ELSEVIER

European Journal of Pharmacology 432 (2001) 187–194

www.elsevier.com/locate/ejphar

The antithrombotic effects of CI-1031 (ZK-807834) and enoxaparin in a canine electrolytic injury model of arterial and venous thrombosis

Thomas B. McClanahan [a,*], Gary W. Hicks [a], Angela L. Morrison [a], Yun-Wen Peng [a], Nancy Janiczek-Dolphin [a], Thomas E. Mertz [a], Mark E. Sullivan [b], John Morser [b], Paul L. Juneau [a], Robert Leadley [a]

[a] *Cardiovascular Pharmacology Section, Pfizer Global Research and Development, Ann Arbor Laboratories, Ann Arbor, MI, USA*
[b] *Berlex Biosciences, Richmond, CA, USA*

Received 15 January 2001; received in revised form 22 May 2001; accepted 29 May 2001

Abstract

Factor Xa is a serine protease positioned at the convergence point of the intrinsic and extrinsic coagulation pathways and is therefore an attractive target in the development of novel anticoagulant drugs. The objective of this study was to evaluate the efficacy of CI-1031 (*N*-[2-[5-amidino-2-hydroxyphenoxy]-6-[3-(1-methyl-1*H*-imidazolin-2-yl)-phenoxy]-3,5-difluoropyrid), a potent and selective inhibitor of Factor Xa, in a canine electrolytic injury model of arterial and venous thrombosis. Enoxaparin (enoxaparin sodium), a low molecular weight heparin currently approved for treatment and prevention of deep vein thrombosis and unstable angina, was also tested for efficacy in this model. CI-1031 was administered intravenously to anesthetized dogs at three doses: 1.25, 2.5 and 5 μg/kg/min ($n=5$ for each group) as a continuous infusion for 5.5 h. The control group ($n=5$) received a continuous infusion of vehicle (3.69 mmol citric acid and 0.9% sodium chloride solution) at a rate of 1 ml/kg/h. Ninety minutes after administration of CI-1031 prothrombin times increased 1.2-, 1.6- and 2.0-fold over baseline values in the 1.25, 2.5 and 5 μg/kg/min groups, respectively. The time to formation of an occlusive thrombus in the femoral arteries averaged 69 ± 5 min in the control group compared to 127 ± 19, 192 ± 33 and 219 ± 15 min in the low-, mid- and high-dose CI-1031 groups. In the femoral veins, occlusion time in the controls averaged 56 ± 11 min compared to 153 ± 22, 137 ± 30 and 214 ± 26 min in the three treatment groups. Thrombus weights in the control arteries averaged 51 ± 4 mg compared to 45 ± 5, 28 ± 10 and 15 ± 3 mg in the CI-1031 treated groups. On the venous side, control thrombus weights averaged 96 ± 18 mg compared to 75 ± 16, 51 ± 16 and 25 ± 4 mg in the low-, mid- and high-dose CI-1031 groups. A plasma CI-1031 concentration of approximately 400 ng/ml was associated with a 50% reduction in thrombus weight relative to control animals. Enoxaparin was administered intravenously at a loading dose of 50, 100 or 200 IU/kg for 1 h followed by a maintenance infusion of 25, 50 or 100 IU/kg/h for 4.5 h. The most dramatic changes in coagulation parameters were observed in thrombin time with virtually no changes in prothrombin time. Enoxaparin elicited a dose-dependent increase in time to thrombotic occlusion and a dose-dependent decrease in thrombus weight similar to that observed with CI-1031. Time to occlusion in the enoxaparin-treated groups averaged 117 ± 33, 188 ± 32 and 217 ± 22 min in the low-, mid- and high-dose groups in the femoral arteries and 84 ± 22, 171 ± 31 and 133 ± 33 min in the femoral veins. Thrombus weights averaged 33 ± 10, 12 ± 5 and 10 ± 4 mg in the arteries and 32 ± 9, 13 ± 2 and 21 ± 6 mg in the veins in the low-, mid- and high-dose groups. Blood loss with CI-1031 tended to be less than enoxaparin at doses that provided comparable efficacy. These results demonstrate that CI-1031, like enoxaparin, is an effective antithrombotic agent in an established canine model of arterial and venous thrombosis. CI-1031 provided dose-dependent efficacy with minimal changes in ex vivo coagulation parameters, suggesting it may be a safe and effective antithrombotic agent for both arterial and venous indications. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Thrombosis; Electrolytic injury; Factor Xa inhibitor; Enoxaparin sodium

