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# Effect of Vorapaxar Alone and in Combination with Aspirin on Bleeding Time and Platelet Aggregation in Healthy Adult Subjects.

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Wrishko, S Zhang, and M S Anderson

ARTICLE

# Effect of Vorapaxar Alone and in Combination with Aspirin on Bleeding Time and Platelet Aggregation in Healthy Adult Subjects

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The effect of the protease-activated receptor-1 (PAR-1) antagonist vorapaxar on human bleeding time is not known. This was a randomized, two-period, open-label trial in healthy men ( $n = 31$ ) and women ( $n = 5$ ). In period 1, subjects received 81 mg aspirin q.d. or a vorapaxar regimen achieving steady-state plasma concentrations equivalent to chronic 2.5 mg q.d. doses, for 7 days. In period 2, each group added 7 days of the therapy alternate to that of period 1 without washout. Bleeding time and platelet aggregation using arachidonic acid, ADP, and TRAP agonists were assessed. Bleeding time geometric mean ratio (90% CI) for vorapaxar/baseline was 1.01 (0.88–1.15), aspirin/baseline was 1.32 (1.15–1.51), vorapaxar + aspirin/vorapaxar was 1.47 (1.26–1.70), and vorapaxar + aspirin/aspirin was 1.12 (0.96–1.30). Unlike aspirin, vorapaxar did not prolong bleeding time compared with baseline. Bleeding time following administration of vorapaxar with aspirin was similar to that following aspirin alone.

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## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?

✓ Aspirin and P2Y12 inhibitors each prolong human bleeding time. The effect of the selective PAR-1 antagonist vorapaxar on human bleeding time is not known. Vorapaxar inhibits TRAP-induced *ex vivo* platelet aggregation but not ADP- or collagen-induced platelet aggregation, suggesting that vorapaxar treatment alone may not prolong bleeding time.

### WHAT QUESTION DID THIS STUDY ADDRESS?

✓ The present study, conducted in healthy volunteers, examined the effect of steady-state vorapaxar on bleeding

time compared with therapy-naive baseline. Aspirin was included as a comparator.

### WHAT THIS STUDY ADDS TO OUR KNOWLEDGE?

✓ This study adds to our knowledge regarding the human bleeding time profile of vorapaxar. Unlike aspirin, bleeding time following vorapaxar was similar to that at baseline. Bleeding time following vorapaxar with aspirin was similar to that following aspirin alone.

### HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS?

✓ The implications of these findings for clinical spontaneous or surgical bleeding await further analyses of clinical trial data.

Vorapaxar sulfate (ZONTIVITY; hereafter referred to as vorapaxar) is a first in class antagonist of the protease-activated receptor-1 (PAR-1), the primary thrombin receptor on human platelets, which mediates the downstream effects of thrombin in hemostasis and thrombosis. Thrombin-induced platelet activation has been implicated in a variety of cardiovascular disorders, including thrombosis, atherosclerosis, and restenosis, following percutaneous coronary intervention. Based on a large, placebo-controlled, phase III study conducted in 26,449 adult patients, the Thrombin-Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischemic Events (TRA 2°P – TIMI 50) trial, vorapaxar administered in addition to standard of care was approved to

reduce the risk of thrombotic cardiovascular events in the United States and European Union in patients with a history of myocardial infarction, and, in the United States, also in patients with peripheral arterial disease.<sup>1,2</sup>

As an inhibitor of the PAR-1 pathway, vorapaxar specifically blocks thrombin-mediated platelet aggregation. Although this mechanism of action provides patient benefit when coadministered with standard of care to reduce the risk of atherothrombotic complications of coronary artery disease, the potential exists that bleeding time due to the inhibition of this hemostatic mechanism may be prolonged. However, as a selective PAR-1 antagonist, vorapaxar inhibits thrombin receptor agonist peptide (TRAP)-induced *ex vivo*

