SCH 602539, a Protease-Activated Receptor-1 Antagonist, Inhibits Thrombosis Alone and in Combination With Cangrelor in a Folts Model of Arterial Thrombosis in Cynomolgus Monkeys

Madhu Chintala, John Strony, Bo Yang, Stan Kurowski, Qiu Li

Objective—To determine the antithrombotic effects of SCH 602539, an analog of the selective protease-activated receptor (PAR)-1 antagonist vorapaxar (formerly SCH 530348) currently in advanced clinical development, and the P2Y12 ADP receptor antagonist cangrelor, alone and in combination.

Methods and Results—Multiple platelet activation pathways contribute to thrombosis. The effects of SCH 602539 and cangrelor alone and in combination on cyclic flow reductions were evaluated in a Folts model of thrombosis in cynomolgus monkeys. The effects of these treatments on ex vivo platelet aggregation and coagulation parameters were also monitored. Dose-dependent inhibition of cyclic flow reductions was observed after treatment with SCH 602539 alone and cangrelor alone (P<0.05 versus vehicle for the 2 highest concentrations of each agent). The combination of SCH 602539 and cangrelor was associated with synergistic antithrombotic effects (P<0.05 versus vehicle for all combinations tested). The 2 highest doses of SCH 602539 inhibited platelet aggregation in response to PAR-1–selective high-affinity thrombin receptor agonist peptide by greater than 80% but did not affect platelet aggregation induced by other agonists; also, they did not affect any coagulation parameters.

Conclusion—The combined inhibition of the PAR-1 and the P2Y12 ADP platelet activation pathways had synergistic antithrombotic and antiplatelet effects. The addition of a PAR-1 antagonist to a P2Y12 ADP receptor antagonist may provide incremental clinical benefits in patients with atherothrombotic disease, both in short- and long-term settings. These hypotheses need to be tested clinically. (Arterioscler Thromb Vasc Biol. 2010;30:00-00.)

Key Words: thrombin ■ thrombosis ■ PAR-1 antagonist ■ antiplatelet ■ platelet activation pathways
Methods

Animals

Given the existence of species differences in the platelet thrombin receptor,8 we used cynomolgus monkeys because they have the same distribution of thrombin receptors (PAR-1 and PAR-4) on their platelets as do humans.10 Animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act, in a program accredited by the American Association for Accreditation of Laboratory Animal Care.

Folts Model of Thrombosis in Anesthetized Monkeys

The procedures used in this study are similar to the acute artery occlusion model developed by Folts and others.9,11–13 Cynomolgus monkeys were sedated with ketamine hydrochloride, 10 mg/kg IM, followed by anesthesia with sodium pentobarbi-
tal, 20-mg/kg IV bolus and 5-mg/kg per hour IV infusion for the duration of the experiment. The body temperature was maintained at 37°C to 39°C, and fluids infused were warmed to body temperature. The right carotid artery was exposed and dissected free of surrounding tissue, and an appropriately sized transonic flow probe was placed around the vessel. A constrictor (Lexan; 5-mm length) was placed onto the carotid artery. The constrictor was sized to abolish reactive hyperemia and to reduce mean carotid blood flow by no more than 50% to 60%. Mechanical damage to the endothelium was induced in the constricted segment of the artery. Cyclic flow reductions (CFRs) because of platelet-dependent thrombus formation occurred shortly after placement of the constrictor over the region of endothelial damage. These gradual declines in carotid blood flow were occasionally interrupted spontaneously but fre-
quently required manual restoration by gently shaking the vessel. Flow reductions would typically return in 3 to 4 minutes. The CFR frequency was calculated as the number of such cycles (CFRs) over a 30-minute period. Based on the pilot studies, CFRs were evaluated with the reinduction of endothelial injury. Study drug or vehicle was administered after stable CFRs were achieved. On the completion of a 30-minute baseline CFR collection period, drug(s) or vehicle was administered incrementally (every 30 minutes) either as a bolus (SCH 602539) or as an infusion (cangrelor); and their effects on CFRs were monitored in the next three 30-minute observation periods. Blood samples, 3 mL, were collected from the femoral arterial catheter at the end of each 30-minute observation period for assessment of ex vivo platelet aggregation and coagulation parameters.

