Thromboxane Antagonism in Experimental Canine Carotid Artery Thrombosis

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Background and Purpose: The two objectives of this study were to assess the potential of BAY U 3405 to prevent arterial thrombosis in response to vessel wall injury and to determine the ability of BAY U 3405 to prevent thrombotic reocclusion after thrombolysis with anisoylated plasminogen streptokinase activator complex.

Methods: Dogs were instrumented with a carotid flow probe, stimulating electrode, and a stenosis. Current (150 μA) was applied to the intimal surface of the right carotid artery, and time to occlusive thrombus formation was noted. BAY U 3405 was administered, and the procedure for thrombus formation was repeated for the left carotid artery.

Results: BAY U 3405 administration prevented occlusive arterial thrombosis formation. Ex vivo platelet aggregation was inhibited, bleeding time increased, and thrombus weight reduced after BAY U 3405 treatment. In a second group, thrombi were formed initially in both carotid arteries, BAY U 3405 was administered as before, and anisoylated plasminogen streptokinase activator complex was infused in the right carotid artery proximal to the occlusive thrombus. BAY U 3405 did not alter the incidence of rethrombosis compared with the lytic agent alone.

Conclusions: BAY U 3405 prevented primary arterial thrombosis, corresponding to inhibition of platelet aggregation, and increased bleeding times. BAY U 3405, however, did not prevent rethrombosis after successful thrombolysis with anisoylated plasminogen streptokinase activator complex, despite the fact that platelet reactivity was inhibited. The data are consistent with the concept that the residual thrombus represents a more effective thrombogenic stimulus as compared with arterial wall injury alone and that the mechanisms associated with primary versus secondary thrombus formation may require separate therapeutic approaches. (Stroke 1993;24:820–828)

KEY WORDS • platelet aggregation • thrombosis • thromboxane antagonists • dogs

Thromboxane A2 (TXA2), an autacoid with proaggregatory and vasoconstricting properties, is formed from the isomerization of prostaglandin endoperoxide (PGH2). Biosynthesis of the autacoid is dependent on the action of the P450 monooxygenase thromboxane synthetase, present in the blood platelet.3 Synthesis of TXA2 at local vascular sites may play an important role in modulating vascular resistance and enhancing platelet reactivity.2,3 The primary trigger of spontaneous reductions in arterial blood flow associated with transient ischemic attacks may be related to fissuring at the site of an atherosclerotic plaque leading to a dynamic process involving platelet accumulation and thrombus formation as has been suggested for the diseased coronary artery.4,5 Despite the participation of endothelium-derived vasoactive autacoids (prostacyclin, nitric oxide, endothelin), platelet activation and aggregation at the site of the fissured plaque plays the dominant role in the sequence of events in intra-arterial thrombotic occlusion.3 The presence of increased urinary excretion of TXA2 in acute ischemic syndromes has been reported.6,7 The data suggest that episodic platelet activation and formation of TXA2 at the site of vessel-wall injury may serve to mediate or amplify the events that culminate in thrombotic vascular occlusion.

BAY U 3405 [(5R)-3-(4-fluorophenylsulfonamido-1,2,3,4-tetrahydro-9-carbazolepropanoic acid] has been reported to effectively inhibit platelet aggregation as well as prevent contractions of vascular and bronchial smooth muscle, actions believed to be associated with its ability to block TXA2 receptor-mediated responses.8–11 The in vivo antithrombotic action of BAY U 3405 was demonstrated in a canine model of coronary artery thrombosis.12 BAY U 3405 is a noncompetitive inhibitor of platelet aggregation when tested against the TXA2 mimetic U 46619.13

The purpose of the present investigation was twofold: first, to assess the potential of BAY U 3405 to prevent in vivo intravascular thrombus formation in the canine carotid artery (primary thrombosis); and second, to determine whether the thromboxane antagonist was effective in preventing reocclusion (secondary thrombosis) of the carotid artery after successful thrombolysis achieved with the local administration of anisoylated plasminogen streptokinase activator complex (APSAC). The in vivo studies were accompanied by the ex vivo assessment of platelet reactivity in response to arachi-
odynamic acid. The results of our investigation demonstrate that BAY U 3405 prevents carotid artery thrombosis in response to vessel wall injury but does not prevent carotid artery reocclusion after successful thrombolysis at a time when ex vivo platelet aggregation in response to arachidonic acid is inhibited. The results indicate that local thromboxane generation may not be the sole factor in initiating reocclusion after thrombolysis and that the mechanism for primary thrombosis may differ from that of secondary arterial thrombosis.

