Animal Models

A novel P2Y\textsubscript{12} adenosine diphosphate receptor antagonist that inhibits platelet aggregation and thrombus formation in rat and dog models


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Summary

Irreversible platelet inhibitors, such as aspirin and clopidogrel, have limited anti-thrombotic efficacy in the clinic due to their bleeding risk. We have developed an orally active reversible P2Y\textsubscript{12} receptor antagonist, BX 667. The aim of this study was to determine if the reversible antagonist BX 667 had a greater therapeutic index than the irreversible P2Y\textsubscript{12} receptor antagonist clopidogrel. Since BX 667 is rapidly converted to its active metabolite BX 048 in rats, we first injected BX 048 intravenously (iv) in a rat arterial venous (A-V) shunt model of thrombosis. BX 048 dose- and concentration-dependently attenuated thrombosis. When administered orally, BX 667 and clopidogrel had similar efficacy, but BX 667 caused less bleeding than clopidogrel. In a rat model of a platelet-rich thrombus induced by vessel injury with FeCl\textsubscript{3}, both BX 667 and clopidogrel exhibited higher levels of thrombus inhibition after oral administration compared to their potency in the A-V shunt model. Again, BX 667 caused less bleeding than clopidogrel. In a dog cyclic flow model, iv injection of either BX 667 or clopidogrel dose-dependently reduced thrombus formation with lower bleeding for BX 667 than clopidogrel. Inhibition of thrombosis was highly correlated with inhibition of ADP-induced platelet aggregation in these animal models. In dogs pre-treated with aspirin, BX 667 maintained its wider therapeutic index, measured by inhibition of platelet aggregation over bleeding, compared to the aspirin-clopidogrel combination. These data demonstrate that the reversible P2Y\textsubscript{12} receptor antagonist, BX 667, has a wider therapeutic index than clopidogrel in experimental models of thrombosis.

Keywords
ADP, platelet aggregation, thrombosis, rat, dog, cyclic flow variation

Introduction

Antiplatelet therapy reduces mortality and non-fatal events in patients with atherothrombotic disease. Aspirin and clopidogrel have both emerged as critical therapies in the treatment of coronary artery disease, inhibiting platelet aggregation by two distinct mechanisms. Aspirin, which inhibits thromboxane-induced platelet aggregation by irreversibly blocking the enzyme cyclooxygenase (1), is modestly efficacious (25% risk reduction for the occurrence of myocardial infarction, stroke or vascular death) and relatively safe, thus, remaining the standard reference compound for oral antiplatelet treatment (2–4). Clopidogrel (Plavix™) undergoes hepatic metabolism (5) to produce active metabolites (6, 7) that irreversibly inhibit ADP-induced platelet aggregation (8) through the platelet ADP receptor P2Y\textsubscript{12} (9). In the CAPRIE trial, clopidogrel was shown to be slightly more effective than aspirin with five fewer thrombotic events per 1,000 patients (8.7% added benefit) (10, 11). When taken with aspirin, clopidogrel further reduced adverse coronary events by 19%, which, however, was accompanied by a 30% increase in major bleeding relative to aspirin treatment alone (12). Nearly half of the major bleeding events were defined as life-threatening, and six per 1,000 patients who were treated with clopidogrel in combination with aspirin required a blood transfusion. Despite the widespread use of these drugs and the obvious benefit of antiplatelet therapy, there are concerns about their use. Clopidogrel has a narrow therapeutic index with modest efficacy at 75 mg/day and higher doses causing unacceptable bleeding (13). Addition-

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ally, clopidogrel has a slow onset of action, requiring more than four days to reach steady-state levels of inhibition of platelet aggregation (14–17). To counteract this deficiency, a 300-mg loading dose of clopidogrel is often used, although there seems to be no significant benefit to this loading dose compared to giving a 75-mg dose only (18, 19). Conversely, clopidogrel irreversibly inhibits platelet aggregation, requiring about five days to return to pretreatment behavior after discontinuing the drug (8, 14–17). While this may be beneficial in terms of patient compliance, it can lead to complications when clopidogrel-treated patients require surgery or other procedures. Furthermore, clopidogrel was reported to cause thrombotic thrombocytopenic purpura, an infrequent but potentially fatal complication also associated with the structurally related ticlopidine (20). In addition, clopidogrel is converted to its active metabolites in the liver by cytochrome P450 3A4, which potentially can cause drug-drug interactions (21, 22). Perhaps a greater concern is the emergence of aspirin and clopidogrel resistance, which may put some patients at risk of therapeutic failure (23–26).

