Antithrombotic Efficacy of a Novel Murine Antihuman Factor IX Antibody in Rats


Abstract—A murine antihuman factor IX monoclonal antibody (BC2) has been generated and evaluated for its capacity to prolong the activated partial thromboplastin time (aPTT) in vitro and ex vivo and to prevent arterial thrombosis in a rat model in vivo. BC2 extended aPTT to a maximum of 60 to 80 seconds at 100 to 1000 nmol/L in vitro (rat and human plasma, respectively) and ex vivo (rat) after dosing of rats up to 6 mg/kg in vivo. BC2, administered as bolus (1 to 6 mg/kg) followed by infusion (0.3 to 2 mg · kg⁻¹ · h⁻¹), dose-dependently prevented thrombosis of an injured rat carotid artery (FeCl₃-patch model), increased time to artery occlusion, and reduced incidence of vessel occlusion. BC2 efficacy in preventing arterial thrombosis exceeded that of heparin (bolus 15 to 120 U/kg followed by infusion 0.5 to 4.0 U · kg⁻¹ · min⁻¹), whereas the latter rendered the blood incoagulable (aPTT>1000 seconds). BC2 demonstrated complete antithrombotic efficacy also as a single bolus given either as prevessel or postvessel injury as evidenced by reduction of thrombus mass (from 4.18±0.3 mg, P<0.001), increasing vessel patency time (from 14.9±0.9 minutes to 58.3±1.7 minutes, P<0.001) and decreasing incidence of vessel occlusion from 100% to 0% in vehicle- versus BC2-treated rats, respectively. BC2 (3 mg/kg, IV) administered in a single bolus resulted in 50% reduction in thrombus mass (P<0.01), extended vessel patency time (P<0.001), extended aPTT only 4-fold, and had no effect on blood loss via a tail surgical wound; heparin, at doses that reduced thrombus mass to a similar extent, extended aPTT beyond 1000 seconds (over 500-fold) and increased blood loss from 1.8±0.7 to 3.3±0.6 mL (P<0.001). These data suggest that BC2 may provide enhanced therapeutic efficacy in humans at lesser interference with blood hemostasis than heparin.


Key Words: thrombosis ■ coagulation ■ factor IX ■ heparin ■ aspirin ■ monoclonal antibodies

Despite substantial efforts to prevent and treat thrombotic events, arterial thrombosis continues to be the major cause of death in adult populations of developed countries. Although contemporary medicine offers several medical strategies to combat thrombosis, the unmet medical need is significant as none of the available therapeutics provides the desired efficacy and safety profile. Aspirin (ASA) remains the primary antplatelet agent of proven efficacy for chronic, secondary prevention of arterial thrombosis,1,2 whereas ticlopidine and clopidogrel (ADP inhibitors) add marginal benefits over ASA. Heparin and coumadin are proven anticoagulants for acute and chronic thrombotic disorders, respectively.5–7 The most recent novel antithrombotic agent introduced for adjunctive treatment of thrombotic events associated with vascular interventions is abciximab (ReoPro18), an antibody that blocks platelet integrin GPIIb/IIIa (the fibrinogen receptor) and hence, platelet aggregation.8,9 However, these anticoagulants and the GPIIb/IIIa antagonists may have restricted therapeutic capacity due to significant adverse effects such as bleeding, thrombocytopenia, and need for careful patient monitoring.7 Thus the state-of-the-art in treatment of acute thrombotic disorders comprises a combination of antithrombotic agents including aspirin, heparin, fibrinolytics (when appropriate), and the GPIIb/IIIa receptor antagonists. This arsenal of agents, however, carries significant liabilities of which bleeding, especially cerebral hemorrhages, remains a major medical problem.7 The persistent untoward consequences of contemporary antithrombotic regimens provide an impetus for exploration for superior antithrombotic agents that extend desired therapeutic efficacy, reduce liabilities, and overall, provide a significant broadening of the therapeutic index. In the past few years, research interest expanded toward additional anticoagulant targets such as specific thrombin, factor Xa, and tissue factor/factor VIIa inhibitors as antithrombotic agents.10,11 We have explored other sites in the coagulation cascade to identify alternative ways to combat the thrombotic process.