**Materials and Methods**

These studies conform to the *Position of the American Heart Association on Research Animal Use* adopted November 11, 1984, by the American Heart Association. The procedures followed in this study were in accordance with the guidelines of the University of Michigan (Ann Arbor) University Committee on the Use and Care of Animals. Veterinary care was provided by the University of Michigan Unit for Laboratory Animal Medicine. The University of Michigan is accredited by the American Association of Accreditation of Laboratory Animal Care, and the animal care and use program conforms to the standards in “The Guide for Care and Use of Laboratory Animals,” Department of Health, Education, and Welfare Publication No. NIH 78-23.

BAY U 3405 was provided by Miles Inc, Pharmaceutical Division (West Haven, Conn). APSAC was provided by SmithKline Beecham (King of Prussia, Pa). Sodium citrate, ADP, arachidonic acid, epinephrine, and any reagent used in the laboratory but not specifically mentioned were purchased from Sigma Chemical Co (St Louis, Mo).

The model used in this study is a modification of one developed by our laboratory for the study of experimentally induced coronary artery thrombosis. The experimental procedure results in the formation of a platelet-rich intravascular thrombus at the site of an electrolytically induced endothelial lesion in close proximity to a distal arterial stenosis. The carotid artery was selected for our experimental model, thereby allowing one vessel to be used as a control and the other to be used after administration of the test agent, which in the current study was the highly specific thromboxane antagonist BAY U 3405. In this manner, each dog could serve as its own control. The carotid artery response to the electrolytic injury is similar to that observed in the canine coronary artery and has the advantage that each dog will have demonstrated the ability to form an occlusive thrombus before administration of the test drug. This allows for a paired analysis and eliminates those animals that may not form thrombi due to causes unrelated to the vessel wall injury and platelet-vessel wall interaction and subsequent occlusive thrombus formation.

Healthy, male mongrel dogs (15 to 17 kg) were anesthetized with sodium pentobarbital (30 mg/kg intravenously), intubated, and ventilated with room air at a tidal volume of 30 mL/kg and a frequency of 12 breaths per minute (Harvard Apparatus, South Natick, Mass). Both common carotid arteries and the right internal jugular vein were exposed. A catheter was inserted into the jugular vein for blood sampling and drug administration. Arterial blood pressure was monitored from the cannulated femoral artery with a blood pressure transducer (Gould Inc, Cardiovascular Prod-
PROTOCOL 1: Prevention of Thrombosis

Start stimulation
Left Carotid Artery (150 µA)
Remove left carotid artery and weigh thrombus

Stop stimulation

BAY U3405 (3.0 or 30.0 mg/kg [i.v.])

5 ml of vehicle

Start stimulation
Right Carotid Artery (150 µA)
Remove right carotid artery, weigh thrombus

PROTOCOL 2: Prevention of Rethrombosis After Lysis with APSAC

APRAC 0.05 U/kg i.a., RCA only

BAY U3405 (3.0 or 30.0 mg/kg [i.v.])

Anesthetize and instrument dog

Bilateral occlusion

Start bilateral stimulation (150 µA)

Remove carotid arteries and weigh thrombi

Figure 1. Schematic representation of protocols used in this study: protocol 1, prevention of thrombus formation in carotid arteries; protocol 2, prevention of rethrombosis after lysis of preformed carotid artery thrombus with local administration of anisoylated plasminogen activator complex (APRAC). RCA, right carotid artery.

length, and the intact thrombus mass was lifted off the intimal surface of the vessel. The weight of the thrombus mass was determined with an analytical balance.

The left carotid artery of each animal served as the test vessel. The vessel was instrumented in a manner identical to that described for the contralateral vessel. BAY U 3405 (3.0 mg/kg IV and 30.0 mg/kg IV) was administered as a single dose slowly over the course of 2 to 3 minutes. The application of the anodal current to the left carotid artery was initiated 15 minutes after the administration of BAY U 3405.