Ex Vivo Platelet Aggregation

Platelet aggregation studies were performed ex vivo on blood samples obtained from the monkeys subjected to the Folts model, using a whole blood aggregometer (model 540VS; ChronoLog, Havertown, Pa). Briefly, 0.5 mL of blood was incubated with 0.5 mL of normal saline at 37°C in a cuvette containing a stir bar for 2 minutes. Platelet agonists used in this study included TRAP, 3 μmol/L; ADP, 10 μmol/L; the thromboxane A2 mimetic U46619, 10 μmol/L; and collagen, 3 μg/mL. Platelet aggregation was monitored for 5 minutes after the addition of the agonist. The peak aggregation response was recorded in ohms. In addition, standard coagulation parameters, including prothrombin time (PT), activated partial thromboplastin time (aPTT), and activated clotting time (ACT), were assessed.

PT and aPTT Evaluation

These assays were performed in plasma obtained from the animals at the indicated time points. The PT assay is used for the detection of deficiencies in the extrinsic coagulation system, especially for factors VII and X. Briefly, a 100-μL plasma sample was warmed to 37°C for 3 minutes. Then, 200 μL of thromboplastin reagent was added to the plasma sample and inserted into the well of the automated instrument (Coag-A-Mate) to evaluate and record the time to the formation of a clot. The aPTT test is used to detect deficiencies in the intrinsic coagulation system, especially for factors VIII, IX, XI, and XII. Briefly, a 100-μL plasma sample was incubated with the aPTT reagent at 37°C for 5 minutes. Then, 100 μL of calcium chloride was added to the plasma sample to initiate clot formation, the speed of which was detected and measured with the automated instrument (Coag-A-Mate).

ACT Evaluation

The ACT measures the clotting time of fresh whole blood and was performed in an automated coagulation timer (ACT II; Medtronic) using the RACT cartridges (Medtronic). Briefly, 0.2 mL of citrated whole blood was added to each well of the cartridge and the test was initiated by inserting the cartridge into the machine (ACT II). The end point, clot formation, was measured by the rate of fall of the plunger-flag mechanism contained in each cartridge. The
plunger assembly falls rapidly through an unclotted sample, but the fibrin web formed during clotting impedes the rate of decent, which can be detected by a photo-optical system and displayed. These measurements were performed in duplicate.

**Evaluation of Synergy**

The in vivo effect of coadministration of PAR-1 and P2Y12 ADP receptor antagonists on the inhibition of CFRs (synergy, additivity, or antagonism) was tested using the criteria described by Berenbaum. Briefly, CFR frequency was plotted versus drug dose administered and fitted to dose-response curves using inhibitory effect $E_{\text{max}}$ modeling. Estimates of the dose regimens needed for 50% reduction in CFRs ($E_{\text{C50}}$) were calculated using the following equation:

$$E = E_{\text{max}} (1 - C / (C + E_{\text{C50}}))$$

where $E$ is the CFR frequency, assuming maximum frequency ($E_{\text{max}}$) is achieved at dose level 0 and 0 frequency at dose level infinity. The $E_{\text{C50}}$ value refers to the concentration of the antiplatelet agent required to reduce maximum CFR frequency by 50%.

Dose-response curves were constructed for SCH 602539 and cangrelor. Equipotent dose values obtained for the tested combinations were fitted to the equation used by Berenbaum for the determination of the synergy factor. Values of less than 1 indicate the presence of synergy, whereas values equal to 1 are indicative of an additive effect.

$$\frac{\text{Dose of } A_{\text{in combination with B}}}{\text{Dose of } A_{\text{alone}}} + \frac{\text{Dose of } B_{\text{in combination with A}}}{\text{Dose of } B_{\text{alone}}}$$

**Results**

Of the 24 monkeys studied, 22 exhibited stable CFRs after instrumentation. There was minimal reduction in blood flow in the context of abolishing hyperemic blood flow. The heart rate, blood pressure, and body temperature were unchanged for the duration of the study.