Factor IX (FIX) is a key coagulation factor essential for amplification of coagulation that results in thrombus formation.12–14 Severe deficiency in FIX leads to bleeding (Hemo-
Publications B, Christmas disease). Lollar and Fass showed that active-site blocked factor IXa (FIXai) inhibits clot formation in vitro. Benedict et al demonstrated that administration of FIXai prevents thrombosis in experimental animal models, indicating that modulation of FIX function can be achieved to gain therapeutic efficacy in thrombosis. Bajaj and co-workers have described antibody mediated inhibition of FIX coagulant activity but did not explore in vivo application of the antibody as an antithrombotic agent. We hereby report a novel murine antihuman FIX/IXa antibody (BC2) that possesses high efficacy in the prevention of thrombosis in a rat arterial thrombosis model. In addition, comparison of BC2 with aspirin (ASA) and heparin or a combination of BC2 with ASA resulted in superior antithrombotic efficacy with limited extension of aPTT and blood loss.

**Materials and Methods**

**BC2 Production and Assay**

Monoclonal antibodies were generated against purified human FIX using female Balb/C mice as described by Jenny et al. Hybridoma medium was assayed in an ELISA format using FIX immobilized onto microtiter plates, and wells containing anti-FIX antibodies were subcloned by limiting dilution. Anti-FIX antibody from the hybridoma, BC2, was produced from ascites tumors in mice and purified by conventional means and stored in buffer containing 0.02 mol/L tris, 0.15 mol/L NaCl, pH 7.4. An MLA Electra 800 Automatic Coagulation Timer (Medical Laboratory Automation, Inc) was used to measure aPTT. Purified human factor IX and IXa were purchased from Hematologic Technologies (Essex Junction, VT). Molecular weight fractionated heparin with an average molecular weight of 15 400 was used for all in vitro experiments. Unfractionated heparin was obtained from A.H. Robbins (Elkin-Sinns, Inc, Cherry Hill, NJ) and was dissolved in normal saline.

**Assay of Factor IXa Activity**

The effect of BC2 on factor IXa activity was determined by measuring the prolongation of clotting times when clotting was initiated by the addition of preactivated factor IXa to factor IX deficient plasma. The factor IXa concentration was adjusted to 30 nmol/L, which gave a clot time of approximately 30 seconds.

**Animal Preparation**

Male Sprague-Dawley rats (Charles River, Raleigh, NC) weighing 300 to 490 g were anesthetized with sodium pentobarbital (55 mg/kg, IP). The rats were placed dorsal on a heated (37°C) surgical board and an incision was made in the neck; the trachea was isolated and cannulated with PE-240, Intramedic tube (Clay Adams, Parsippany, NJ). The left carotid artery and jugular vein were then isolated. A Parafilm M sheet (4 mm², American National Can) was placed under the carotid artery, and an electromagnetic blood flow probe (Carolina Medical) was placed on the artery to measure blood flow. A cannula (Tygon, 0.02x0.04 in, Norton Performance Plastics) was inserted into the jugular vein for drug administration. The left femoral artery was then isolated and cannulated for measurement of blood pressure and collection of blood samples.

**Procedure of Carotid Artery Lesion**

To initiate thrombosis in the carotid artery, a 6.5-mm diameter circular patch of glass micro-filter paper saturated with FeCl₃ solution (50%) was placed on the carotid artery downstream from the flow probe and kept for 10 or 15 minutes as described previously. In this well characterized model, thrombus formation is usually completed within 15 to 20 minutes.

**Measurement of aPTT and Prothrombin Time**

One mL of arterial blood was drawn from the femoral artery into 3.8% citrate solution and centrifuged; aPTT and prothrombin time were measured.

**Figure 1.** Experimental design of the rat arterial thrombosis model. R, indicates time of drug administration; BS, time of blood sample withdrawn; TM, time of thrombus extraction from the carotid vessel; and aPTT, activated partial thromboplastin time. The period of injury represents the time of exposure of the vessel to the patch soaked with 50% FeCl₃.