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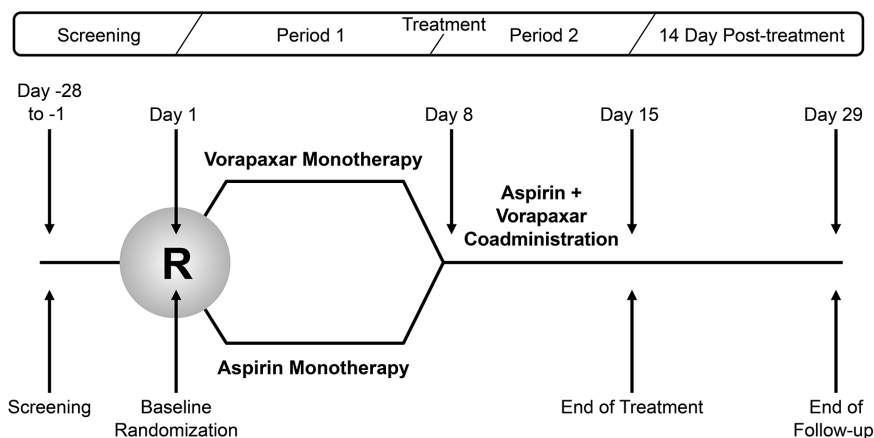


Figure 1 Study design.

platelet aggregation without affecting ADP- or collagen-induced platelet aggregation, suggesting that vorapaxar treatment alone may not prolong bleeding time due to the continued integrity of these alternative homeostatic pathways. The template bleeding time is a reproducible and sensitive method to measure the processes that form an initial platelet plug upon external injury to the vasculature.<sup>3</sup> Bleeding time assesses platelet function in the context of damaged skin and microvasculature as well as the interaction of platelets with the coagulation and fibrinolytic system. The present study, conducted in healthy volunteers, evaluated the potential for steady-state vorapaxar to prolong bleeding time compared with therapy-naïve baseline using a standard templated methodology for this assessment. Aspirin was included as a comparator in this study as it is commonly used as standard of care background therapy in the patient populations for which vorapaxar is indicated.

## METHODS AND MATERIALS

### Subjects

The study enrolled healthy male and female subjects 18 to 65 years of age. Exclusion criteria included: significant intolerance to aspirin; anticoagulant therapy; bleeding or spontaneous bruising diathesis; or a platelet transfusion within 7 days. All subjects provided written informed consent prior to participating in the trial.

### Study design

This was a randomized, active controlled, parallel group, two-period, assessor-blinded, open-label trial (identified as Protocol No. 046). The study design is summarized in **Figure 1** and the specific vorapaxar and aspirin doses administered are shown in **Table 1**. In period 1, subjects received either 81 mg aspirin once-daily for 7 days ( $N = 18$ ), or a 7-day regimen of vorapaxar ( $N = 18$ ) achieving steady-state plasma concentrations equivalent to chronic 2.5 mg once-daily doses. In period 2, each group added 7 days of the therapy alternate to that of period 1, without washout. Subjects fasted from all food and drink (except water) for at least 4 h prior to all platelet aggregation blood sample collections. All subjects refrained from consuming high or moderate fat

foods and drinks for at least 8 h prior to platelet aggregation sample collections. Consumption of low fat foods and drinks was permitted up to 4 h prior to the platelet aggregation blood draws. All bleeding time and platelet aggregometry operators were blinded to subject allocation, and were not involved with any other aspects of study conduct that would allow them to review subject source documents.

The study protocol was reviewed and approved by an independent institutional review board (Thomas Jefferson University Institutional Review Board). The study was conducted in accordance with the guidelines on good clinical practice and with ethical standards for human experimentation established by the Declaration of Helsinki.