Antithrombotic Effects in the Folts Model of Thrombosis

The results of 22 experiments with the vehicle are shown in Figure 2A. The stability and utility of the surgical model were demonstrated by the consistent and reproducible CFRs achieved in animals treated with vehicle for the 2-hour study period. In the 22 experiments, the control frequency of the CFRs was a mean ± SEM of 8.9 ± 1.8 per 30 minutes (range, 7–13) (Figure 2A).

Intravenous bolus doses of SCH 602539 reduced the CFR frequency from baseline by approximately 50%, 70%, and 90% with the 0.1-, 0.2-, and 0.3-mg/kg doses, respectively (Figure 2B). The reductions achieved with the 2 highest doses were statistically significant versus vehicle. The intermediate and highest doses of cangrelor completely prevented CFRs in 3 and 5 of 6 animals, respectively. No complete suppression was evident in any of the animals treated with the lowest dose of cangrelor.

To assess the antithrombotic effects of SCH 602539 in combination with cangrelor, doses of each agent that provided only modest inhibition of CFRs when used alone were chosen (SCH 602539, 0.1 mg/kg; and cangrelor, 0.1 µg/kg per minute). Doses of SCH 602539 and cangrelor that were estimated not to affect CFR frequency when administered alone (SCH 602539, 0.05 mg/kg; and cangrelor, 0.05 µg/kg...
Evaluation of Synergy Resulting From Dual P2 Years₁₂ and PAR-1 Inhibition

The Folts model of CFRs, resulting from fixed stenosis of an arterial bed, is the result of the in vivo interplay of platelet-sensitive vasoactive molecules, which closely mimics the clinical scenario of unstable angina. The mean±SEM EC₅₀ values calculated from dose-response curves are 0.100±0.042 mg/kg and 0.099±0.039 μg/kg per minute for SCH 602539 and cangrelor, respectively.

Five experiments were conducted for each of the 3 combinations partnering SCH 602539 and cangrelor. The corresponding equipotent doses for each compound compared with the different dose combinations were calculated from the respective dose-response curve (Figure 2D and Figure 3). The in vivo CFR inhibition exerted by SCH 602539 or cangrelor, when combined with its partner, was greater than that of the individual components administered alone.

The presence of a synergistic interaction is suggested by the 50% reduction in CFR frequency with SCH 602539, 0.05 mg/kg, in combination with cangrelor, 0.05 μg/kg per minute; and the complete extinction of CFR production in 4 of 5 animals receiving SCH 602539, 0.1 mg/kg, plus cangrelor, 0.1 μg/kg per minute. Similar antithrombotic effects were also observed with the combination of SCH 602539, 0.15 mg/kg, plus cangrelor, 0.1 μg/kg per minute, administered in the subsequent 30-minute period. The CFR frequency for baseline and each of the 3 doses administered, along with the calculated doses, are shown for SCH 602539 and cangrelor in Figure 2D and Figure 3, respectively.

Synergism was confirmed because the synergistic factor was less than 1 in 12 of 15 combination experiments (data not shown) and was seen in the lowest tested dosage combination of 0.05 plus 0.05. By using the model of Berenbaum,¹⁵ the calculated mean±SEM synergistic factor for all 15 combination experiments was 0.41±0.17.