**Figure 2.** Effect of BC2 on in vitro clotting time. A, Rat citrated plasma (●) or human citrated reference plasma (○) (100 uL) were incubated with 100 uL of Dade Actin Activated Cephaloplastin Reagent plus BC2 at the indicated concentrations for 3 minutes at 37°C. Samples of human plasma were also incubated with heparin (■). Coagulation was initiated by addition of 100 uL of 0.02 mol/L Ca²⁺, and the time to clot formation was measured with an MLA Automatic Coagulation Electra 800 Timer. B, Increasing concentrations of BC2 were added to samples of Factor IX deficient human plasma. Clotting was initiated by addition of 30 nmol/L Factor IXa and 0.005 mol/L Ca²⁺, and the prolongation of the clotting time was measured.
(PT) were monitored by a fibrometer (BB1L, Baxter Dade) with standard procedures. aPTT and PT values are represented in seconds.

Monitoring Blood Loss From Surgical Wound

To monitor blood loss, a tail surgical cut model was used as described previously.23 Briefly, the rat tail was cut at 30% of its length from its end using a new 21 surgical blade (Bard-Parker). The proximal end of the tail was placed into a tube and blood was permitted to drip freely into a reservoir of 3.8% citrate solution (1 mL) for 15 minutes. This procedure followed the 60-minute period of the experiment proper. The animals were then overdosed with sodium pentobarbital.

Scanning Electron Microscopy of Rat Thrombosis Model

Segments of rat carotid artery were collected from sham, FeCl₃ injury, or FeCl₃+6 mg/kg BC2 injected rats 15 minutes before injury. The arteries were perfusion-fixed with formaldehyde and ligated above and below the lesioned area. Fixed arteries were dehydrated with graded ethanol (30% to 100%), incubated in hexamethyldisilazane, and dried in a desiccator. Dried arteries were opened lengthwise, placed on scanning electron microscopy (SEM) stubs, and sputter-coated with gold.

Experimental Design for Assessment of Therapeutic Interventions

Pretreatment Experiment

Figure 1 depicts the experimental design of studies aimed to explore the effect of BC2 administered as bolus + infusion before the onset of injury. This protocol was also used for comparison of the BC2 effect with that obtained with heparin, ASA, and combination of BC2 or heparin with ASA. In all studies, ASA was administered at 5 mg/kg, IV bolus 15 minutes before vessel injury, whereas heparin or BC2 were administered as bolus followed by infusion as depicted in Figure 1. Heparin or BC2 pretreatments started 15 minutes before placement of the FeCl₃ patch on the carotid artery. All drug infusions continued to the end of the experimental period, 60 minutes from start of vessel injury. Blood samples were collected for aPTT and PT assay at 60 minutes (end of study). Carotid artery blood flow was continuously monitored. At the end of the experiment, the thrombus was extracted from the carotid artery and weighed.
Postinjury Protocol
The same experimental procedures were used as in the pretreatment procedure except that heparin and BC2 administration commenced at the end of the injury period. ASA, when administered, was given 15 minutes before injury. Only bolus administration of BC2 was used in the postinjury treatment experiments, whereas heparin was always administered as bolus followed by infusion. In this series of experiments, the injury period (FeCl3, patch placement) was confined to 10 minutes only.

Data Analysis
All data in the text and figures are mean group values ± standard error of mean for the indicated number of rats in each group. ANOVA and Bonferroni tests for multiple comparisons were used for between group analyses and a value *P*<0.05 accepted as significant.

Results
Effect of BC2 on aPTT, PT, and Activated Factor IX In Vitro
Figure 2 demonstrates a steep dose-dependent effect of heparin on aPTT in vitro. In contrast to heparin, which prolonged clotting times of normal human plasma to >350 seconds at 200 nmol/L, the anti-FIX mAb, BC2, extended the aPTT from 18 seconds to a plateau of 60 seconds in rat plasma and from 28 seconds to 85 to 90 seconds in human plasma at >200 nmol/L antibody. In parallel experiments, anti-FIX antibody was found to cause no change in the PT. ELISA experiments show that BC2 reacts with FIX and with activated factor IX (FIXa) but not with factor X, factor XI, or prothrombin, demonstrating the specificity of BC2.

To identify potential animal species for use in the in vivo analysis of BC2, plasma from rabbit, dog, rat, guinea pig, pig, baboon, and cynomolgus monkey were screened using the aPTT assay. Of these species, only plasma from rat (Figure 2) and the nonhuman primates were inhibited by the antibody, indicating that FIX from rat and nonhuman primates cross-reacts with BC2.