1. Introduction

The activated serine protease Factor Xa plays an important role in the blood coagulation cascade because of its central location at the point where the extrinsic and intrinsic pathways converge. Factor Xa inhibitors prevent the generation of thrombin, thereby disrupting the thrombin feedback loop which amplifies thrombin production (Gardell and Sanderson, 1998; Al-Obeidi and Ostrem, 1999). By reducing thrombin generation, inhibitors of Factor Xa may also attenuate platelet activity and clot stabilization. Several preclinical studies have demonstrated an enhanced efficacy-

* Corresponding author. 2800 Plymouth Rd., Ann Arbor, MI 48105, USA. Tel.: +1-734-622-2886; fax: +1-734-622-1480.
E-mail address: tom.mcclanahan@pfizer.com (T.B. McClanahan).

0014-2999/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(01)01090-1

to-bleeding ratio with Factor Xa inhibitors compared to other antithrombotic strategies (Lynch et al., 1994; Nicolini et al., 1995; Lefkovits et al., 1996; Sato et al., 1998a; Vlasuk et al., 1991). Therefore, the development of new antithrombotic agents that target Factor Xa may provide greater efficacy and reduced bleeding risk than current therapeutic approaches to thrombosis.

Low molecular weight heparins mediate inhibition of Factor Xa by enhancing antithrombin III activity and have been shown to provide more reliable anticoagulation with fewer side effects than standard unfractionated heparin (Zed, 1999). Enoxaparin is a low molecular weight heparin that is currently approved for the prevention and treatment of deep vein thrombosis and, more recently, to prevent ischemic events in patients with unstable angina (Brener, 2000). A number of limitations with low molecular weight heparins, including the inability to inactivate fibrin-bound thrombin and the risk of heparin-induced thrombocytopenia and osteoporosis, leave significant room for the development of safe, effective anticoagulants (Weitz, 1997).

Direct inhibitors of Factor Xa, in contrast to low molecular weight heparin, bind directly to the active site of Factor Xa and have been proven to be effective anticoagulants in experimental models of thrombosis (Weitz and Hirsh, 1998). CI-1031 (*N*-[2-[5-amidino-2-hydroxyphenoxy]-6-[3-(1-methyl-1*H*-imidazolin-2-yl)-phenoxy]-3,5-difluoropyrid), a potent and selective Factor Xa inhibitor with a K_i of 0.11 nM (Phillips et al., 1998; Subramanyam et al., 1998), has previously been shown to attenuate thrombus progression in a rabbit model of thrombosis (Abendschein et al., 2000). The objective of this study was to evaluate CI-1031 and establish a dose–response relationship for antithrombotic efficacy versus bleeding risk. The study also compares the efficacy of CI-1031 with enoxaparin in an established canine model of arterial and venous thrombosis.

2. Materials and methods

2.1. Surgical procedure

This study was conducted in compliance with the Animal Welfare Act Regulations and with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Mongrel dogs of either sex weighing 11–19 kg were anesthetized with 30 mg/kg intravenous sodium pentobarbital and intubated and ventilated with room air (Harvard dog ventilator, South Natick, MA). Arterial blood gasses were measured periodically to verify that pO_2 exceeded 100 mm Hg and that pCO_2 and pH were within normal limits (Radiometer ABL510, Copenhagen, Denmark). The left common carotid artery was cannulated with a polyethylene catheter, and was used for continuous measurement of arterial blood pressure using a P23Db Gould transducer (Gould Instruments Systems, Valley View, OH) as well as for blood sampling. Two polyethylene catheters were placed in the left internal jugular vein, one for administration of CI-1031 or vehicle, and the other for maintenance doses of pentobarbital. The left femoral artery and right femoral vein were dissected and a 3–4-cm section of each vessel was carefully exposed. Each vessel was instrumented with a 2.5-mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) for continuous measurement of blood flow. A Goldblatt clamp, positioned downstream from the flow probe, was used to produce a flow-limiting stenosis. Development of an occlusive thrombus in the femoral artery and vein was induced by applying a 300-μA anodal DC current to the intimal surface of the vessel in the presence of the stenosis. The stimulation electrode was constructed from a 25-gauge hypodermic needle attached to a 30-gauge, Teflon-insulated silver-coated copper wire. The needle was inserted into the lumen of the vessel between the flow probe and the Goldblatt clamp. The position of the needle was inspected at the conclusion of the experiment to ensure contact with the endothelial surface of each vessel.