Effects on Ex Vivo Platelet Aggregation

The effects of SCH 602539 alone, cangrelor alone, and the combination of SCH 602539 and cangrelor on ex vivo platelet aggregation mediated by various agonists (eg, haTRAP, ADP, thromboxane A₂ mimetic U46619, and collagen) in the Folts model of thrombosis in anesthetized cynomolgus monkeys were also evaluated. Table 1 outlines the inhibition of platelet aggregation noted at each of the tested dosing regimens of study drug. The 2 highest doses of SCH 602539, 0.3 and 1 mg/kg, were associated with potent (>80%) and dose-related inhibition of ex vivo platelet aggregation induced by 3-μmol/L haTRAP but did not affect the aggregation induced by 10-μmol/L ADP, 10-μmol/L thromboxane A₂ mimetic U46619, and 3-μg/mL collagen, demonstrating selectivity of SCH 602539 for the PAR-1 receptor pathway. The 2 highest doses of cangrelor, 0.2 and 0.3 μg/kg per minute, inhibited ADP-mediated platelet aggregation by approximately 50% to 60% (as targeted) but did not interfere with the aggregation stimulated by 3-μmol/L haTRAP, 10-μmol/L thromboxane A₂ mimetic U46619, or 3-μg/mL collagen. Finally, the combination of SCH 602539 and cangrelor inhibited the aggregation induced by 3-μmol/L haTRAP and 10-μmol/L ADP in a dose-related manner; and the level of inhibition was the same as for each agent used alone. No inhibition of platelet aggregation induced by 10-μmol/L thromboxane A₂ mimetic U46619 or 3-μg/mL collagen was observed. These findings suggest that the ex vivo inhibition of haTRAP- and ADP-mediated platelet aggregation pathways is not predictive of the synergistic effect of combined therapy with SCH 602539 and cangrelor on the reduction of CFRs.

Effects on Coagulation Parameters

The administration of SCH 602539 alone, cangrelor alone, and the combination of SCH 602539 and cangrelor had no effect on the coagulation parameters, including PT, aPTT, and ACT (Table 2). These findings are consistent with the fact that both SCH 602539 and cangrelor are antiplatelet agents that interact with specific platelet receptors and do not interfere with the activity of the coagulation cascade.

Discussion

In the present study, we have demonstrated the antithrombotic effects of PAR-1 antagonism with SCH 602539 in a Folts model of thrombosis. More important, we have demon-
Chintala et al PAR-1 Antagonist in Folts Model of Thrombosis

Table 1. Effects of SCH 602539, Cangrelor, and Their Combination on Ex Vivo Inhibition of Platelet Aggregation Induced by Various Agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Baseline</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13±3</td>
<td>12±2</td>
<td>17±4</td>
<td>14±5</td>
</tr>
<tr>
<td>TRAP</td>
<td>15±3</td>
<td>14±2</td>
<td>16±3</td>
<td>13±4</td>
</tr>
<tr>
<td>ADP</td>
<td>14±3</td>
<td>12±3</td>
<td>19±4</td>
<td>21±5</td>
</tr>
<tr>
<td>U46619</td>
<td>20±5</td>
<td>17±3</td>
<td>24±3</td>
<td>29±5</td>
</tr>
<tr>
<td>Collagen</td>
<td>27±4</td>
<td>26±2</td>
<td>24±3</td>
<td>32±7</td>
</tr>
<tr>
<td>SCH 602539</td>
<td>Baseline</td>
<td>0.1 μg/kg/min</td>
<td>0.2 μg/kg/min</td>
<td>0.3 μg/kg/min</td>
</tr>
<tr>
<td>TRAP</td>
<td>13±3</td>
<td>12±3</td>
<td>12±3</td>
<td>9±3</td>
</tr>
<tr>
<td>ADP</td>
<td>14±3</td>
<td>10±2</td>
<td>7±1</td>
<td>6±1</td>
</tr>
<tr>
<td>U46619</td>
<td>15±3</td>
<td>12±3</td>
<td>10±4</td>
<td>10±4</td>
</tr>
<tr>
<td>Collagen</td>
<td>24±3</td>
<td>20±3</td>
<td>20±4</td>
<td>20±3</td>
</tr>
<tr>
<td>SCH 602539 plus cangrelor</td>
<td>Baseline</td>
<td>0.05 mg/kg + 0.05 μg/kg/min</td>
<td>0.1 mg/kg + 0.1 μg/kg/min</td>
<td>0.15 mg/kg + 0.1 μg/kg/min</td>
</tr>
<tr>
<td>TRAP</td>
<td>23±4</td>
<td>14±3</td>
<td>10±2</td>
<td>0</td>
</tr>
<tr>
<td>ADP</td>
<td>14±1</td>
<td>10±1</td>
<td>9±2</td>
<td>7±2</td>
</tr>
<tr>
<td>U46619</td>
<td>22±2</td>
<td>22±3</td>
<td>15±5</td>
<td>20±3</td>
</tr>
<tr>
<td>Collagen</td>
<td>26±3</td>
<td>30±3</td>
<td>20±6</td>
<td>22±4</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. The agents used included the following: haTRAP, 3 μmol/L; ADP, 10 μmol/L; U46619, 10 μmol/L; and collagen, 3 μg/mL.