While the beneficial effects of oral antiplatelet compounds are well established, the limitations of aspirin and clopidogrel underscore the need for new agents with improved efficacy and safety profiles. Both aspirin and clopidogrel are irreversible inhibitors of platelet aggregation. We have discovered and developed an orally active and reversible small-molecule ADP P2Y_{12} receptor antagonist, BX 667, which may overcome the deficiencies of clopidogrel by exhibiting a wider therapeutic index, allowing higher levels of platelet inhibition, faster onset and offset of action, and a low potential for drug-drug interaction. The aim of the present study was to test whether a reversible ADP receptor antagonist will have a wider therapeutic index than an irreversible antagonist by comparing the efficacy versus bleeding risk of BX 667 to clopidogrel in combination with or without aspirin in models of thrombosis.
Methods

Chemicals
BX 667, “(S)-4-({4-[1-(Ethoxycarbonyl)-1-methylethoxy]-7-methyl-2-quinolyl} Carbamoyl)-5-[4-(ethoxycarbonyl) piperazin-1-yl]-5-oxopentanoic acid”, and BX 048, “(S)-4-([4-(1-Carboxy-1-methylethoxy)-7-methylquinolin-2-yl]carbonyl) amino)-5-[4-(ethoxycarbonyl) piperazin-1-yl]-5-oxopentanoic acid”, were used in this study. BX 048 is the acid form of the ester BX 667 (Fig. 1). The details of chemical synthesis, as well as the pharmacological, chemical and physical properties of BX 667 and BX 048 will be reported in separate publications. Plavix tablets (List No. 1171–40, NDC 63653–1171–4, Bristol-Myers Squibb/Sanofi Pharmaceuticals Partnership) were crushed into a powder using a pestle and mortar, from which clopidogrel was extracted and purified.

Animal models
All animal experimental procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH).

Rat arterial venous shunt (A-V shunt) model
Male Sprague-Dawley rats (350–450 g, 7–18 animals/group) were anesthetized with an intraperitoneal injection of Nembutal (65–75 mg/kg). As illustrated in Figure 2A, the left carotid artery and the right jugular vein were each cannulated with siliconized PE-50 tubing (8 cm). Fifty minutes (min) after anesthesia, when the animal’s condition was stabilized after surgery, the arterial and venous catheters were connected by a shunt tube (Tygon S-50-HL, 6 cm) containing a silk thread (6–0 silk suture, 10 cm) coated with collagen (Horm, 100 µg/ml) to form an arterial venous (A-V) shunt. Blood was allowed to flow through the shunt for 10 min. At the end of the experiment, the shunt tube was removed and the amount of thrombus deposited on the silk thread was measured as dry weight after 24 hours (h) at room temperature. BX 048 (1, 3 and 10 mg/kg) or vehicle (15% dimethyl sulfoxide [DMSO] in saline, 1 ml/kg) was injected via the jugular vein catheter 5 min before the A-V shunt. Blood samples (1 ml) were taken immediately before dosing and at the end of the experiment for measurements of ex-vivo platelet aggregation. The last blood sample was also used for measurement of plasma drug levels.