2.2. Experimental protocol

A total of 35 dogs were used in the study to evaluate the effects of CI-1031 and enoxaparin on thrombus formation. Dogs were assigned to one of seven treatment groups with five animals in each group. CI-1031 was dissolved in a citric acid (3.69 mmol) and sodium chloride (0.9%) solution, and the pH was adjusted with NaOH to approximately 5.0. The animals received vehicle (the citric acid and sodium chloride solution, 1 ml/kg/h) or CI-1031 at doses of 1.25, 2.5 or 5 μg/kg/min for 5.5 h. Enoxaparin (Lovenox, Aventis Pharmaceuticals, Bridgewater, NJ), was diluted with saline (0.9% sodium chloride solution) and administered intravenously as a loading dose of either 50, 100 or 200 IU/kg for 1 h followed by a maintenance infusion of 25, 50 or 100 IU/kg/h for 4.5 h, respectively.

Baseline measurements were obtained prior to the administration of drug or vehicle. The Goldblatt clamp on each vessel was adjusted to reduce flow approximately 50% in the femoral artery and 70% in the femoral vein resulting in flows averaging 60 and 30 ml/min, respectively. After 90 min of drug or vehicle infusion, a 300-μA anodal current was applied to each vessel for a total of 2 h. Time to occlusion was recorded when flow remained at 0 ml/min for five consecutive minutes without reperfusion. The time to occlusion was reported as 240 min if an occlusive thrombus did not form during the 4 h after initiation of current. At the end of the experiment the vessel segments were ligated both proximal and distal to the point of injury and excised. The vessels were opened longitudinally and the thrombus was removed and weighed.

2.3. Coagulation parameters and bleeding risk

Coagulation parameters were tested at baseline, 30, 60, 90, 150, 210, 270 and 330 min of drug infusion. Blood

samples were collected in sodium citrate tubes from the carotid artery catheter and centrifuged for 10 min at $3000 \times g$ and 3 °C to obtain plasma for analysis. Plasma coagulation was assessed by determining activated partial thromboplastin time (APTT, STA®-PTT, Diagnostica Stago, Asnieres, France), thrombin time (TT, STA®-Thrombin, Diagnostica Stago), and prothrombin time (PT, STA®-Neoplastine®, Diagnostica Stago) with an ST4 Diagnostica Stago coagulation instrument (Parsippany, NJ), and by measuring activated clotting time (ACT) with a Medtronic ACTII instrument (Parker, CO). Arterial blood samples were also collected in EDTA tubes and centrifuged for 10 min at $3000 \times g$ and 3 °C. This plasma was collected and stored at −70 °C for subsequent analysis of CI-1031 concentration. Concentrations were determined by LC/MS/MS analysis. The lower limit of quantitation was 10 ng/ml.

Two methods were used to assess the risk of bleeding. At baseline, 30, 60, 90, 150, 210, 270 and 330 min of drug infusion, a Surgicutt® automated incision device (Edison, NJ) was used to make a standardized incision 5 mm long and 1 mm deep on the upper lip. The wound was carefully blotted with filter paper every 30 s until bleeding stopped. The time required for the wound to clot was recorded as the template bleeding time. In addition, clean, dry sponges were weighed and placed into the surgical incisions exposing the carotid artery, jugular vein, and the right and left femoral vessels to collect blood loss from the sites. These sponges were collected periodically and stored in a sealed container to be weighed immediately after the experiment.