### Pharmacokinetic assessments

Blood was drawn for vorapaxar pharmacokinetic (PK) analysis predose on day 1 of each treatment period and at selected time points through 2 h following the day 7 dose in period 1 and ~24 h following the day 7 dose in period 2. The plasma concentration of vorapaxar was analyzed by means of ultraperformance liquid chromatography and tandem mass spectrometry detection using positive ion electrospray. The lower limit of quantitation was 1 ng/mL. The analytical range was 1–1000 ng/mL. For each vorapaxar treatment period, the plasma concentration of vorapaxar was estimated for all subjects at trough ( $C_{\text{trough}}$ ) during the last two administered doses as well as at peak plasma concentration ( $C_{\text{max}}$ ) and 24 h following the day 7 dose ( $C_{24}$ ).

## PHARMACODYNAMIC ASSESSMENTS

### Platelet aggregation

Platelet aggregation was assessed three times: predose in periods 1 (baseline) and 2, and 24 h after the last dose in period 2. Blood samples for whole blood aggregometry were collected into BD Vacutainer tubes containing citrate (Becton Dickinson). Platelet-rich plasma was prepared by centrifuging the anticoagulated whole blood samples and transferring the platelet-rich plasma layer into a separate tube. The platelet-rich plasma (225  $\mu\text{L}$ ) was transferred to a cuvette containing a magnetic stir bar. After incubation for 3 min at 37°C, an electrode probe assembly was inserted into the cuvette. Following establishment of a baseline measurement,

**Table 1** Summary of vorapaxar and aspirin doses administered

Treatment regimen	No. of patients	Period 1 <sup>a</sup>	Period 2 <sup>a</sup>
1	18	Vorapaxar alone <ul style="list-style-type: none"> <li>• Day 1 am: 7.5 mg vorapaxar single dose</li> <li>• Day 1 pm: 10 mg vorapaxar single dose<sup>b</sup></li> <li>• Day 2 am: 5 mg vorapaxar single dose<sup>a</sup></li> <li>• Day 2 pm: 7.5 mg vorapaxar single dose<sup>a,b</sup></li> <li>• Days 3–5: 5 mg vorapaxar once-daily<sup>a</sup></li> <li>• Days 6–7: 2.5 mg vorapaxar once-daily<sup>a</sup></li> </ul>	Aspirin coadministered with vorapaxar 81 mg aspirin coadministered with 2.5 mg vorapaxar, both once-daily × 7 days <sup>b</sup>
2	18	Aspirin alone 81 mg aspirin once-daily × 7 days <sup>a</sup>	Vorapaxar coadministered with aspirin <ul style="list-style-type: none"> <li>• Day 1 am: 7.5 mg vorapaxar + 81 mg aspirin, single doses</li> <li>• Day 1 pm: 10 mg vorapaxar single dose<sup>b</sup></li> <li>• Day 2 am: 5 mg vorapaxar + 81 mg aspirin, single doses<sup>a</sup></li> <li>• Day 2 pm: 7.5 mg vorapaxar single dose<sup>a,b</sup></li> <li>• Days 3–5: 5 mg vorapaxar q.d. + 81 mg aspirin once-daily<sup>a</sup></li> <li>• Days 6–7: 2.5 mg vorapaxar q.d. + 81 mg aspirin once-daily<sup>a</sup></li> </ul>

<sup>a</sup>Dose(s) on days 2–6 were to be administered approximately the same time as the dose(s) on day 1, ± 2 h.

<sup>b</sup>The second (pm) vorapaxar dose on days 1 and 2 was to be administered ~12 h ± 2 h after the morning (am) dose.

25 μL of one of the following agonists was added to achieve the final indicated concentration: adenosine diphosphate (ADP; 10 μM), arachidonic acid (500 μg/mL), or TRAP-6 (15 μM). Platelet aggregometry was assessed using a Chrono-Log Model 700 aggregometer (Chrono-Log, Havertown, PA, USA). Changes in sample impedance were recorded for a total of 6 min. A single technician performed all of the platelet aggregometry assays.

### Coagulation parameter assessment

Blood samples for an assessment of coagulation function were collected prior to day 1 study drug administration in periods 1 and 2, and 24 h after the period 2 day 7 study drug administration. Standard measures of coagulation assessed in this study included prothrombin time (PT) and activated partial thromboplastin time (aPTT).