To determine whether BC2 can block the activity of FIXa, BC2 was mixed with 30 nmol/L FIXa and added to FIX deficient plasma. As shown in Figure 2B, increasing concentrations of BC2 prolonged the clotting time from 30 seconds (no BC2) to 65 seconds at 800 nmol/L antibody demonstrating that BC2 binding blocks factor X activation by FIXa.

SEM of Carotid Thrombus
SEM of sham arteries revealed an essentially normal endothelium with rare scattered platelets (Figure 3B, inset). Few breaks in the endothelium are noted, probably the result of mechanical damage during surgery. No evidence of thrombus formation was observed in the sham rats.

SEM of the arteries treated with FeCl3 revealed mural thrombi that occupied a large portion of the lumen of the vessel (Figure 3A). The thrombi were composed of aggregated platelets, red blood cells, and amorphous and fibrillar proteinaceous material. The proteinaceous material is consistent with fibrin. The endothelium of the arteries was mostly obscured by the large thrombi (Figure 3). Where visible, the endothelium overlying the region treated with FeCl3 was covered by numerous adherent platelets and amorphous proteinaceous material.

SEM of the arteries treated with FeCl3 from rats treated with BC2 revealed the lumen of the vessels to be largely free of thrombus (Figure 3B). The endothelium overlying the region treated with FeCl3 showed extensive damage. Some areas were covered by adherent platelets and some platelet aggregates, but there was little or no proteinaceous material.

Selection of BC2 Dose Based on aPTT Time/Dose Relationships
Figure 4 depicts the effect of various IV bolus doses of BC2 on aPTT throughout the duration of the experimental protocol. The study demonstrates that only a dose of 3 mg/kg and above resulted in extension of aPTT and that 6 mg/kg maintained aPTT extension of 3.5- to 4-fold over basal levels throughout the designated experimental protocol.

Effect of Pretreatment With BC2, Heparin, ASA, or BC2+ASA on Thrombus Weight, aPTT, PT, and Vessel Occlusion in FeCl3-Induced Arterial Thrombosis
Figure 5 illustrates the effects of heparin (dose-response, 15 to 120 U/kg, bolus+0.5 to 4 U · kg⁻¹ · min⁻¹, infusion), aspirin (5 mg/kg), BC2 (1 to 6 mg/kg), bolus+0.3 to 2 mg · kg⁻¹ · h⁻¹ (infusion), and combination of BC2 or heparin plus ASA on thrombus mass. Heparin dose-dependently suppressed thrombus formation in the carotid artery as reflected by thrombus weight at the end of the experimental period. However, the residual thrombus found in the vessels of rats treated with the highest dose of heparin was still approximately 20% of that in the vehicle-treated rats. ASA per se did not reduce the thrombus mass significantly, whereas the combination of heparin (30 U/kg+1 U · kg⁻¹ · min⁻¹) and
ASA (5 mg/kg) resulted in a residual thrombus that equaled that found in the heparin group alone. BC2 at 3 or 6 mg/kg bolus followed by 1 or 2 mg \( \cdot \) kg\(^{-1} \cdot \) h\(^{-1} \) effectively reduced thrombus mass as compared with its vehicle control; in fact, the residual thrombus mass at the highest BC2 dose was negligible. Interestingly, the lowest dose of BC2, 1 mg/kg bolus (a dose that had no significant effect when given alone), when combined with ASA (5 mg/kg, a dose of no effect per se) resulted in complete abolishment of thrombus formation.

Figure 6 demonstrates the incidence of occlusion (%), as determined by the absence of measurable blood flow, of the carotid arteries in the various treatment groups. Heparin treatment resulted in reduction of the incidence of vessel occlusion from 100% in the vehicle group to no occlusion at the highest dose. ASA alone had a tendency to reduce the incidence of occlusion (not significant), whereas ASA and heparin (30 U/kg \( \cdot \) min\(^{-1} \)) significantly reduced the incidence of vessel occlusion. BC2 at the medium and higher doses, as well as the combination of the lowest dose and ASA, completely prevented vessel occlusion.