2.4. Data analysis

Recordings were obtained during each experiment on a Gould eight-channel recorder and the analog signals were digitized on a Po-Ne-Mah digital acquisition system (Gould Instrument Systems). Hemodynamic variables included heart rate, mean arterial blood pressure, left femoral arterial flow and right femoral venous flow. Results are reported as mean values ± the standard error of the mean. The data were analyzed for statistical differences by a one-way analysis of variance for group differences followed by a Dunnett's post hoc test to determine the level of significance. A paired Student's *t*-test was used to assess differences within a group over time. A value of $P < 0.05$ was considered to be statistically significant.

2.5. Pharmacodynamic modeling

The effect of CI-1031 on thrombus formation was described by a sigmoid E_{max} model (Derendorf and Hochaus, 1995):

$$E = \frac{E_{max} C^S}{EC_{50}^S + C^S}$$

Plasma CI-1031 concentrations (C) at the start of the current (90 min into the infusion) were used in this analysis. Effect (E) was calculated as the percent reduction in thrombus weight for each animal relative to the mean thrombus weight in the control group. E_{max} was estimated as the maximum percent reduction in thrombus weight, EC_{50} as the drug concentration corresponding to 50% of the maximum reduction in thrombus weight, and S as the constant reflecting the shape of the effect–concentration curve.

3. Results

3.1. Time to occlusion and thrombus weights

The time to thrombotic occlusion in the control group was 69 ± 5 min in the femoral artery compared to 127 ± 19, 192 ± 33 and 219 ± 15 min in the 1.25, 2.5 and 5 μg/kg/min CI-1031 groups, respectively (Fig. 1). In the femoral veins, control occlusion times averaged 56 ± 11 min compared to

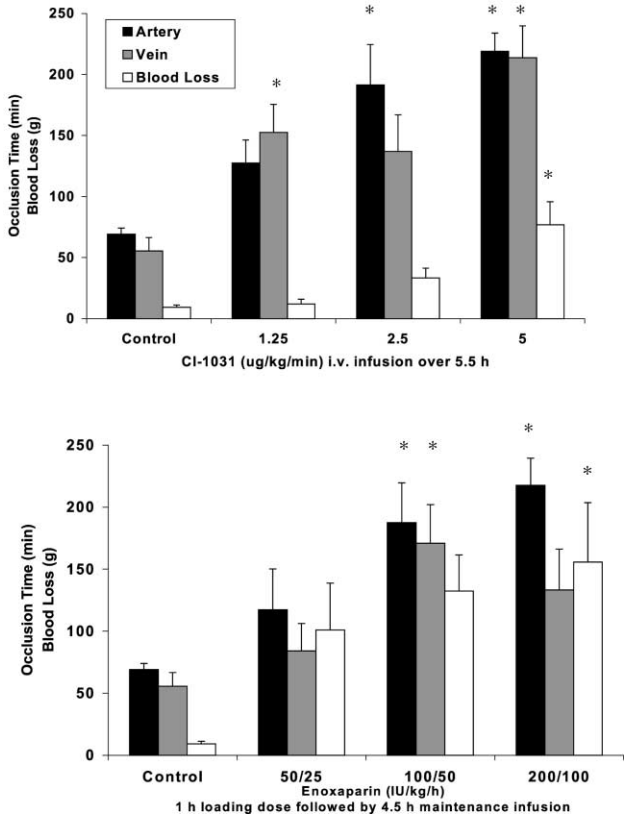

Fig. 1. Effect of CI-1031 and enoxaparin on time to formation of an occlusive thrombus and on blood loss. CI-1031 was administered intravenously at doses of 1.25, 2.5 and 5 μg/kg/min as an infusion over 5.5 h. Enoxaparin was administered at doses of 50/25, 100/50 and 200/100 load/maintenance IU/kg/h. The loading dose was given for 1 h followed by the maintenance infusion for the remainder of the study. Values are mean ± S.E.M.; $n = 5$ for each group. * $P < 0.05$ from control group.

153 ± 22, 137 ± 30 and 214 ± 26 min in the respective CI-1031 treatment groups. In the arteries, the times to occlusion were significantly greater than control values in the 2.5 and 5 μg/kg/min groups. In the veins, the 1.25 and 5 μg/kg/min groups were significantly ($P < 0.05$) greater compared to controls.