A second protocol was used to evaluate the effects of thrombomodulin antagonism for the prevention of rethrombosis after lysis of a preexisting thrombus. The injury current was applied to both carotid arteries simultaneously for a maximum period of 3 hours or was terminated 30 minutes after blood flow in each vessel had remained stable at zero flow velocity to substantiate formation of a stable occlusive thrombus (Figure 1). BAY U 3405 (3.0 or 30.0 mg/kg IV) was administered as a single dose slowly over the course of 2 to 3 minutes. Ten minutes after BAY U 3405 was administered, APRAC (0.05 U/kg) was infused intra-arterially, directly proximal to the thrombus (right carotid artery only) to achieve lysis in that vessel only without disturbing the thrombus in the contralateral vessel. The local intra-arterial dose of APRAC was administered over 90 seconds. The dose of APRAC was selected to avoid alteration in the plasma clotting factors as determined by measurements of activated partial thromboplastin time (aPTT), thrombin time (TT), and prothrombin time (PT).

An additional group of heartworm-negative dogs was screened for baseline platelet aggregation to assess platelet responsiveness to arachidonic acid. The initial screening procedure identified six animals whose platelets showed a positive response to arachidonic acid at concentrations of 0.65 and 0.325 mmol/L. Each of the dogs satisfying the inclusion criteria received 30 mg/kg IV BAY U 3405. Platelet aggregations were determined daily for the next 7 days at the same hour each day to avoid any influence of diurnal variations on platelet reactivity.

Blood (20 ml) was withdrawn for platelet studies from the jugular cannula into a plastic syringe containing 3.2% sodium citrate as the anticoagulant (1:10 citrate/blood [vol:vol]). The platelet count was determined with an H-10 cell counter (Texas International Laboratories, Inc, Houston, Tex). Platelet-rich plasma (PRP), the supernatant present after centrifugation of anticoagulated whole blood at 1,000 rpm for 5 minutes (140 g), was diluted with platelet-poor plasma (PPP) to achieve a platelet count of 200,000/mm³. PPP was prepared after the PRP was removed by centrifuging the remaining blood at 12,000g for 10 minutes and discarding the bottom cellular layer. Ex vivo platelet aggregation was measured by established spectrophotometric methods with a four-channel aggregometer (BioData-PAP-4, Bio Data Corp, Hatboro, Pa) by recording the increase in light transmission through a stirred suspension of PRP maintained at 37°C. Aggregation was induced with arachidonic acid (0.65 and 0.325 mmol/L). A subaggregatory dose of epinephrine (550 mmol/L) was used to prime the platelets before stimulation. Values were expressed as percentage of aggregation, representing the percentage of light transmission standardized to PRP and PPP samples yielding 0% and 100% light transmission, respectively.

To assess the anticoagulation state of the animals, PT, aPTT, and TT were determined using a Hemochron (Technidyne, Edison, NJ) and the reagents supplied by the manufacturer. Citrated whole blood was used for these determinations. Values are represented as a percentage of baseline readings.

Bleeding times were measured in anesthetized dogs with a Simplate device by making incisions on the tongue and blotting the wound with filter paper at 30-second intervals until blood no longer was transferred to the filter paper.

Animals to be included in the final protocol had to satisfy all of the following preestablished criteria: 1) a circulating platelet count of not less than 100,000 per cubic millimeter, 2) demonstrated ability for platelets to aggregate in response to arachidonic acid before administration of BAY U 3405, 3) thrombotic occlusion of the right carotid artery (control vessel) within 3 hours from the onset of vessel wall injury with a 150-µA direct anodal current, and 4) absence of heartworms at final postmortem examination.16-18

Data are expressed as mean ± SEM. The design of the protocol permitted each animal to serve as its own control. The right carotid artery was compared with the contralateral vessel with respect to time to occlusion, blood flow velocity, and resulting thrombus mass. Ex vivo platelet aggregation in response to arachidonic acid was assessed before and after administration of BAY U...
3405. The data were analyzed by paired or group analysis using Student's *t* test when applicable, and differences were considered significant for *P* < .05.