### Bleeding time

Bleeding time was assessed predose in periods 1 (baseline) and 2, and 24 h after the last dose in period 2. A blood pressure cuff was placed on the subject's arm above the elbow and inflated to 40 mm Hg throughout the procedure. A target incision site without superficial veins approximately in the middle of the muscular area of the forearm was selected and cleansed. A small incision wound of 5-mm length and 1-mm depth was made at the target site with a sterile disposable template bleeding time device (Surgicutt for adults; International Technidyne Corporation, Edison, NJ, USA).<sup>4</sup> A stopwatch was started at the appearance of the first drop of blood. The edge of a 3.5" circular filter paper was used to blot the blood through capillary action by gently touching the drop every 30 seconds. The wound itself was not disturbed. The blood pressure gauge was removed once bleeding stopped spontaneously and a sterile dressing was applied. The end point was reached when blood could no longer be blotted from the forearm incision. Bleeding times >30 min were truncated and the measurement reported as >30 min. The same operator performed the bleeding time test on the same subject throughout the course of the study.

### Statistical analysis

Descriptive statistics were provided for the plasma PK of vorapaxar at trough during the last two administered doses of each period, at  $C_{max}$  after dosing on day 7 of each period, and 24 h after the day 7 dose in each period. Arithmetic mean and SD based on the raw scale were provided. In addition, the percent coefficient of variation were also provided and calculated according to the following formula:  $100 \times \sqrt{\frac{s^2}{\bar{x}^2}} - 1$ , where  $s^2$  is the observed variance on the natural log-scale.

Bleeding time data were analyzed using a linear mixed effects model with fixed effect for treatment (baseline, vorapaxar alone, aspirin alone, and vorapaxar + aspirin). An unstructured covariance matrix was used to allow for unequal treatment variances and to model the correlation between the measurements within each subject using the REPEATED statement in SAS PROC MIXED. The Kenward and Roger's method were used to calculate the DDFM = KR. The geometric mean ratio (GMR) for bleeding time: (a) steady-state vorapaxar alone vs. baseline; (b) steady-state vorapaxar coadministered with steady-state aspirin compared with steady-state aspirin alone; (c) steady-state vorapaxar coadministered with steady-state aspirin compared with steady-state vorapaxar alone; and (d) steady-state aspirin compared with baseline was estimated and a 90% confidence interval (CI) of the GMR was provided. The prespecified GMR lower and upper equivalence bounds for these four comparisons were 0.67 and 1.50, respectively. Assuming a pooled within-subject SD for bleeding time of 0.2637, and if the true GMR for bleeding time of steady-state vorapaxar alone at period 1, day 7, 24 h vs. baseline was between 0.82 and 1.21, then a sample size of 36 subjects in which at least one-half of the sample ( $N = 18$ ) achieved steady-state vorapaxar levels would provide 90% probability that the 90% CI for this comparison was between 0.67 and 1.50.

For the other PD end points, TRAP-, arachidonic acid (AA)-, ADP-induced platelet aggregation as well as PT and aPTT, the same linear mixed effects model described above for bleeding time was used.

**Table 2** Plasma PKs of vorapaxar administered alone or with aspirin 81 mg, once-daily for 7 days

Regimen	Treatment	Period	Day	No.	C <sub>trough</sub> (ng/mL) <sup>a</sup>	C <sub>max</sub> (ng/mL)	C <sub>24</sub> (ng/mL) <sup>b</sup>
1	Vorapaxar days 1–7	1	6	18	51.1 (41.0)	NA	NA
			7	18	50.6 (38.8)	67.3 (40.3)	47.9 (39.1)
	Vorapaxar coadministered with aspirin days 1–7	2	6	17 <sup>c</sup>	50.9 (27.9)	NA	NA
			7	17 <sup>c</sup>	51.1 (30.9)	55.1 (24.5)	51.0 (31.3)
2	Vorapaxar coadministered with aspirin days 1–7	2	6	18	47.4 (21.2)	NA	NA
			7	18	47.6 (19.0)	54.0 (24.1)	44.1 (21.0)