In a separate experiment, BX 667, clopidogrel or vehicle (94% PEG 300, 4% ethanol and 2% water) was administered by gavage in conscious rats. The pharmacokinetic (PK)/pharmacodynamic (PD) profiles for BX 667 and clopidogrel are quite different. Our previous PK/PD studies in rats showed that following a single oral dose of BX 667, peak plasma drug concentration of its metabolite BX 048 and platelet inhibition were achieved in 30–60 min followed by a slow decline. In contrast, intravenously and BX 667 orally. The plasma drug concentrations were obtained at the end of the experiment 15 minutes after intravenous injection of BX 048 or an average value of 1.5 and 2 hours after oral administration of BX 667. The data for BX 048 were obtained from the experiment presented in Figure 2C. D) Comparison of the effects of BX 667 and clopidogrel on tail bleeding at the oral doses that reduced thrombosis by 25% (ED$_{25}$) and 50% (ED$_{50}$) in anesthetized rats.

![Figure 3: Inhibition of thrombus formation by oral administration of BX 667 or clopidogrel in the A-V shunt model of thrombosis in anesthetized rats. Number of animals in each dosing group was indicated by the numbers in parenthesis.](https://thrombosis-online.com/18-05-06/ID:1001066444/IP:54.70.40.11)
or oral administration of clopidogrel (10 mg/kg) required 2 to 4 h to reach maximum platelet inhibition, and this effect could persist for several days (5, 8). Since clopidogrel undergoes a complex metabolism to produce active metabolites that are unstable and difficult to detect in plasma (6), inhibition of platelet aggregation is used as an indirect way of measuring clopidogrel’s effect. To obviate such PK and metabolic differences and to compare the drugs’ effects at a stable plasma level, BX 667 was given 1 h before and clopidogrel 3 h before anesthesia as illustrated in Figure 3A. Blood samples (0.5 ml) were taken 20 min before the A-V shunt (0.5 h after anesthesia) and at the end of the experiment for measurement of plasma levels of BX 048. Since the conversion of BX 667 to its active metabolite BX 048 is complete and immediate in rats, no BX 667 can be detected following oral dosing. Dose-response curves were constructed, and effective doses that reduced thrombus weight by 25% (ED$_{25}$) or 50% (ED$_{50}$) were calculated using the curve fitting method.

In order to compare the bleeding tendency between BX 667 and clopidogrel at equivalent efficacy, ED$_{25}$ or ED$_{50}$ doses for both compounds were given by gavage (n = 14 – 20/group) using the identical protocol described above (Fig. 3A). The tip of the tail was excised 1 mm from the end and immediately immersed in 25 ml of isotonic saline at 37°C. Bleeding was allowed to proceed for 30 min. Clotting of the collected blood was prevented by continuous stirring during the experiment and by addition of 5 ml of 5% EDTA in saline at the end of the experiment. Blood loss was quantified by counting the red blood cells (RBC, 10$^6$/µl) using a blood cell counter (Baker-9200+).

Rat carotid artery injury (FeCl$_2$) model
The arterial injury model has been well-characterized as a platelet-rich thrombosis model (27, 28) and was run as described in detail in our previous publication (29). The experimental protocol used for drug application in the A-V shunt model was also used in this experiment (Fig. 4A). A bleeding test was carried out in the same animals simultaneously with efficacy study. Five min after removal of FeCl$_2$, a small incision was made on the back of the tongue using a standard cutting device (Surgicutt Jr., International Technidyne Corp., Edison, NJ, USA). The time required for bleeding to stop was recorded as bleeding time, and the blood loss was collected and weighed on a pre-weighed piece of blotting paper. Blood samples were also taken at the end of the experiment to measure plasma levels of BX 048.

Dog cyclic flow variation model
Male beagle dogs (8–12 kg) were anesthetized by inhalation of isoflurane (1–3%) via an intubated tracheal tube before isolation of the femoral artery and placement of a flow probe (2.5SB, Transonic Systems, Ithaca, NY, USA) around the vessel(s). A three-lumen catheter was inserted into the right jugular vein. A Millar pressure transducer (2 Fr.) was inserted into the right brachial artery to measure blood pressure and heart rate. After stabilization of the hemodynamic parameters, cyclic flow variation was initiated in the femoral artery with mechanical injury of the endothelium by clamping the vessels six times with a pair of 8 mm wide hemostatic forceps whose teeth were covered with paper tape to prevent vessel tears. Next, the mean femoral blood flow was reduced to 20 ml/min by applying a constrictor around the vessel. After cyclic flow variation had been established, a blood