ASA (5 mg/kg) resulted in a residual thrombus that equaled that found in the heparin group alone. BC2 at 3 or 6 mg/kg bolus followed by 1 or 2 mg \( \cdot \) kg\(^{-1} \cdot \) h\(^{-1} \) effectively reduced thrombus mass as compared with its vehicle control; in fact, the residual thrombus mass at the highest BC2 dose was negligible. Interestingly, the lowest dose of BC2, 1 mg/kg bolus (a dose that had no significant effect when given alone), when combined with ASA (5 mg/kg, a dose of no effect per se) resulted in complete abolishment of thrombus formation. Figure 6 demonstrates the incidence of occlusion (%), as determined by the absence of measurable blood flow, of the carotid arteries in the various treatment groups. Heparin treatment resulted in reduction of the incidence of vessel occlusion from 100% in the vehicle group to no occlusion at the highest dose. ASA alone had a tendency to reduce the incidence of occlusion (not significant), whereas ASA and heparin (30 U/kg \( \cdot \) min\(^{-1} \)) significantly reduced the incidence of vessel occlusion. BC2 at the medium and higher doses, as well as the combination of the lowest dose and ASA, completely prevented vessel occlusion.

Figure 7A shows the effect of the various pretreatments on the aPTT, which was performed with a limitation of 1000 seconds. A dashed line denoting a 3.5-fold increase over baseline aPTT is depicted. Heparin dose-dependently increased aPTT; the 2 highest doses rendered the plasma incoagulable as the aPTT reached 1000 seconds in every animal. The medium dose (H30) that failed to maintain vessel patency in the majority of animals extended aPTT 10-fold over baseline. ASA had no effect on aPTT but potentiated the extension of the aPTT by the medium dose of heparin (H30). BC2 at 3 or 6 mg/kg doses extended aPTT but even at the highest doses did not exceed a 3.5-fold prolongation of aPTT; interestingly, in contrast to the synergy ASA had on heparin.
extension of aPTT, no such augmentation was observed when BC2 was administered with ASA.

Figure 7B depicts the effect of the various treatments on PT. Neither ASA nor BC2 treatment regimens extended PT. All doses of heparin, and especially the 2 highest doses, significantly prolonged PT.

Effect of BC2 Administered as a Single Bolus Before Carotid Lesion on Thrombus Weight, Incidence of Vessel Occlusion, and Patency Time and aPTT

Comparison between a single bolus injection of BC2 with heparin infusion at a dose that produced maximal extension of aPTT (Figure 8) demonstrates that both treatments resulted in similar efficacy in reducing thrombus mass and extension of vessel perfusion time: BC2 was somewhat more effective in preventing vessel occlusion (10% compared with 27% in the heparin group) (Figure 8B). A marked difference was clearly noted in the effect of BC2 versus heparin on aPTT (Figure 8C); BC2 increased aPTT by <3.5-fold, whereas heparin rendered the plasma incoagulable (aPTT > 1000 seconds).

Effect of Murine IgG Administration on aPTT, PT, and Blood Loss of Anesthetized Rats

To control for possible effects of murine immunoglobulin on hemostatic parameters of rats, a bolus dose of murine IgG at 6 mg/kg was administered IV as per the protocol described for pretreatment studies. Figure 9 demonstrates that a 6 mg/kg, IV bolus dose of a murine IgG, corresponding to the highest dose of BC2, does not result in alteration of thrombus weight, time to occlusion, aPTT, or PT.

Effect of BC2 or Heparin Administered After Completion of Carotid Injury on Thrombus Mass, Vessel Patency Time, Incidence of Carotid Artery Occlusion, aPTT and PT

Figures 10A and 10B compare the effect of BC2 or heparin administered after the completion of the FeCl3 patch injury, on thrombus mass, incidence of vessel occlusion, and carotid artery patency time. In vehicle-treated control rats, complete vessel occlusion occurs approximately 5 minutes after the end of the injury period. BC2 administered as a single bolus (3 mg/kg) significantly inhibited thrombus formation in the vessel and reduced the occlusion rate by 67%. Furthermore, vessel patency was extended to almost the complete duration of the experiment (60 minutes). A dose-response study of heparin administered after the completion of the injury demonstrated that heparin treatment also reduced thrombus mass and increased vessel patency time and occlusion frequency, as demonstrated in the pretreatment experiments. Whereas BC2 prolonged the aPTT to 64 seconds, heparin (at all doses) dramatically extended the aPTT (600 to 1000 seconds) (Figure 11A). Furthermore, at the 2 highest doses of
heparin, a significant extension of PT was also observed (Figure 11B). In marked contrast, BC2 (3 mg/kg) had no effect on PT.

