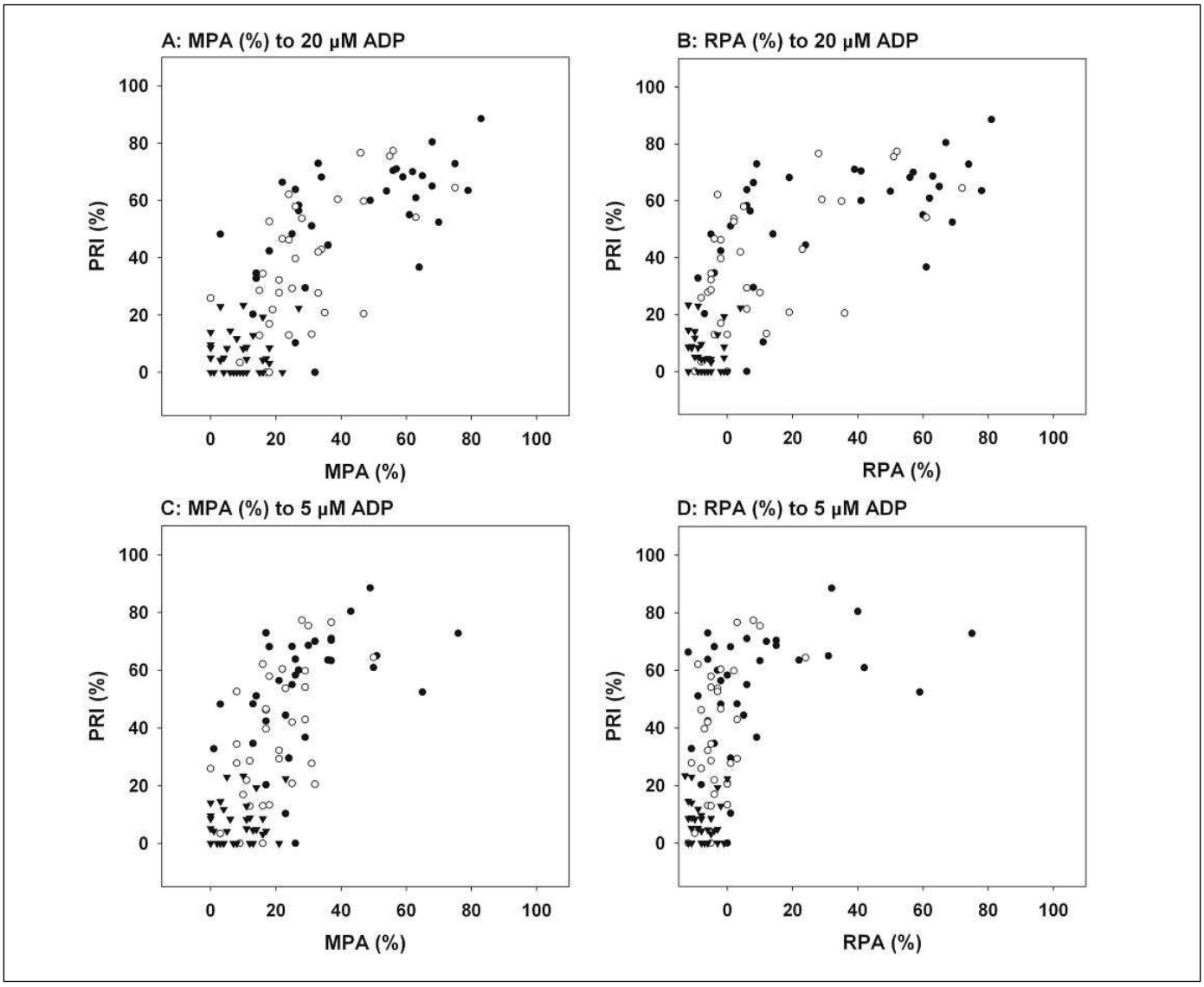

Figure 4: Plot of individual VASP (Platelet reactivity index, %) vs. LTA at 24 h after LD. A) Maximal platelet aggregation (MPA, %) responses to 20 μM ADP; B) Residual Platelet Aggregation (RPA, %) responses to 20 μM ADP; C) MPA (%) responses to 5 μM ADP; and, D) RPA (%) responses to 5 μM ADP, all data from 24 hours after thienopyridine loading doses. Symbols represent clopidogrel 300 mg (●), clopidogrel 600 mg (❍), and prasugrel 60 mg (▼) doses, respectively.

closely reflect $P2Y_{12}$ function, as measured by VASP, than do MPA and RPA with 5 μM ADP.

Exploratory PD response analyses

Table 3 summarizes the exploratory analysis of data from this study with regard to thienopyridine PD poor responders according to five published definitions (10, 26–27, 32, 41) and three separate PD measures (VASP; ΔMPA; RPA). Based upon these measures and definitions, up to 61% and 31% of healthy subjects in this crossover study would be classified as PD poor responders after a clopidogrel 300 mg or 600 mg LD, respectively, whereas none would satisfy the published definitions of a PD poor responder after a prasugrel 60 mg LD.