![Figure 4: Oral administration of BX 667 or clopidogrel prevented carotid artery occlusion in a rat model of injury-induced thrombosis caused by exposure to FeCl$_2$ on the carotid artery in anesthetized rats. A) Experimental protocol. B) Representative original recordings of blood flow measured in the isolated carotid artery topicaly applied with FeCl$_2$ in rats treated with vehicle (control) or BX 667 at 3 mg/kg. C) Dose-response curves of carotid artery blood flow at 30 minutes (min) (top) and time to occlusion (bottom) following application of FeCl$_2$. Since the experimental duration was 30 min, when the time to occlusion reached 30 min, the treatment completely prevented FeCl$_2$-induced occlusion. The data are obtained from the same experiment in Figure 5A.](https://www.thrombosis-online.com)
sample was taken for determining basal platelet activity in an ex-vivo platelet aggregation assay. Basal bleeding time was also measured from the tongue using a standardized cutting device (Surgicutt Jr., International Technidyne Corp., Edison, NJ, USA). After 30 min of stable cyclic flow variations, BX 667, clopidogrel or phosphate buffered saline (PBS) was injected as a bolus into the jugular vein. Subsequently, bleeding time was determined and blood samples were collected for measuring ex-vivo platelet aggregation and drug concentrations at 1, 30, 60, 90, 120 and 180 min after drug or vehicle administration. The total number of cyclic flow variations occurring in each 30-min period was counted.

Ex-vivo platelet aggregation and plasma drug concentration

Ex-vivo inhibition of ADP-induced platelet aggregation was measured in rats and dogs undergoing efficacy studies using an impedance aggregometer (Model # 592, Chronolog Corporation). Heparinized (10 U/ml) whole blood was diluted 1:1 with 0.9% saline and incubated at 37°C in a cuvette, which was then transferred to an impedance measuring well. A submaximal concentration of 5 µM ADP was added that had been shown in a pilot study to induce platelet aggregation at 90% of the maximal level in control blood (EC90). The extent of platelet aggregation was calculated by measuring the area under the curve (AUC) over 15 min and expressing it as % inhibition compared to the baseline level in each animal determined in the blood obtained before drug treatment. Plasma concentrations of BX 667 and its active metabolite BX 408 were measured by LC/MS/MS.

Data analysis and statistics

Data are presented as mean and standard error of the mean unless specified otherwise. Statistical comparisons among the treatments were performed by Student t-test for two groups or analysis of variance (ANOVA) for more than two groups, followed, if significant, by the Student-Newman-Keuls test. A p-value less than 0.05 was used to determine statistical significance.

Results

Rat A-V shunt model

Intravenous bolus injection of BX 048 dose-dependently inhibited platelet aggregation and thrombus formation (Fig. 2). The responses nearly reached the plateau level of ~80% inhibition of thrombus formation at 3 mg/kg but were not further increased at 10 mg/kg (Fig. 2B). Both the inhibition of platelet aggregation and thrombus formation were dependent on plasma drug concentration measured at the end of the experiment (Fig. 2C). In addition, inhibition of thrombus formation also correlated with inhibition of platelet aggregation (Fig. 2D).

Following oral administration of BX 667, plasma concentration of its active metabolite, BX 048, remained constant from 1.5 to 2 h after administration (Fig. 3A), during which thrombus formation was dose-dependently reduced (Fig. 3B). Clopidogrel also dose-dependently reduced thrombus formation, however, with a much steeper dose-response curve. The ED25 and ED50 were 11.2 and 49.3 mg/kg for BX 667, and 2.6 and 7.6 mg/kg for clopidogrel, respectively. The efficacy of BX 667 correlated with plasma concentrations of the active metabolite BX 048 with an
EC₅₀ value of 22 µM. The relationship between plasma concentrations of BX 048 and thrombus inhibition is similar whether it was administered intravenously as BX 048 or metabolized following oral dosing with BX 667 (Fig. 3C).