strated that the in vivo antithrombotic effects of PAR-1 antagonism, in combination with P2 years12 ADP receptor antagonism, are synergistic. The inhibitory activity of SCH 602539 was specific for platelet aggregation induced by haTRAP. The aggregation induced by other agonists, such as ADP, thromboxane A2 mimetic U46619, and collagen, was not affected, demonstrating the specificity and selectivity for the PAR-1 receptor. Cangrelor specifically inhibited aggregation induced by ADP and demonstrated a modest numeric reduction of thrombosis demonstrated that the addition of oral aspirin to oral clopidogrel was associated with potent antithrombotic effects, and also additive bleeding effects, possibly related to the combined inhibitory activity of 2 antiplatelet agents on collagen-induced platelet aggregation. The additive antithrombotic effects of combined inhibition of the thromboxane A2 and P2 years12 ADP receptor platelet activation pathways with aspirin and clopidogrel observed in these studies are consistent with significant reductions in ischemic events with dual antiplatelet therapy over aspirin alone reported in large clinical trials.2,3,20–22 These findings suggest that in the vascular bed, platelet activation leading to thrombosis is a complex matrix mediated by multiple pathways.

The present study is the first direct demonstration of in vivo synergism with the combination of a PAR-1 antagonist and a P2 years12 ADP receptor antagonist. Although the binding of thrombin to the PAR-1 receptor represents 1 of the most potent platelet activation pathways leading to thrombosis, neither aspirin nor P2 years12 ADP receptor antagonists (including cangrelor) significantly inhibit the PAR-1 pathway. For this reason, the addition of a PAR-1 antagonist to a P2 years12 ADP receptor antagonist and aspirin can be expected to provide even greater antithrombotic efficacy and a further reduction in ischemic events. The presence of a synergistic effect, resulting from the combined administration of a P2 years12 antagonist with the direct thrombin inhibitor melagatran, has been previously described.23

There are 2 possible explanations for this synergism. The first is via the concurrent inhibition of the G protein–coupled agents interact with specific platelet receptors and do not interfere with the coagulation cascade. SCH 602539 is structurally related to vorapaxar, a PAR-1 antagonist being studied in clinical trials.2,3,20–22 These findings suggest that in the vascular bed, platelet activation leading to thrombosis is a complex matrix mediated by multiple pathways.

As expected, neither SCH 602539 nor cangrelor (nor the combination of the 2) had any notable effect on coagulation parameters, a finding that is consistent with the fact that these agents interact with specific platelet receptors and do not interfere with the coagulation cascade. SCH 602539 is structurally related to vorapaxar, a PAR-1 antagonist being evaluated in 2 large ongoing clinical trials (secondary prevention and acute coronary syndromes; clinicaltrials.gov identifiers: NCT00526474 and NCT00527943).

Synergistic antithrombotic effects similar to those observed with the combination of SCH 602539 and cangrelor in this study have been reported with other combinations of antiplatelet agents18,19 that target distinct platelet activation pathways contributing to thrombosis. In the Folts model of thrombosis in pigs, the combination of low oral doses of clopidogrel, 0.1 mg/kg, and aspirin, 1 mg/kg, completely eliminated CFRS at 90 minutes, whereas the higher doses of each agent alone (clopidogrel, 5 mg/kg, and aspirin, 7 mg/kg) reduced, but did not completely abolish, the CFRS.18 A separate study19 in rabbits that used several different models of thrombosis demonstrated that the addition of oral aspirin to oral clopidogrel was associated with potent antithrombotic effects, and also additive bleeding effects, possibly related to the combined inhibitory activity of 2 antiplatelet agents on collagen-induced platelet aggregation. The additive antithrombotic effects of combined inhibition of the thromboxane A2 and P2 years12 ADP receptor platelet activation pathways with aspirin and clopidogrel observed in these studies are consistent with significant reductions in ischemic events with dual antiplatelet therapy over aspirin alone reported in large clinical trials.2,3,20–22 These findings suggest that in the vascular bed, platelet activation leading to thrombosis is a complex matrix mediated by multiple pathways.