Effect of BC2 or Heparin on Blood Loss from Surgical Cut

The effect of BC2 (3 mg/kg, IV bolus) or heparin (dose-response) on blood loss is illustrated in Figure 12. Blood volume was monitored over a 15-minute period by free flow after the completion of experimental protocol, ie, 60 minutes post injury. BC2 treatment did not result in an increase in blood loss as compared with vehicle control. In contrast, heparin treatments (60 to 240 U/kg bolus plus 1, 2, 4, or 8 U · kg⁻¹ · min⁻¹ infusion) resulted in a significant increase in blood loss over the prespecified experimental period. The dose of heparin that did not result in increased blood loss also failed to reduce thrombus mass or diminish the incidence of vessel occlusion (see Figure 10A).

To confirm that BC2 administration as a bolus followed by infusion does not result in excessive blood loss, we have repeated the experiment vide supra using a bolus treatment (2 mg/kg) followed by infusion of 1 mg · kg⁻¹ · h⁻¹ throughout the experimental period. As depicted in Figure 13, the extended infusion paradigm has not resulted in blood loss over that in the vehicle-treated rats, although aPTT was significantly extended in accord with previous data with bolus dose of BC2 (see Figures 8 and 11).

Figure 10. Effect of BC2 single bolus or heparin infusion on thrombus mass, incidence of occlusions, and carotid patency time in post-injury treatment study. Rats were treated with BC2 (3 mg/kg single IV bolus) or heparin (30, 60, 120, or 240 U/kg bolus plus 1, 2, 4, or 8 U · kg⁻¹ · min⁻¹ infusion) at the end of the 10 minute FeCl₃ patch injury period. A, Thrombus mass at the end of the 60-minute protocol. Values above the dashed horizontal line represents incidence of vessel occlusion. B, Carotid patency time. Number of rats=6 to 14. Asterisks denote statistical significance compared with vehicle control. *P<0.01, **P<0.001.

Figure 11. Effect of BC2, single bolus, or heparin infusion on aPTT and PT in the post-injury treatment protocol. Rats were treated with BC2 (3 mg/kg single IV bolus) or heparin (30, 60, 120, or 240 U/kg bolus plus 1, 2, 4, or 8 U · kg⁻¹ · min⁻¹ infusion) at the end of the 10-minute FeCl₃ patch injury period. A, aPTT determined at the end of the 60-minute protocol; B, PT. Number of rats=6 to 14. Asterisks denote statistical significance compared with vehicle control. *P<0.01, **P<0.001.

Effect of BC2 on FIX Activity

Because aPTT may not linearly relate to FIX activity, we have explored the effect of BC2 on FIX activity versus aPTT extension in the same experiment. BC2 was dosed in vivo and FIX activity and aPTT monitored ex vivo. Figure 14 indicates that significant reduction in Factor IX activity is associated with minor change in aPTT and almost complete reduction in FIX (>90%) extends aPTT by approximately 3.5- to 4-fold. In the context of the efficacy studies, significant blockade of thrombus formation and 90% reduction of vessel occlusion

Figure 12. Effect of BC2, single bolus, or heparin infusion on blood loss from surgical wound in the post-injury protocol. Blood loss after a surgical cut to the rat tail was performed as described under Methods. Rats were treated with BC2 (3 mg/kg single IV bolus) or heparin (30, 60, 120, or 240 U/kg bolus plus 1, 2, 4, or 8 U · kg⁻¹ · min⁻¹ infusion) at the end of the 10-minute FeCl₃ patch injury period. Number of rats=6 to 14. Asterisks denote statistical significance compared with vehicle control. *P<0.01, **P<0.001.
incidence can be achieved with 3 mg/kg doses of BC2, which decrease FIX activity levels to approximately 50% of the normal levels.

Effect of Human FIX on BC2-Induced aPTT Extension In Vivo

To assess the capacity of human FIX (hFIX) to serve as an antidote to BC2, rats were administered BC2 and aPTT monitored 30 minutes later. At that time, rats were injected with human FIX (3 or 5 mg/kg, IV bolus) or vehicle and aPTT assayed 30 minutes later. Figure 15 depicts extension of aPTT from 17.6 ± 0.3 to 45.2 ± 2.3 seconds and 44.8 ± 1.6 seconds (*P < 0.001) at 30 and 60 minutes post BC2, respectively. Human FIX dose-dependently reduced aPTT toward baseline, although incompletely (aPTT at 60 minutes was 24.7 ± 0.5 seconds after injection of 5 mg/kg hFIX).