In order to compare bleeding at the same efficacy between BX 667 and clopidogrel, ED₂₅ and ED₅₀ doses of BX 667 (11.2 and 49.3 mg/kg) and clopidogrel (2.6 and 7.6 mg/kg) or vehicle were given by gavage to separate groups of rats (n=14–20), following an identical experimental protocol to that used in the efficacy study (Fig. 3A). Both BX 667 and clopidogrel dose-dependently increased blood loss (Fig. 3D). However, clopidogrel resulted in a steeper dose-response curve compared to BX 667. Thus, a ~three-fold increase in the dose of clopidogrel (from 2.6 to 7.6 mg/kg) resulted in a ~ten-fold increase in blood loss, whereas a ~4.4-fold increase in the dose of BX 667 (from 11.2 to 49.3 mg/kg) only resulted in a ~two-fold increase in bleeding. At the ED₅₀ dose clopidogrel resulted in significantly more blood loss than BX 667 (Fig. 3D).

**Rat carotid artery injury (FeCl₃) model**

Following topical application of FeCl₃ on the outside of the isolated carotid artery, a thrombus gradually formed inside the vessel reducing carotid blood flow and eventually causing thrombotic occlusion in all control rats treated with vehicle (Fig. 4B). Pre-treatment with either BX 667 or clopidogrel dose-dependently increased carotid artery blood flow measured at 30 min (Fig. 4C, top) and the time to vessel occlusion (Fig. 4C, bottom), as well as attenuated thrombus formation measured as clot weight (Fig. 5A). Although the ED₅₀ of BX 667 (0.9 mg/kg) was comparable to clopidogrel (0.6 mg/kg), the dose-response curve was steeper for clopidogrel than for BX 667. In rats treated with BX 667, thrombus inhibition was correlated with the plasma concentrations of BX 048 with an EC₅₀ value of 0.71 µM (Fig. 5B, top). Although bleeding time (Fig. 5A, top) and blood loss (Fig. 5A, bottom) were also dose-dependently increased for both compounds, they were significantly less in rats treated with BX 667 than with clopidogrel. In rats treated with BX 667, the bleeding time was not significantly prolonged until the plasma concentration of BX 048 exceeded 10 µM, a level ten-fold higher than the EC₅₀ of the efficacious concentration (Fig. 5B, bottom).

The therapeutic index can be expressed as the area between the dose response curve for efficacy and the dose response curves for either bleeding time (Fig. 5A, top) or blood loss (Fig. 5A, bottom). When measured by bleeding time, BX 667 (AUC = 5456) exhibited an over eight-fold greater therapeutic index than clopidogrel (AUC=641). When measured by blood loss, BX 667
(AUC = 7,500) exhibited an over six-fold greater therapeutic index than clopidogrel (AUC = 1,275). Another way of calculating the therapeutic index is to divide the effective dose that extends bleeding time two-fold (ED$_{2\times}$) or three-fold (ED$_{3\times}$) by the efficacious dose at ED$_{50}$. Thus, the ED$_{2\times}$/ED$_{50}$ was 8 for BX 667 and only 3 for clopidogrel. The ED$_{3\times}$/ED$_{50}$ was 37 for BX 667 and only 5 for clopidogrel. By this calculation the therapeutic index is three- to seven-fold greater for BX 667 when compared to clopidogrel.

**Dog cyclic flow variation model**

The canine cyclic flow variation model has been widely used for characterization of antiplatelet compounds (30–35). In this model vascular injury and stenosis are employed to induce platelet-dependent thrombus formation resulting in intermittent thrombotic occlusion or cyclic blood flow variations characterized by a transient reduction of flow to zero followed by spontaneous resumption of blood flow (Fig. 6). The pattern of cyclic flow variations was stable for over 4 h and was not interrupted by intravenous injection of vehicle. In some experiments, we simultaneously recorded blood flows in both of the femoral arteries. A very similar pattern of cyclic flow variations was observed between the left and right femoral arteries with no changes in blood pressure and heart rate (Fig. 6A). There was also no obvious difference in response to drug treatment between the two vessels. Therefore in subsequent experiments, we only used the data obtained from one femoral artery. Intravenous bolus injection of BX 667 immediately abolished the cyclic flow variation, which recurred later as plasma drug levels declined, demonstrating that the effect of BX 667 is reversible (Fig. 6B). The duration of action of BX 667 was dose-dependent (Fig. 6D). In contrast, the onset of the effect was much slower for clopidogrel (Fig. 6C), although faster at higher doses (Fig. 6D). In addition, the effects of clopidogrel were irreversible. Once the cyclic flow variations were abolished by clopidogrel, there was no recurrence during the remainder of the study (Fig. 6C, D).