The time to formation of an occlusive thrombus in the enoxaparin-treated animals generally increased in a dose-dependent manner. In the arteries, occlusion times were 117 ± 33, 188 ± 32 and 218 ± 22 min in the 50/25, 100/50 and 200/100 IU/kg/h enoxaparin groups compared to 69 ± 5 min in control animals. Occlusion times in the veins were 84 ± 22, 171 ± 31 and 133 ± 33 min in the low-, mid- and high-dose groups compared to 56 ± 11 min in the untreated controls.

Fig. 2 illustrates the dose-dependent reduction in thrombus weights in CI-1031 and enoxaparin-treated animals. Thrombus weights in control arteries averaged 51 ± 4 mg compared to 45 ± 5, 28 ± 10 and 15 ± 3 mg in the groups treated with 1.25, 2.5 and 5 μg/kg/min of CI-1031, respectively. Thrombus weights in the veins averaged 96 ± 18 mg in the control group compared to 75 ± 16, 51 ± 16 and 25 ± 4 mg in the 1.25, 2.5 and 5 μg/kg/min groups. Thrombus weights in the CI-1031-treated animals were significantly less in the arteries for the 2.5 and 5 μg/kg/min groups and in the veins for the 5 μg/kg/min group compared to controls.

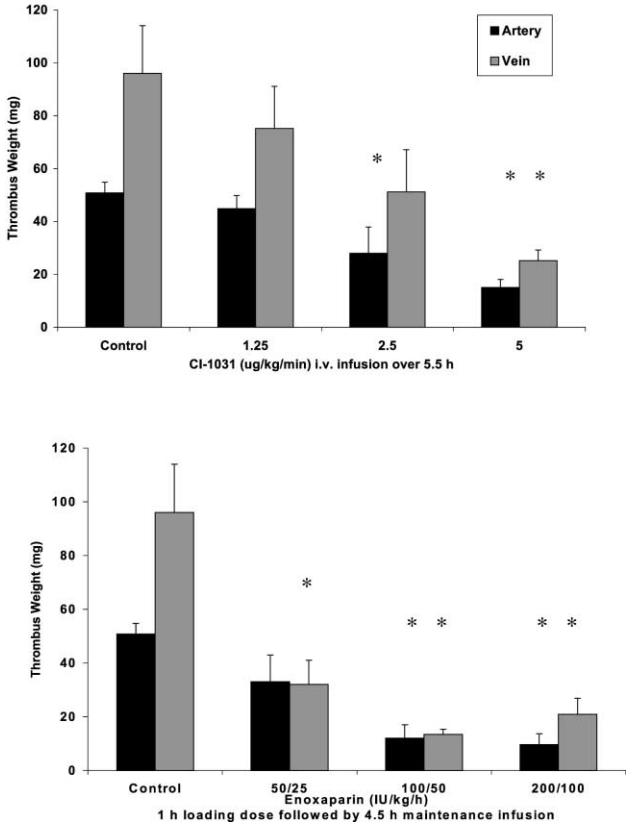

Fig. 2. Effect of CI-1031 and enoxaparin on thrombus weights. Values are mean ± S.E.M.; $n = 5$ for each group. * $P < 0.05$ from control group.

Thrombus weights in the arteries averaged 33 ± 10, 12 ± 5 and 10 ± 4 mg in the low-, mid- and high-dose enoxaparin groups compared to 51 ± 4 mg in the control group. In the veins, thrombus weights averaged 32 ± 9, 13 ± 2 and 21 ± 6 mg in the enoxaparin-treated groups compared to 96 ± 18 mg in the control group.