The present study is the first direct demonstration of in vivo synergism with the combination of a PAR-1 antagonist and a P2 years12 ADP receptor antagonist. Although the binding of thrombin to the PAR-1 receptor represents 1 of the most potent platelet activation pathways leading to thrombosis, neither aspirin nor P2 years12 ADP receptor antagonists (including cangrelor) significantly inhibit the PAR-1 pathway. For this reason, the addition of a PAR-1 antagonist to a P2 years12 ADP receptor antagonist and aspirin can be expected to provide even greater antithrombotic efficacy and a further reduction in ischemic events. The presence of a synergistic effect, resulting from the combined administration of a P2 years12 antagonist with the direct thrombin inhibitor melagatran, has been previously described.23

There are 2 possible explanations for this synergism. The first is via the concurrent inhibition of the G protein–coupled

strated that the in vivo antithrombotic effects of PAR-1 antagonism, in combination with P2 years12 ADP receptor antagonism, are synergistic. The inhibitory activity of SCH 602539 was specific for platelet aggregation induced by haTRAP. The aggregation induced by other agonists, such as ADP, thromboxane A2 mimetic U46619, and collagen, was not affected, demonstrating the specificity and selectivity for the PAR-1 receptor. Cangrelor specifically inhibited aggregation induced by ADP and demonstrated a modest numeric reduction of thrombosis demonstrated that the addition of oral aspirin to oral clopidogrel was associated with potent antithrombotic effects, and also additive bleeding effects, possibly related to the combined inhibitory activity of 2 antiplatelet agents on collagen-induced platelet aggregation. The additive antithrombotic effects of combined inhibition of the thromboxane A2 and P2 years12 ADP receptor platelet activation pathways with aspirin and clopidogrel observed in these studies are consistent with significant reductions in ischemic events with dual antiplatelet therapy over aspirin alone reported in large clinical trials.2,3,20–22 These findings suggest that in the vascular bed, platelet activation leading to thrombosis is a complex matrix mediated by multiple pathways.

The present study is the first direct demonstration of in vivo synergism with the combination of a PAR-1 antagonist and a P2 years12 ADP receptor antagonist. Although the binding of thrombin to the PAR-1 receptor represents 1 of the most potent platelet activation pathways leading to thrombosis, neither aspirin nor P2 years12 ADP receptor antagonists (including cangrelor) significantly inhibit the PAR-1 pathway. For this reason, the addition of a PAR-1 antagonist to a P2 years12 ADP receptor antagonist and aspirin can be expected to provide even greater antithrombotic efficacy and a further reduction in ischemic events. The presence of a synergistic effect, resulting from the combined administration of a P2 years12 antagonist with the direct thrombin inhibitor melagatran, has been previously described.23

There are 2 possible explanations for this synergism. The first is via the concurrent inhibition of the G protein–coupled
Thrombin-mediated platelet activation requires G protein–coupled receptors. The PAR-1 and PAR-4 transmembrane receptors, respectively.24 G receptor $G_{\text{q}}$, which mediates both P2 years$_1$ and PAR-1 transmembrane signaling. $G_{\text{q}}$ and $G_{\text{q}}$ are the principal secondary intracellular signals for ADP and are localized to the P2 years$_1$ and P2 years$_{12}$ receptors, respectively.24 $G_{\text{q}}$ is the more potent of the 2 G protein–coupled receptors. Thrombin-mediated platelet activation requires $G_{\text{q}}$ localized to the PAR-1 and PAR-4 transmembrane receptors.5 Blockade of PAR-1 may directly block $G_{\text{q}}$ signaling, rendering the platelet incapable of reacting to either thrombin or P2 years$_1$-mediated ADP stimulation. However, $G_{\text{q}}$ receptor responsiveness itself is not affected by the direct inhibition of the transmembrane PAR-1 or P2 years$_1$ receptors, making it less likely that such an intracellular phenomenon occurs. The second possible mechanism relies on the platelet’s paracrine effect on ADP secretion and the signal amplification mechanism resulting from such secretion. Thrombin, the most potent platelet agonist, activates platelets directly by phospholipase C–mediated calcium release and Rho-mediated platelet shape change. However, thrombin also generates activation amplification through the secondary release of ADP from the dense granules. Blocking thrombin-mediated platelet secretion may result in a reduction in the total ADP pool available to the localized site. This would result in less agonist/antagonist competition for P2 years$_{12}$ receptors and a greater level of inhibition of ADP-mediated platelet aggregation. Such triple inhibition may allow for lower levels of ADP receptor blockade using available agents while increasing efficacy and reducing the risk of bleeding. In addition, triple therapy may obviate the perceived clinical need for potent P2 years$_{12}$ ADP receptor antagonists.