**Results**

Fifty-eight animals were used in this study. Sixteen dogs were excluded for the following reasons: failure to form an occlusive thrombus (*n* = 11), low platelet counts and failure to demonstrate ex vivo aggregation (*n* = 2), inability to lyse a preformed thrombus with APSAC (*n* = 2), or presence of heartworms at postmortem examination (*n* = 1).

Basal carotid artery blood flow velocity did not differ between control animals (37.3 ± 3.0 cm/sec), dogs treated with 3.0 mg/kg BAY U 3405 (38.6 ± 4.9 cm/sec), and dogs treated with 30.0 mg/kg BAY U 3405 (32.6 ± 5.3 cm/sec). Blood pressure and heart rate were not different across groups at baseline. The administration of BAY U 3405 did not alter blood pressure or heart rate at either dose tested. BAY U 3405 was effective in preventing occlusive thrombus formation in response to electrolytic vessel wall injury at both 3.0 and 30.0 mg/kg IV. Figure 2 shows the effects of electrolytic vessel wall stimulation on carotid artery blood flow in control and drug-treated animals. Dogs receiving BAY U 3405 did not show evidence of occlusive thrombus formation in contrast to control animals that had a mean time to occlusion of 150.7 ± 16.3 min.

Ex vivo aggregation in response to arachidonic acid was inhibited at both 30 minutes and 3 hours after administration of BAY U 3405 (Figure 3). Coagulation factors and bleeding times were measured at baseline and 3 and 6 hours after BAY U 3405 (30 mg/kg IV) administration (Table 1). BAY U 3405 had no effect on PT, TT, or aPTT. The template bleeding time was prolonged at 3 hours to 3.5 times baseline and returned to 1.4 times baseline at 6 hours. Thrombi were removed intact from the carotid arteries of control and BAY U 3405–treated dogs at the conclusion of the study and weighed. BAY U 3405 pretreatment was associated with a dose-dependent reduction in thrombus weight (Figure 4).

The results from the initial protocol indicate that BAY U 3405 in doses of 3.0 and 30.0 mg/kg prevents primary thrombus formation in response to vessel wall injury. The in vivo efficacy of BAY U 3405 correlates with its ability to inhibit ex vivo platelet aggregation in response to arachidonic acid.

The second protocol was conducted to ascertain the ability of BAY U 3405 to prevent thrombus formation in response to a more intense and protracted thrombogenic stimulus, ie, after lysis of a preexisting thrombus. Baseline carotid artery blood flow velocity did not differ between control and drug-treated groups. Administration of BAY U 3405 before induction of thrombolysis with locally administered APSAC did not influence the time to lysis and the reestablishment of carotid artery

**Table 1. Effects of 30 mg/kg IV BAY U 3405**

<table>
<thead>
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<th></th>
<th>Baseline</th>
<th>3 Hours</th>
<th>6 Hours</th>
</tr>
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<tbody>
<tr>
<td><strong>Prothrombin time</strong></td>
<td>30.4 ± 1.8</td>
<td>32.6 ± 2.4</td>
<td>30.4 ± 1.6</td>
</tr>
<tr>
<td><strong>Thromboplastin time</strong></td>
<td>39.4 ± 1.6</td>
<td>38.0 ± 1.2</td>
<td>37.2 ± 4.7</td>
</tr>
<tr>
<td><strong>Activated partial thromboplastin time</strong></td>
<td>32.0 ± 1.9</td>
<td>32.6 ± 1.6</td>
<td>32.6 ± 1.7</td>
</tr>
<tr>
<td><strong>Bleeding time</strong></td>
<td>123.4 ± 10.6</td>
<td>428.4 ± 83.5</td>
<td>166.6 ± 18.0</td>
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</table>

All values are in seconds.