Both BX 667 and clopidogrel dose-dependently inhibited cyclic flow variations (Fig. 7A) and platelet aggregation (Fig. 7B), with a similar potency measured by an ED$_{50}$ of 0.21 and 0.25 mg/kg, respectively, and dose-dependently prolonged tongue bleeding time (Fig. 7A, B). The bleeding time was significantly longer (p < 0.05) in the dogs treated with clopidogrel (5.5 ± 0.6 fold) than BX 667 (3.5 ± 0.7 fold) at 3 mg/kg, but not at 0.3 and 1 mg/kg where bleeding was minimal. Thus, the therapeutic index was greater for BX 667 than clopidogrel. In separate groups of dogs, 20 mg/kg aspirin was orally administered for three days, which abolished arachidonic acid-, but not ADP-induced platelet aggregation, and prolonged the baseline bleeding time 45% (from 2.21 ± 0.16 to 3.24 ± 0.32 min, p < 0.01). Further treatment with BX 667 or clopidogrel in these dogs led to an enhanced inhibition of platelet aggregation and prolongation of bleeding time in a dose-dependent manner (Fig. 7C). Yet, the therapeutic index, measured as the ratio of bleeding time to platelet aggregation, was still greater with the aspirin-BX 667 than with the aspirin-clopidogrel combination.
In studies with BX 667, there was a good correlation between plasma levels of drug (summation of BX 667 and BX 048 levels) and efficacy measured either by inhibition of cyclic flow variation or by platelet aggregation with an EC_{50} of 0.17 and 0.34 µM, respectively (Fig. 7D). In addition, there was also a good correlation between the inhibition of cyclic flow variation and platelet aggregation for both BX 667 and clopidogrel, demonstrating that platelet aggregation is a good pharmacodynamic marker for predicting efficacy in this model of thrombosis (Fig. 7E).

**Discussion**

In the present studies, we demonstrated that a novel orally active and reversible small-molecule P2Y_{12} receptor antagonist, BX 667, inhibited ADP-induced platelet aggregation and thrombus formation in three different animal models of thrombosis. Compared to the irreversible clopidogrel, BX 667 showed a greater therapeutic index, rapid onset and reversibility of action. Even when given with aspirin in the dog model, BX 667 still maintained a wider therapeutic index than clopidogrel. These results are consistent with reports in a canine thrombosis model that a reversible P2Y_{12} receptor antagonist inhibited thrombus with minimal bleeding prolongation (36–39).

The present data also showed that antithrombotic efficacy was highly correlated with inhibition of ADP-induced platelet aggregation, and at least 80% inhibition of platelet aggregation was required to give near maximal antithrombotic efficacy in animal models of thrombosis. In contrast, the current clinical dose of clopidogrel of 75 mg/day only inhibits platelet aggregation by 40–50 % with higher doses causing excessive bleeding. Thus, the greater therapeutic index of BX 667 in animal models of thrombosis would allow dosing to achieve higher inhibition of platelet aggregation without increasing bleeding risk, leading to greater therapeutic efficacy. Reversible P2Y_{12} receptor antagonists have been shown in humans to inhibit platelet aggregation by 80% with minimal effects on bleeding, demonstrating a greater therapeutic index than either aspirin or clopidogrel (38, 40, 41). Furthermore, intravenous administration of cangrelor, a reversible P2Y_{12} receptor antagonist, in patients undergoing percutaneous coronary intervention achieved rapid, reversible inhibition of platelet aggregation with less bleeding time prolongation compared to the glycoprotein IIb/IIIa receptor antagonist, abciximab (42).