3.2. Template bleeding time and blood loss

There were no significant changes in template bleeding times in any of the CI-1031 treatment groups. The 2.5 and 5 μg/kg/min groups exhibited only a maximal 1.2-fold increase over baseline values. In the control animals, template bleeding time was 133 ± 5 s at baseline and 131 ± 13 s 90 min after vehicle administration. In the CI-1031 treatment groups, baseline bleeding times averaged 143 ± 7, 126 ± 3 and 136 ± 7 s compared to 143 ± 18, 154 ± 10 and 163 ± 15 s 90 min after drug administration in the 1.25, 2.5 and 5 μg/kg/min groups, respectively. Blood loss measured by gauze sponges collected from surgical sites increased in a dose-dependent manner, but was significantly greater compared to control ($P < 0.05$) only for the 5 μg/kg/min group (Fig. 1). Blood loss in the control group was 9 ± 2 g compared to 12 ± 4, 33 ± 8 and 77 ± 19 g in the 1.25, 2.5 and 5 μg/kg/min groups, respectively.

There were no significant changes in template bleeding times in any of the enoxaparin treatment groups. Template bleeding times at baseline averaged 143 ± 13, 139 ± 10 and 141 ± 13 s compared to 171 ± 12, 158 ± 10 and 173 ± 15 s 90 min after administration of 50/25, 100/50 and 200/100 IU/kg/h of enoxaparin. Blood loss from surgical sites tended to be much more variable in the enoxaparin-treated groups and was significantly greater than the control group ($P < 0.05$) at the 200/100 IU/kg/h group. Blood loss averaged 101 ± 38, 132 ± 29 and 156 ± 48 g in the 50/25, 100/50 and 200/100 IU/kg/h groups.

3.3. Coagulation parameters

Table 1 summarizes the coagulation parameters in the control and the CI-1031-treated groups. There were no significant differences among groups in activated partial thromboplastin time, thrombin time, activated clotting time or prothrombin time prior to the administration of drug or vehicle.

Coagulation parameters increased in a dose-dependent manner in the three CI-1031 treatment groups. Activated partial thromboplastin time increased 1.3-, 1.4- and 2.0-fold over baseline values 90 min after drug administration in the 1.25, 2.5 and 5 μg/kg/min groups, respectively. In general, CI-1031 produced only minor changes in thrombin time during the study. Administration of CI-1031 increased activated clotting times 1.2- and 1.6-fold over baseline in the 2.5 and 5 μg/kg/min groups after 90 min of infusion.