Discussion

These results demonstrate that there is a good correlation between estimates of the effect of thienopyridines on platelet function obtained by the VASP and LTA assays. These observations confirm and extend the findings of Aleil et al. and Pampuch et al., who demonstrated a correlation through a more limited dose response range (25, 27). As has been previously reported, a clopidogrel 600 mg LD results in greater inhibition of platelet function than a 300 mg LD, and a prasugrel 60 mg LD results in greater inhibition of platelet function than either of the LDs of clopidogrel (11, 16, 32–33). Indeed, the very low VASP-PRI following a prasugrel 60 mg LD suggests that this dose maximally inhibits $P2Y_{12}$ function, indicating that the correlation between

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Table 3: Poor pharmacodynamic response [number (%)] at 24 hours after treatment with a thienopyridine LD.

Definition	Clopidogrel 300 mg LD	Clopidogrel 600 mg LD	Prasugrel 60 mg LD	Citation
MPA (5 μM ADP) > 75th percentile of baseline at any time after a thienopyridine dose	0/36 (0%)	0/35 (0%)	0/35 (0%)	10
RPA (5 μM ADP) ≥ 15% at any time after a thienopyridine dose	7/36 (19.4%)	1/33 (3.0%)	0/35 (0%)	32
ΔMPA (20 μM ADP) <15% at 4 hours or 24 hours after a thienopyridine LD	9/34 (26.5%)	2/33 (6.1%)	0/34 (0%)	41
VASP (PRI, %) > baseline – 2-fold SD from baseline at 24 hours after a LD	12/33 (36.4%)	4/33 (12.1%)	0/34 (0%)	27
VASP PRI >50% after a thienopyridine dose	22/33 (66.7%)	11/33 (33.3%)	0/34 (0%)	26

methods extends throughout the potential dose-response range for thienopyridines.

The effect of thienopyridines on the platelet aggregation (LTA) response to ADP can be reported in several different ways, including IPAmax, IPAresid, MPA, RPA or ΔMPA. As demonstrated in Table 3, each of these methods correlated well with the VASP assay, indicating that each parameter can reflect the effect of thienopyridines on platelet function. In general, the correlation between LTA and VASP-PRI was higher when 20 μM ADP was used as the agonist compared to 5 μM ADP. Indeed, Figure 4 suggests that the LTA response to 5 μM ADP may overestimate the degree of inhibition of $P2Y_{12}$ function, at least as measured by the VASP assay. By providing a wider window of response, 20 μM ADP may be a more useful agonist concentration for LTA assessment of thienopyridine effect on platelet function.

As seen in previous investigations, the response to both loading doses of clopidogrel demonstrated wide interindividual variability by both LTA and the VASP assay (9–11, 26–27, 31–34, 41). As the VASP assay is a more direct measure of $P2Y_{12}$ function, this would suggest that the variability in response to clopidogrel is due to different levels of $P2Y_{12}$ inhibition rather than potential differences in baseline platelet aggregation response, as has been suggested by others (13). This variability in response to clopidogrel has led to a number of definitions for a PD poor response to clopidogrel, but these definitions have usually not been based on clinical outcomes.

Several clinical studies have now demonstrated a relationship between the magnitude of inhibition of platelet aggregation as measured by LTA during clopidogrel administration and the relative risk of adverse cardiovascular events (8–11, 14, 30–34, 42–43). Patients with a lower level of platelet inhibition (higher MPA) appear to be at a significantly increased risk of adverse outcomes. More recently, with the development of the VASP assay to more directly assess $P2Y_{12}$ blockade, preliminary data suggest a similar relationship between high VASP-PRI and adverse cardiovascular events after percutaneous coronary interventions, including stent thrombosis (26, 43–46). The results from the present study confirm the concordance between LTA and VASP data and support the possible use of VASP-PRI to identify criteria for assessing the clinical adequacy of the response to thienopyridines as has been proposed by Barragan et al. (26). Thus, this may be considered an enabling technology since the VASP assay is more amenable to performance by a reference clinical laboratory than LTA is. Notably, in the current study, blood samples were shipped and assayed over a 72-hour period.

In this light, it was noteworthy that the lower limit of normal in the drug-free state for the VASP assay obtained in this study was quite similar to that observed by Aleil et al., where samples were assayed within 48 hours (27). This suggests that the VASP assay may be reproducible between participating laboratories, with the possibility of obtaining a degree of standardization that is more difficult with LTA.

This study was conducted at a single clinical site. VASP measurements were conducted at a single centralized laboratory facility with expertise in flow cytometry methods and consistent operational procedures. LTA measurements were conducted on one aggregometer by a single laboratory facility. The three-way crossover design permitted each subject to serve as her/his own control, thereby allowing intrasubject comparison of these treatments by the two methods. The study was designed to explore the relationship between VASP and LTA data during the LD phase; VASP data were not collected during maintenance dosing with clopidogrel or prasugrel, thus leaving this important dosing phase unexplored. The study enrolled healthy volunteers, with no aspirin therapy, thereby limiting direct application of these findings to patients with cardiovascular disease.

Our results confirm the utility of the VASP assay for measuring platelet $P2Y_{12}$ receptor function and its inhibition by established and investigational thienopyridines that provide a wide range of receptor blockade.

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Abbreviations

ADP, adenosine diphosphate; MPA, maximal platelet aggregation; IPAmax, inhibition of platelet aggregation, maximal; IPAresid, inhibition of residual platelet aggregation; PRI, platelet reactivity index; RPA, residual platelet aggregation; VASP, vasodilator stimulated phosphoprotein.

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