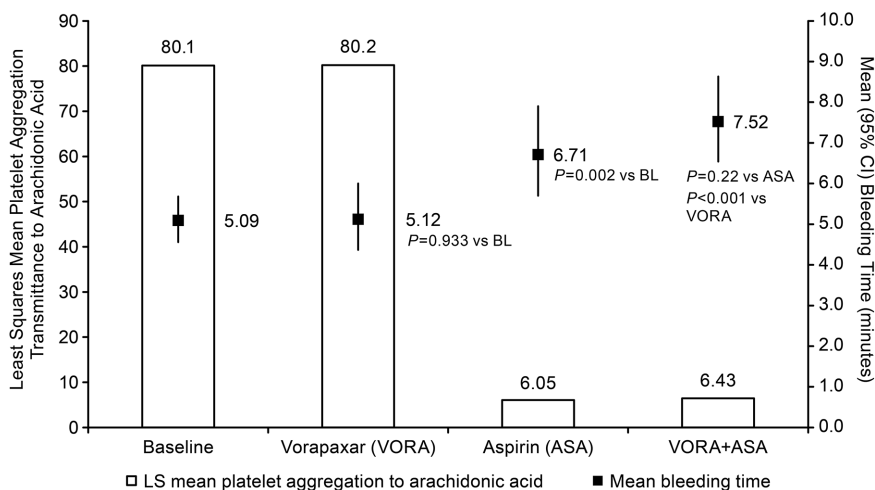
NA, values are not applicable; PK, pharmacokinetic.

C<sub>trough</sub>, C<sub>max</sub>, and C<sub>24</sub> data expressed as geometric mean (% coefficient of variation).

Treatment regimen 1: Vorapaxar for 7 days to steady-state in period 1; aspirin coadministered with steady-state vorapaxar for 7 days in period 2.

Treatment regimen 2: Aspirin for 7 days to steady-state in period 1; vorapaxar coadministered with steady-state aspirin for 7 days in period 2.

<sup>a</sup>Pre-dose (0 h) timepoint. <sup>b</sup>C<sub>24</sub> = Hour 24 post-dose timepoint. <sup>c</sup>Data collected after period 2 day 2 for one patient were excluded from analyses due to a minor protocol deviation

**Figure 2** Comparison of platelet aggregation to arachidonic acid and bleeding time. BL, baseline.

No multiplicity adjustment was performed because there is only one formal primary hypothesis (on vorapaxar) and one primary end point of interest (bleeding time). Thus, all the  $p$  values showed in this article were unadjusted nominal  $p$  values.

A natural log-transformation was applied to all the bleeding time data as well as PT and aPTT prior to analyses, and final results were reported on the original scale after back-transformation. Data for TRAP-, AA-, and ADP-induced platelet aggregation were not transformed. The assumption of normality was tested by the Shapiro-Wilk statistics and an examination of the Q-Q plot. Although minor departure for normality was observed in the bleeding time data based on the Shapiro-Wilk statistics ( $p < 0.05$ ), further examination of the residuals from the Q-Q plot suggested that the deviation was relatively minor, thus the log-transformation was retained as the primary analysis. The homogeneity of variance assumption was tested by the Levene's test and was satisfied ( $p > 0.050$ ).

## RESULTS

### Demographics

Thirty-six healthy male ( $N = 31$ ) and female ( $N = 5$ ) subjects were enrolled into the study at a single clinical site.

The mean age of the subjects was 41 years (range, 21–54 years). Twenty-seven subjects (75%) were black, eight subjects (~22%) were white, and one (~3%) was of mixed race. All 36 subjects completed the study; however, data collected after period 2 day 2 for one patient were excluded from analyses due to a minor protocol deviation.