Discussion

The present experiments describe the in vitro and in vivo anticoagulant and antithrombotic properties of BC2, a novel antihuman FIX monoclonal antibody that blocks the coagulant activity of FIX and FIXa. Initial studies indicate that BC2 binds to the Gla domain of human FIX, and the observation that BC2 cross-reacts with rat FIX suggests that the epitope is conserved between human and rat FIX. More detailed epitope mapping studies will be required to define the specific sequence recognized by BC2. The observation that BC2 inhibits rat FIX enabled the development of an extensive portfolio of in vivo animal pharmacology.

In vivo efficacy of BC2 was demonstrated in a standard arterial thrombosis model in rats where severe vessel wall (and endothelial cells) injury is rapidly induced by Fe³⁺-mediated oxygen radical formation. This has been clearly confirmed in our study by performing SEM and rapid and complete occlusion of the vessel as directly measured arterial blood flow in the carotid artery. BC2 was most efficacious in blocking thrombus formation when administered either before or shortly after the injury to the vessel. The effects of
BC2 were dose-dependent and showed potential synergy with ASA (the latter had little effect in blocking thrombosis when administered on its own in this model). The antithrombotic efficacy of BC2 was reflected by thrombus mass reduction, increased reversal time, and reduced incidence of vessel occlusion. In both preinjury or postinjury protocols, BC2 efficacy equaled or exceeded that of heparin, even when the latter was provided at very high doses. However, although heparin extended aPTT and PT and increased (doubled) the volume of blood loss after a surgical cut, BC2 had no effect on PT nor did BC2 treatment result in increased blood loss from the surgical wound. Indeed, complete protection against thrombosis was only achieved at heparin doses that rendered the plasma incoagulable (aPTT>1000 seconds). Most interesting, BC2 extension of aPTT ex vivo was limited to 60 to 70 seconds, which was similar to its plateau effect in vitro. Finally, the specificity of BC2 in blocking FIX and the reversibility of anti-FIX antibody therapy was proven by the administration of its antidote, FIX. Indeed, an almost 3-fold extension of the aPTT induced by BC2 infusion in rats was rapidly and dose-dependently reversed by human FIX administered at the peak of BC2 effect. The reason for the incomplete reversal of BC2-induced aPTT extension may be the result of species specificity of human FIX interacting with the rat proteins that comprise the factor X activating complex.

Despite the central location of FIX in the coagulation cascade, where it is activated by tissue factor/factor VIIa complex and by factor Xla and is the primary activator of factor X on activated platelets,13 factor IXa/Ixa has received little attention as a potential target for therapeutic intervention in thrombotic disorders.25,26 Our studies with this novel anti-FIX antibody clearly demonstrate the capacity of specific inhibition of FIX to prevent acute arterial thrombosis. These studies thus extend previous studies reporting antithrombotic efficacy of FIXa in canine models of arterial thrombosis and cardiopulmonary bypass surgery.17,27 Preliminary studies conducted in our laboratory also demonstrate that treatment with BC2 results in extended patency of the blood vessel; thus a single bolus injection of BC2 not only increased the incidence of vessel patency 60 minutes after BC2 administration but also 24 hours later at a time when aPTT was within normal range. This phenomenon may reflect the capacity of the vessel wall to initiate repair when coagulation is interrupted at FIX/Ixa limiting the generation of factor Xa and thrombin. Taken together, our studies with BC2, as well as those with FIXa/Ixa, demonstrate that efficacy in blocking thrombosis can be achieved with limited prolongation of the aPTT and with less resultant increase in bleeding compared with heparin.

Acknowledgment

The authors wish to acknowledge the excellent technical contribution of J. Vasko-Moser and S. Tirri for preparation of the manuscript.

References

Antithrombotic Efficacy of a Novel Murine Antihuman Factor IX Antibody in Rats

doi: 10.1161/01.ATV.19.10.2554

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 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/19/10/2554

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/