The challenges clinically are to determine whether the addition of a PAR-1 antagonist to the standard of care of aspirin and a P2 years$_{12}$ ADP receptor antagonist will provide incremental clinical benefit without incremental bleeding risk and to determine the optimal dose for each agent. The combined inhibition of thromboxane A$_2$ and P2 years$_{12}$ ADP receptor antagonist provides more potent antithrombotic activity than either agent alone and has been documented to reduce the rate of ischemic outcomes compared with aspirin alone.2,3 Recent studies of a more potent P2 years$_{12}$ ADP receptor antagonist, prasugrel, resulted in an incremental 19% reduction in clinical events; however, there remains a 10% prevalence of clinical events complicated with a 32% increased bleeding risk, including major hemorrhage during coronary artery bypass grafting and intracranial hemorrhage during stroke.2 A conceptual therapeutic window model of P2 years$_{12}$ inhibition has been proposed by Gurbel and Tantry; this model defines the delicate balance between efficacy and bleeding. Such a model would establish a warfarin international normalized ratio–like range that factors the type of ADP inhibition and the presence of PAR-1 inhibition. These data would suggest that in the context of the model of Gurbel and Tantry, PAR-1 inhibition would improve therapeutic outcomes, widen the therapeutic range of ADP inhibition, and reduce ADP-mediated risk of bleeding by allowing lower levels of ADP-mediated inhibition of platelet aggregation.

The clinical potential of PAR-1 antagonists as a novel class of oral direct-acting antplatelet agents for the management of atherothrombosis is supported by the results of 2 recent phase 2 trials with the PAR-1 antagonist vorapaxar, which showed strong trends toward reduced incidence of ischemic events without an accompanying increase in bleeding.14,26 Two large ongoing trials (Clinicaltrials.gov identifiers: NCT00526474 and NCT00527943) are investigating the clinical efficacy and safety of vorapaxar in combination with standard antiplatelet therapy among patients presenting with an acute coronary syndrome and those with a history of a coronary artery, cerebrovascular, or peripheral artery disease.

In conclusion, this study demonstrates that combined P2 years$_{12}$ and PAR-1 antagonism results in the synergistic inhibition of CFRs in the Folts model of fixed arterial stenosis, suggesting the possible clinical benefit of blocking multiple platelet pathways. Such a concept is being tested in the ongoing phase 3 megatrials of vorapaxar.

**Acknowledgments**

We thank Subharao Vemulapalli, PhD, and Arthur Brown, both formerly of Schering-Plough Corporation, for their contributions to...
the development of the manuscript; and Joshua Barbach, MA, and Gina Fusaro, PhD, for their assistance with the electronic submission and editorial support of the manuscript.

Sources of Funding
This study was supported by Schering-Plough Corporation (now Merck).

Disclosures
All authors declare that they are full-time employees of Schering-Plough Corporation (now Merck).

References


SCH 602539, a Protease-Activated Receptor-1 Antagonist, Inhibits Thrombosis Alone and in Combination With Cangrelor in a Folts Model of Arterial Thrombosis in Cynomolgus Monkeys

Madhu Chintala, John Strony, Bo Yang, Stan Kurowski and Qiu Li

Arterioscler Thromb Vasc Biol. published online August 26, 2010;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2010/08/26/ATVBAHA.110.203414.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at: http://atvb.ahajournals.org//subscriptions/