*P* < .05 vs control by paired *t* test, *n* = 6.
blood flow. Despite the ability of BAY U 3405 to prevent primary thrombosis, pretreatment with the compound did not prevent carotid artery reocclusion after successful thrombolysis had been achieved by local administration of APSAC. The incidence of carotid artery reocclusion is shown in Figure 5. The inability to maintain carotid artery patency occurred despite the continued efficacy of the drug in the prevention of ex vivo platelet aggregation in response to arachidonic acid. The ex vivo platelet aggregation results are summarized in Figure 6. Administration of the lytic agent APSAC did not effect platelet aggregation. As demonstrated in protocol 1, platelet aggregation in response to arachidonic acid was inhibited by both doses of BAY U 3405. Although ex vivo platelet aggregation studies suggest that BAY U 3405 effectively inhibited the endoperoxide/thromboxane receptor, this alone was not sufficient to prevent rethrombosis after successful thrombolysis. The failure to prevent rethrombosis occurred despite a tenfold increase in the dose of BAY U 3405 necessary to prevent primary thrombosis.

BAY U 3405 (30 mg/kg) was administered intravenously to each of six heartworm-negative, healthy, unanesthetized dogs. Ex vivo platelet aggregation studies in response to arachidonic acid and platelet counts were done once daily for 7 consecutive days after administration of a single intravenous dose of BAY U 3405. The results are summarized in Figure 7A. BAY U 3405 produced a persistent and significant inhibition of arachidonic acid–induced platelet aggregation that extended to 6 days after a single administration of the compound. By the seventh day ex vivo platelet aggregation determinations began to return to baseline values. Platelet counts during this time period remained unchanged from the predrug values (Figure 7B).

**Discussion**

The purpose of this study was twofold. The first intent was to examine the effectiveness of endoperoxide/TXA²
antagonism with BAY U 3405 on carotid artery thrombus formation in response to endothelial vessel wall injury. Secondly, we examined the ability of BAY U 3405 to prevent rethrombosis after lysis of a preexisting arterial thrombus under conditions that avoided alterations in the systemic coagulation mechanism. The latter was accomplished through the local administration of APSAC at the site of the occlusive arterial thrombus. The dose of APSAC selected for this purpose did not produce measurable changes in circulating clotting factors (APTT, TT, and PT), alter template bleeding time, or influence ex vivo platelet reactivity in response to arachidonic acid. The action of APSAC, as assessed in the study protocol, was confined to local dissolution of the occlusive carotid artery thrombus.

Pretreatment with BAY U 3405 prevented carotid artery thrombus formation, inhibited ex vivo platelet aggregation to arachidonic acid, reduced thrombus weight in a dose-dependent manner, and increased template bleeding time. Endoperoxide/TXA2 antagonism by BAY U 3405, however, was insufficient to protect against rethrombosis after thrombolysis with APSAC. Additionally, we observed inhibition of ex vivo platelet aggregation to arachidonic acid over a period of 6 days in dogs treated with a single intravenous dose of BAY U 3405. The mechanism for the prolonged inhibitory effect of BAY U 3405 against arachidonic acid–induced platelet aggregation remains unexplained. However, the failure of BAY U 3405 to prevent reocclusion after thrombolysis cannot be attributed to dissipation of the drug’s effect on the platelet endoperoxide/TXA2 receptor.

The observations provide compelling evidence that a difference exists between the underlying mechanisms involved in primary and secondary thrombus formation. After the induction of thrombolysis, reocclusion of the vessel will be dependent on an interaction among the blood elements, the coagulation system, and the residual thrombus mass (all of the “reactants” in the process of reocclusion being derived from endogenous sources representative of the natural physiological processes). Furthermore, alterations in the local pattern of arterial blood flow resulting from a stenotic region or the presence of a residual thrombus would encourage thrombus formation due to an enhancement in shear rate and turbulence at the site of vessel narrowing. Shear rate is implicated as a determinant of platelet and fibrin deposition on altered arterial surfaces. The present model does not involve the use of anticoagulants during the experimental procedure and thus does not negate the important role of the coagulation system in the overall response to injury and rethrombosis. The major deficiency in the experimental model, and one that confronts all investigators in the field, is the lack of an atherogenic component to the cell-cell interaction leading to thrombus development. Therefore, as with many experimental models, interpretation of the results must focus on the possible mechanisms by which pharmacological agents are acting under the prescribed experimental conditions. This must be done without drawing conclusions regarding the relevancy of such interpretations to human clinical conditions involving intravascular thrombosis. The present model, involving the electrolytically damaged canine carotid artery, provides a pathophysiological substrate (albeit lacking in some elements) for the initiation of endogenous events associated with the development of occlusive arterial thrombosis. Shebuski and colleagues reported that endoperoxide/TXA2 receptor inhibition with sulotroban was capable of decreasing the time to lysis and reducing the rate of rethrombosis after tissue plasminogen activator (t-PA)–induced thrombolysis in the canine coronary artery. However, it must be appreciated that coronary artery thrombolysis was achieved with systemic administration of t-PA. The effect of the lytic agent on circulating clotting factors was not reported. Thrombolysis with recombinant t-PA (rt-PA) or streptokinase is reported to activate platelets, increase the synthesis of platelet–derived thromboxane, and increase local thrombin formation. It is therefore possible that alterations in the systemic clotting factors contributed to the apparent efficacy of sulotroban. Furthermore, a different pharmacological intervention was being considered (sulotroban), with the possibility that unrecognized pharmacodynamic factors may have influenced the experimental findings.