### Pharmacokinetics

**Table 2** and **Figure 2** summarize the vorapaxar PK results. On day 7 of the vorapaxar treatment periods, the geometric mean C<sub>max</sub> levels ranged from 54 to 67 ng/mL and the geometric mean C<sub>24</sub> levels ranged from 44 to 51 ng/mL. On days 6 and 7 of the vorapaxar treatment periods, the geometric mean C<sub>trough</sub> levels ranged from 47 to 51 ng/mL and 48 to 51 ng/mL, respectively.

### Platelet aggregation

**Table 3** and **Figure 2** summarize the platelet aggregation assessment results. TRAP-induced platelet aggregation was inhibited 93.6% (90% CI = 90.3–96.9) relative to baseline after 7 days of vorapaxar treatment alone and was unchanged after 7 days of treatment with vorapaxar coadministered with aspirin. AA-induced platelet aggregation was inhibited 92.4% (90% CI = 88.0–96.8) relative to baseline after 7 days of aspirin treatment alone.



**Table 3** TRAP-, AA-, and ADP-induced platelet aggregation (% inhibition) 24 h following 7 days of treatment with vorapaxar or aspirin, and 24 h following 7 days treatment with vorapaxar + aspirin

TRAP-induced platelet aggregation		
Treatment	No.	LS mean (95% CI) % inhibition of platelet aggregation
Aspirin	18	10.3 (6.99–13.6)
Vorapaxar + aspirin	35	93.7 (92.8–94.6)
Vorapaxar	18	93.6 (90.3–96.9)
Treatment comparison	Difference (90% CI)	p value
Vorapaxar vs. aspirin	83.3 (79.4–87.3)	<0.001
Vorapaxar + aspirin vs. aspirin	83.4 (80.5–86.4)	<0.001
Vorapaxar + aspirin vs. vorapaxar	0.1 (–2.8 to 3.0)	0.949
AA-induced platelet aggregation		
Treatment	No.	LS mean (95% CI) % inhibition of platelet aggregation
Aspirin	18	92.4 (88.0–96.8)
Vorapaxar + aspirin	35	92.0 (90.1–93.9)
Vorapaxar	18	–1.2 (–5.7 to 3.2)
Treatment comparison	Difference (90% CI)	p value
Vorapaxar vs. aspirin	–93.6 (–98.9 to –88.4)	<0.001
Vorapaxar + aspirin vs. aspirin	–0.4 (–4.4 to 3.7)	0.877
Vorapaxar + aspirin vs. vorapaxar	93.3 (89.2–97.3)	<0.001
ADP-induced platelet aggregation		
Treatment	No.	LS mean (95% CI) % inhibition of platelet aggregation
Aspirin	18	10.2 (6.1–14.3)
Vorapaxar + aspirin	35	9.4 (6.8–12.0)
Vorapaxar	18	–2.6 (–6.7 to 1.6)
Treatment comparison	Difference (90% CI)	p value
Vorapaxar vs. aspirin	–12.8 (–17.1 to –8.4)	<0.001
Vorapaxar + aspirin vs. aspirin	–0.8 (–3.9 to 2.3)	0.655
Vorapaxar + aspirin vs. vorapaxar	11.9 (8.8–15.1)	<0.001

AA, arachidonic acid; CI, confidence interval; LS mean, least squares mean; TRAP, thrombin receptor agonist peptide.

Coadministration of aspirin with vorapaxar did not detectably alter this interaction. ADP-induced platelet aggregation was unaffected by vorapaxar treatment as compared with baseline. ADP-induced platelet aggregation was inhibited modestly by aspirin treatment relative to baseline either in the absence (10.2% inhibition; 90% CI = 6.09–14.3) or presence (9.39% inhibition; 90% CI = 6.78–12.0) of vorapaxar.

### Coagulation parameters

Neither vorapaxar nor aspirin nor their combined administration altered aPTT or PT (data not shown).