Table 1
Coagulation parameters with CI-1031

CI-1031 (μg/kg/min)	Baseline	30 min	60 min	90 min	150 min	210 min	270 min	330 min
APTT (s)								
Control	21.0 ± 1.8	24.4 ± 4.1	21.7 ± 2.9	21.0 ± 3.1	22.6 ± 2.8	21.9 ± 3.0	23.3 ± 3.9	23.8 ± 4.1
1.25	22.4 ± 1.4	28.5 ± 2.0[a]	28.5 ± 2.9[a]	30.6 ± 2.7[a]	31.0 ± 1.0	32.2 ± 2.6[a,b]	32.0 ± 4.0[a]	38.4 ± 1.4[a,b]
2.5	22.1 ± 1.6	28.3 ± 1.8[a]	29.2 ± 2.9[a]	31.8 ± 2.3[a,b]	32.3 ± 2.6[a,b]	31.9 ± 2.2[a,b]	26.9 ± 2.3	29.4 ± 7.8[a]
5	20.2 ± 0.8	34.0 ± 3.0[a]	35.8 ± 2.9[a,b]	41.2 ± 2.3[a,b]	39.8 ± 2.3[a,b]	48.4 ± 4.2[a,b]	45.7 ± 2.6[a,b]	60.0 ± 8.2[a,b]
TT (s)								
Control	11.8 ± 0.5	15.7 ± 2.3[a]	12.1 ± 0.3	11.9 ± 0.3	12.2 ± 0.4	12.2 ± 0.7	11.7 ± 0.4	11.9 ± 0.4
1.25	12.4 ± 0.3	13.5 ± 0.3[b]	13.3 ± 0.1	13.5 ± 0.5	12.8 ± 0.2	13.4 ± 0.5	12.6 ± 0.6	12.7 ± 0.2
2.5	12.1 ± 0.3	12.5 ± 0.4[b]	12.4 ± 0.3	12.2 ± 0.5	12.6 ± 0.4	12.3 ± 0.5	12.4 ± 0.3	12.8 ± 0.3
5	12.5 ± 0.3	12.9 ± 0.4[b]	13.2 ± 0.4	13.2 ± 0.4	13.2 ± 0.6	13.2 ± 0.5	13.4 ± 0.7	13.3 ± 0.7
ACT (s)								
Control	77 ± 2	76 ± 3	69 ± 3	61 ± 3[a]	68 ± 2	64 ± 4	63 ± 3[a]	63 ± 3[a]
1.25	88 ± 3	93 ± 6[b]	90 ± 5[b]	91 ± 7[b]	81 ± 7	92 ± 6[b]	92 ± 8[b]	98 ± 3[b]
2.5	78 ± 2	105 ± 2[a,b]	99 ± 5[a,b]	99 ± 4[a,b]	103 ± 7[a,b]	98 ± 8[a,b]	93 ± 11[a,b]	105 ± 8[a,b]
5	85 ± 4	118 ± 9[a,b]	130 ± 11[a,b]	136 ± 5[a,b]	131 ± 2[a,b]	156 ± 4[a,b]	160 ± 5[a,b]	162 ± 7[a,b]
PT (s)								
Control	7.3 ± 0.1	7.3 ± 0.2	7.4 ± 0.1	7.2 ± 0.1	7.6 ± 0.2	7.8 ± 0.3	7.8 ± 0.2	7.9 ± 0.4
1.25	7.1 ± 0.2	9.9 ± 0.5	11.1 ± 0.7[a]	12.2 ± 0.8[a]	14.1 ± 0.9[a,b]	16.3 ± 1.5[a,b]	18.3 ± 1.6[a,b]	20.4 ± 2.9[a,b]
2.5	6.7 ± 0.5	10.7 ± 1.5[a]	12.3 ± 1.7[a]	13.2 ± 2.0[a,b]	14.5 ± 2.4[a,b]	16.3 ± 3.3[a,b]	17.6 ± 4.4[a,b]	18.7 ± 4.3[a,b]
5	7.1 ± 0.2	13.9 ± 0.4[a,b]	16.8 ± 0.6[a,b]	18.5 ± 0.6[a,b]	20.8 ± 0.8[a,b]	22.1 ± 1.3[a,b]	23.5 ± 1.6[a,b]	25.3 ± 1.9[a,b]

APTT, activated partial thromboplastin time; TT, thrombin time; ACT, activated clotting time; PT, prothrombin time; 1.25, 2.5 and 5 μg/kg/min, dose of CI-1031 administered intravenously; Baseline, baseline values prior to the administration of CI-1031 or vehicle; 30, 60, 90,150, 210, 270 and 330 min, time in minutes after start of drug infusion.
$N = 5$ for each group.
Values are expressed as mean ± S.E.M.
[a] $P < 0.05$ vs. baseline.
[b] $P < 0.05$ vs. control.

Prothrombin times at 90 min were 1.7-, 1.9- and 2.5-fold over baseline in the three treatment groups.

Table 2 summarizes the changes in coagulation parameters in the enoxaparin-treated animals. All coagulation parameters except PT increased in a dose-dependent manner in the three enoxaparin-treated groups. After 90 min of drug infusion, activated partial thromboplastin time increased 1.2-, 1.6- and 2.1-fold over baseline in the 50/25, 100/50 and 200/100 IU/kg/h groups. The greatest change in coagulation parameters was observed in thrombin time, which increased 17-, 21- and 70-fold over baseline after 90 min of drug infusion. Activated clotting times at 90 min increased 1.1-, 1.4- and 1.7-fold over baseline values in the three respective groups. There was no effect on prothrombin time in any of the enoxaparin treatment groups.

3.4. Plasma concentration

Plasma concentrations increased in a dose-dependent manner in animals treated with CI-1031. Fig. 3 illustrates the plasma concentration-time profiles for each dose. Plasma concentrations were near steady-state by 90–150 min following the start of the infusion of the lower two doses of CI-1031, which has a half-life of approximately 1 h in conscious dogs (Subramanyam et al., 1998). In the high dose group, plasma concentration of CI-1031 gradually increased during the infusion.