The method of achieving thrombolysis in the present study involves the local administration of the lytic agent at the site of the occlusive thrombus formation. There-

![Graph A](image1.png)

**Figure 7.** A shows platelet aggregations in response to ADP and arachidonic acid over a period of 8 days after treatment with a single 30.0-mg/kg IV dose of BAY U 3405. Comparisons were made by analysis of variance. *P < .05 vs baseline values; n = 6. B shows platelet counts determined in platelet-rich plasma over 8 days after a single 30-mg/kg IV dose of BAY U 3405. n = 6.
fore, induction of a systemic lytic effect is minimized or prevented entirely. Chances of inducing a systemic lytic effect are increased when thrombolysis is achieved by intravenous administration of the lytic agent. Although APSAC was administered in close proximity to the occlusive arterial thrombus, the residual drug (not bound to the thrombus mass) would eventually enter the systemic circulation upon reperfusion. However, the amount of APSAC entering the general circulation was not sufficient to lyse the occlusive thrombus in the contralateral carotid artery. Although we cannot state with absolute certainty that changes in circulating coagulation factors did not occur, it is evident that BAY U 3405 did not prevent reocclusion of the carotid artery after successful thrombolysis. Failure to restore flow in the contralateral carotid vessel demonstrates that the systemic concentration of APSAC was less than that necessary for dissolution of the occlusive thrombus.

Previous investigators have shown that thromboxane antagonism by itself, using BAY U 3405 or other inhibitors of the thromboxane receptor, are not sufficient to prevent thrombotic reocclusion after thrombolysis. Platelets maintain the capacity to undergo aggregation despite TXA2 receptor blockade. Not all platelet agonists depend on activation of the arachidonic acid pathway to produce in vivo or in vitro platelet aggregation. Evidently, the combination of a deep arterial wall injury combined with a residual thrombus present after thrombolysis presents a more effective thrombogenic stimulus than did the original vessel wall injury. Platelet aggregation, as determined ex vivo, was not enhanced after APSAC, which suggests that if the platelets are stimulated by the lytic agent, it either is not detectable ex vivo or does not occur when the lytic agent is applied locally to the site of the occlusive thrombus, thus circumventing a systemic lytic effect. The observations of this study are consistent with the results demonstrated with other thromboxane antagonists. The present study, in contrast to those cited previously, avoided the intravenous administration of the lytic agent, thus minimizing or preventing alterations in the circulating coagulation factors. The direct effects of TXA2 inhibition by BAY U 3405 upon reocclusion could therefore be assessed without the complications introduced by alternations in circulating clotting factors.