**Table 4** Bleeding time at baseline, 24 h following 7 days of treatment with vorapaxar or aspirin and 24 h following 7 days treatment with vorapaxar + aspirin

Treatment	No.	LS mean (95% CI) bleeding time (min)
Baseline	36	5.09 (4.56–5.68)
Vorapaxar	18	5.12 (4.37–6.00)
Aspirin	17	6.71 (5.70–7.90)
Vorapaxar + aspirin	35	7.52 (6.54–8.63)
Treatment comparison	GMR (90% CI)	p value
Vorapaxar vs. baseline	1.01 (0.88–1.15)	0.933
Vorapaxar + aspirin vs. aspirin	1.12 (0.96–1.30)	0.219
Aspirin vs. baseline	1.32 (1.15–1.51)	0.002
Vorapaxar + aspirin vs. vorapaxar	1.47 (1.26–1.70)	< 0.001

CI, confidence interval; GMR, geometric mean ratio, transformed back from log-scale; LS mean, least squares mean.

### Bleeding time

**Table 4** summarizes the bleeding time assessment results. Aspirin alone increased least squares (LS) mean bleeding time from 5.09 min to 6.71 min ( $p = 0.002$ ), whereas vorapaxar had no effect, with an LS mean bleeding time of 5.12 min ( $p = 0.933$ ). Aspirin and vorapaxar together demonstrated an LS mean bleeding time of 7.52 min, different from vorapaxar alone ( $p < 0.001$ ). There was a slight numerical increase in bleeding time with vorapaxar coadministered with aspirin compared with aspirin alone, but the difference was not significant ( $p = 0.219$ ). Bleeding time results were not available for one of 18 subjects in the aspirin group.

### Safety

No subject reported a bleeding-related adverse event.

### DISCUSSION

Vorapaxar administered in addition to standard of care reduces thrombotic cardiovascular events in patients with a history of myocardial infarction or with peripheral artery disease.<sup>1,2</sup> Although its PAR-1-mediated mechanism of action provides patient benefit when coadministered with standard of care, the potential exists that bleeding time, due to the inhibition of this hemostatic mechanism, may be prolonged. A properly conducted capillary bleeding time assessment is considered a reproducible and sensitive method to measure the effects of treatment on the initial phase of hemostasis, formation of a platelet plug. The present study in healthy volunteers evaluated the potential for vorapaxar to prolong bleeding time using a recognized standard methodology.

The present study assessed steady-state bleeding time by a regimen producing, after 5 days' dosing, plasma exposures of vorapaxar equivalent to those at steady-state. Population PK model-based simulations assuming the demographic

characteristics (gender, race, and body weight) of subjects enrolled in this study show that subjects receiving a 2.5 mg once-daily dose are expected to have steady-state average  $C_{max}$  and trough plasma concentration ( $C_{min}$ ) of 64.8 ng/mL (95% CI = 58.3–72.2 ng/mL) and 42.2 ng/mL (95% CI = 36.7–48.6 ng/mL), respectively. The plasma concentrations of vorapaxar on days 6 and 7 measured in each period of the present study ( $C_{max}$  = 54–67 ng/mL;  $C_{trough}$  day 6 and 7 = 47–51 ng/mL in this study) confirmed attainment of appropriate steady-state conditions. Results from previous phase I clinical trials of vorapaxar in healthy volunteers also support that day 6 and 7 vorapaxar concentrations in this study are consistent with steady-state concentrations for 2.5 mg once-daily dose.<sup>5,6</sup>

The inhibition of platelet aggregation was measured by various methods to confirm the anticipated action of the administered aspirin and vorapaxar. Administration of vorapaxar alone resulted in inhibition of TRAP-induced platelet aggregation without an effect on either ADP- or AA-mediated activation of platelet aggregation, consistent with vorapaxar's PAR-1-specific mechanism of action. Aspirin alone inhibited AA-induced platelet aggregation and also, to a small extent, that of ADP-induced aggregation. The combination of vorapaxar and aspirin demonstrated these combined effects without further alteration. These results were fully consistent with the administered agents, demonstrating the engagement of vorapaxar at the PAR-1 receptor in subjects treated with vorapaxar for 7 days in the presence or absence of aspirin as well as inhibition of the thromboxane B<sub>2</sub> receptor after administration of aspirin in the presence or absence of vorapaxar.