Pharmacokinetic and metabolic studies showed that in animals, the ester form, BX 667, is hydrolyzed to the carboxylic acid form, BX 048, without a significant change in binding affinity and platelet inhibitory potency. Such conversion is immediate and complete in rats; thus, only BX 048 but not BX 667 can be measured in circulating blood. In the rat A-V shunt experiment, both intravenous BX 048 and oral BX 667 administrations resulted in a similar efficacy with a similar pharmacodynamic relationship between the plasma concentration of BX 048 and thrombus inhibition for both compounds even given by different routes of administration (Fig. 3C). In dogs, both compounds can be measured in circulating blood as the conversion from BX 667 to BX 048 is gradual and incomplete. Since both compounds have a similar biological activity and potency, the summation of the plasma concentrations of both compounds was used to construct concentration-response curves. In the dog cyclic flow variation model, the EC_{50} (0.34 µM) for thrombus inhibition was comparable with the EC_{50} (0.71 µM) in the rat model of platelet-rich thrombosis. The slightly higher potency of BX 048 and BX 667 in the dog than rat model is consistent with the in-vitro potency in platelet aggregation assays (details will be published in separate publications).

The rat A-V shunt model produces a fibrin-rich thrombus, which is more sensitive to an anticoagulant than an antplatelet agent (43, 44). Alternative arterial injury models, such as the rat FeCl_{2} (27–29, 45) and dog cyclic flow variation (30–35) models, induce a platelet-rich thrombus, and thus are more sensitive to antplatelet agents. This explains why the potency of both BX 667 and clopidogrel was much higher in both rat and dog arterial injury models compared to that in the rat A-V shunt model in the present study. In all these models, BX 667 had much shallower dose-response curves for both antithrombotic efficacy and bleeding compared to clopidogrel. This feature of BX 667 is similar to that of FPL-67085, both of which are reversible platelet ADP-receptor antagonists, suggesting that their reversibility allows a wider safe range of dose or plasma concentration for a more predictable efficacy than the irreversible clopidogrel (37, 41). Drug-target residence time is important for both the efficacy and potential side effects of a drug (46). In the case of P2Y_{12} antagonists efficacy and bleeding are both due to the blockade of the receptor. Thus, while a long drug-target residence time improves efficacy, it would also inevitably increase bleeding. Clopidogrel, being an irreversible inhibitor would exacerbate the bleeding side effects, especially in situations where the patient receives a hemostatic challenge or needs surgery.

Although the combination of clopidogrel and aspirin showed an additional 20% relative risk reduction for future events (composite of death from cardiovascular causes, non-fatal myocardial infarction, or stroke) in patients with acute coronary syndromes, it was associated with a 30% increase in major bleeding, compared to aspirin treatment alone (12). In the present study, pretreatment with aspirin itself increased bleeding by 45%. Co-treatment of either BX 667 or clopidogrel together with aspirin further increased bleeding compared to that of either individual treatment. However, the therapeutic index of the BX 667-aspirin combination was still wider than that of the clopidogrel-aspirin combination. This result further demonstrates the relative safety of BX 667 compared with clopidogrel even when the bleeding risk has already been increased by co-treatment with aspirin, and suggests that a combination therapy consisting of the irreversible inhibitor, aspirin, and a reversible P2Y_{12} inhibitor may be a better therapeutic option than the combination of two irreversible platelet inhibitors in the treatment of cardiovascular disease.

In summary, although currently used oral platelet inhibitors (aspirin, ticlopidine, clopidogrel) are relatively safe, they are only modestly effective in preventing arterial thrombosis. The present data provide strong evidence that an orally active and reversible ADP-receptor antagonist could represent a new medical entity for platelet inhibition with a wider therapeutic index than other platelet inhibitors currently on the market.
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Abbreviations
ADP, adenosine diphosphate; A-Vs, anteroposterior; IV, intravenous; PO, oral.

References