BAY U 3405 did not potentiate the effects of the APSAC as used in the present study. The time to clot lysis did not differ between the control and drug-treated groups, a result that is in contrast to a previous report. The latter investigators examined the effect of BAY U 3405 (3 mg/kg IV) on t-PA–induced thrombolysis of experimentally induced coronary artery thrombi in anesthetized dogs. BAY U 3405 given immediately before an infusion of rt-PA reduced time to reperfusion by more than 50% compared with vehicle-treated controls. After cessation of the rt-PA infusion, BAY U 3405 reduced the reocclusion rate compared with that in controls. The results were interpreted as indicating that BAY U 3405 improves rt-PA–induced coronary thrombolysis in a canine model. The studies differ with regard to time of observation and the choice of thrombolytic agent as well as its mode of administration. In the experiments reported by Rounding and Fiedler, the observation period in which they report a decreased rate of reocclusion after thrombolysis was limited to 60 minutes. The possibility exists that a substantial degree of systemic lysis may have added to the effects of BAY U 3405 and together they contributed to the apparent beneficial effects with respect to rethrombosis. The presence of a systemic lytic effect may mask for a time the enhanced reactivity of the residual thrombus. An extended monitoring period may have resulted in a different conclusion regarding the prevention of rethrombosis and one more consistent with the observations in the present study. The major difference between the two studies, however, is the use of local thrombolysis as opposed to the use of the systemic route of administration.

We observed a long-lasting inhibition of platelet aggregation in response to arachidonic acid after a single 30-mg/kg dose of BAY U 3405. The circulating life span of a platelet is approximately 7 days, with new platelets entering the circulation after the drug is cleared from the plasma. Based on a predicted plasma half-life of 1.5 hours (Dr Alexander Scriabine, Miles Inc, Pharmaceutical Division, personal communication), all of the administered dose should have been eliminated by 8 hours. Despite this, the platelets continued to be inhibited for approximately 6 days after a single intravenous dose of BAY U 3405. The responsible mechanism involves more than simply inhibition of the circulating platelet pool and may be indicative of a drug action upon the site of platelet formation or the megakaryocyte. The inhibition of platelet aggregation is reversible, and ex vivo aggregation responses began to approach baseline values as a function of platelet production by the megakaryocytes. The precise mechanism accounting for the prolonged effect on ex vivo platelet reactivity is as yet unknown. Our present working hypothesis is that BAY U 3405 acts on endoperoxide/TXA2 receptor of the circulating platelet as well as the receptor on the megakaryocyte. It is the latter site of action that may be influencing the subsequent activity of newly released platelets into the general circulation.

In summary, we have demonstrated that BAY U 3405, a noncompetitive inhibitor of TXA2, is capable of preventing occlusive thrombus formation in response to electrolytic injury of the canine carotid artery. The protective effect is accompanied by inhibition of ex vivo platelet aggregation using arachidonic acid as the agonist. Despite the prolonged action of BAY U 3405 on ex vivo platelet function, the drug does not prevent rethrombosis when a previously occlusive arterial thrombus has undergone successful thrombolysis with locally applied APSAC. The observations demonstrate that the lysed thrombus serves as a more intense thrombogenic surface to the circulating blood than the original vessel wall injury. The preclinical assessment of antiplatelet agents as adjunctive agents for prevention of rethrombosis must be examined by appropriate models that differ from those used to assess pharmacological interventions for the prevention of primary thrombosis in response to vessel injury. Treatment with BAY U 3405 did not prevent rethrombosis despite the inhibition of ex vivo platelet activation in response to arachidonic acid. Multiple agonists participate in vivo in initiating platelet activation and thrombus formation. The ability of BAY U 3405 to prevent primary arterial thrombosis in response to vessel wall injury but not secondary thrombosis after thrombolysis suggests that different mechanisms are responsible for the proaggregatory events.
The development of pharmaceutical interventions for the prevention of primary and secondary thrombosis may require discrete approaches for prevention.

Acknowledgments

This work was supported by National Institutes of Health (Heart, Lung, and Blood Institute) grant HL-19782-14 and by an educational grant from Miles Institute for Preclinical Pharmacology, West Haven, Conn.

References

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Editorial Comment

The biochemical mechanisms underlying arterial thrombosis continue to be under active investigation. Of practical importance is the possibility that interference with key steps in the mechanisms involved might inhibit arterial thrombosis.

In the article above, Rote and colleagues found that the administration of a thromboxane A2 antagonist inhibited platelet aggregation and prevented occlusive arterial thrombosis induced by injury to the arterial wall. In contrast, the arterial thrombosis that occurred...
Thromboxane antagonism in experimental canine carotid artery thrombosis.
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Stroke. 1993;24:820-827
doi: 10.1161/01.STR.24.6.820

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