The AA-induced platelet aggregation inhibition of 92.4% (90% CI = 88.0–96.8) relative to baseline by aspirin treatment alone was consistent with literature values (80% inhibition with 50-mg at steady-state, and 87% inhibition with 324-mg aspirin at steady-state).<sup>7</sup> Coadministration of aspirin with vorapaxar did not detectably alter this effect. ADP-induced platelet aggregation was unaffected by vorapaxar treatment as compared with baseline. However, ADP-induced platelet aggregation was inhibited modestly by aspirin treatment relative to baseline in the absence of vorapaxar (10.2% inhibition; 90% CI = 6.09–14.3) or presence of vorapaxar (9.39% inhibition; 90% CI = 6.78–12.0). Aspirin treatment is known to have modest inhibitory effects on the aggregation sensitivity of the ADP-receptor in response to stimulus by ADP, as demonstrated by Payne *et al.*,<sup>8</sup> who reported that healthy subjects administered a single 150 mg dose of aspirin inhibited ADP-induced platelet aggregation by ~33% compared with an aspirin-naïve baseline. In this study, aPTT and PT values were not changed upon administration of vorapaxar alone, aspirin alone, or vorapaxar coadministered with aspirin over the 7-day dosing intervals, consistent with observations during development of vorapaxar and as has been demonstrated for aspirin.<sup>7</sup>

With respect to the bleeding time assessment in this study, aspirin 81 mg once-daily administered for 7 days was included as a positive sensitivity control. A prior study<sup>9</sup> showed a quantifiable prolongation in bleeding time following administration of aspirin 81 mg once-daily for 7 days: GMR (aspirin 81 mg/baseline) = 1.58; 90% CI = 1.33–1.88), and

was generally consistent, although slightly longer numerically compared with the prolongation observed in the present study with aspirin 81 mg once-daily for 7 days: GMR (aspirin 81 mg/baseline) = 1.32; 90% CI = 1.15–1.51). The bleeding time GMR and 90% CI for vorapaxar alone compared with baseline was 1.01 (90% CI = 0.88–1.15), which was within the prespecified GMR equivalence bounds of 0.67 and 1.50. Thus, it can be concluded that administration of vorapaxar to steady-state plasma levels does not prolong bleeding time significantly as compared with baseline. Similarly, the GMR and 90% CI for vorapaxar coadministered with aspirin compared with aspirin alone was 1.12 (90% CI = 0.96–1.30), which was also within the prespecified GMR equivalence bounds. Thus, it can be concluded that the bleeding time at steady-state plasma vorapaxar coadministered with aspirin 81 mg once-daily for 7 days is similar to that of aspirin 81 mg once-daily for 7 days alone. The present findings are in agreement with those of previously reported vorapaxar single-dose rising and multiple-dose rising studies,<sup>10</sup> which used a puncture device rather than a cutting device for template bleeding time assessment, and which demonstrated that bleeding time was not meaningfully prolonged with vorapaxar.

In conclusion, bleeding time assessed following administration of aspirin to steady-state was significantly elevated relative to bleeding time assessed at baseline. In contrast to aspirin, bleeding time assessed following administration of vorapaxar to steady-state was similar to that assessed at baseline. Bleeding time assessed following administration of steady-state vorapaxar coadministered with steady-state aspirin was similar to that assessed for aspirin at steady-state alone. The implications of these findings for clinical spontaneous or surgical bleeding await further analyses of clinical trial data.

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**Conflict of Interest.** J.H.G., D.L.C., F.G., B.M.W., J.H., R.E.W., S.Z., and M.S.A. are current or former employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Kenilworth, NJ, and may own stock and/or own stock options in the company. W.K.K., S.N., and U.P.N. have nothing to disclose.

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