In the arteries, the CI-1031 concentration associated with a 50% reduction in thrombus weight, as compared to control animals, was 407 ± 50 ng/ml. Thrombus weights in the veins tended to be more variable; approximately 50% thrombus weight reduction was achieved at concentrations ranging from 250 to 400 ng/ml.

3.5. Hemodynamic parameters

There were no significant differences in mean arterial blood pressure or heart rate among groups in the CI-1031-treated animals at baseline. Mean arterial blood pressure tended to rise slightly over the course of the study but there were no significant differences over time. Heart rate tended to be more variable and decreased in all groups by the end of the study. At the final timepoint of the study, there was a drop of 36 beats per minute (bpm) ($P < 0.05$ vs. baseline) in the control group compared to 45, 24

Table 2
Coagulation parameters with enoxaparin

Enoxaparin load/maintenance (IU/kg/h)								
	Baseline	30 min	60 min	90 min	150 min	210 min	270 min	330 min
APTT (s)								
Control	21.0 ± 1.8	24.4 ± 4.1	21.7 ± 2.9	21.0 ± 3.1	22.6 ± 2.8	22.0 ± 3.0	23.3 ± 3.9	23.8 ± 4.1
50/25	18.6 ± 0.6	20.7 ± 1.3	22.8 ± 1.6	22.8 ± 1.2	23.1 ± 1.5	24.3 ± 1.9	25.7 ± 2.3	22.3 ± 1.9
100/50	21.9 ± 0.6	28.0 ± 2.5	34.7 ± 1.8[a]	35.6 ± 2.4[a]	38.5 ± 3.3[a]	40.0 ± 3.8[a]	42.6 ± 4.6[a,b]	44.2 ± 6.7[a,b]
200/100	23.3 ± 1.9	42.5 ± 5.6[a]	49.6 ± 10.8[a,b]	50.1 ± 7.8[a,b]	57.7 ± 11[a,b]	65.4 ± 13.8[a,b]	74.3 ± 4.6[a,b]	79.3 ± 12.3[a,b]
TT (s)								
Control	11.8 ± 0.5	15.7 ± 2.3	12.1 ± 0.3	11.9 ± 0.3	12.2 ± 0.4	12.2 ± 0.7	11.7 ± 0.4	11.9 ± 0.4
50/25	13 ± 0.5	19 ± 3.2	133 ± 106	217 ± 196	221 ± 195	104 ± 72	231 ± 191	41 ± 18
100/50	13 ± 0.5	37 ± 8.0	298 ± 176	281 ± 181	333 ± 171[a]	518 ± 203[a,b]	692 ± 198[a,b]	680 ± 203[a,b]
200/100	12 ± 0.5	180 ± 97	688 ± 191[a,b]	864 ± 135[a,b]	999 ± 0[a,b]	999 ± 0[a,b]	999 ± 0[a,b]	999 ± 0[a,b]
ACT (s)								
Control	77 ± 4	76 ± 3	69 ± 3	61 ± 3	68 ± 3	64 ± 4	63 ± 3	63 ± 3
50/25	82 ± 4	84 ± 4	89 ± 10	95 ± 10[b]	93 ± 10[b]	91 ± 10[b]	97 ± 9[b]	88 ± 9[b]
100/50	84 ± 4	90 ± 7	123 ± 4[a,b]	120 ± 5[a,b]	122 ± 5[a,b]	131 ± 6[a,b]	133 ± 8[a,b]	134 ± 14[a,b]
200/100	90 ± 4	117 ± 10[a,b]	144 ± 8[a,b]	155 ± 9[a,b]	155 ± 8[a,b]	154 ± 9[a,b]	154 ± 7[a,b]	158 ± 10[a,b]
PT (s)								
Control	7.3 ± 0.2	7.3 ± 0.2	7.4 ± 0.1	7.5 ± 0.1	7.6 ± 0.2	7.8 ± 0.3	7.8 ± 0.2	7.9 ± 0.4[a]
50/25	6.7 ± 0.2	6.7 ± 0.2	6.9 ± 0.2	6.9 ± 0.2	6.9 ± 0.2	7.0 ± 0.2	6.9 ± 0.5	6.5 ± 0.2[b]
100/50	6.4 ± 0.2	6.4 ± 0.3	6.6 ± 0.3	6.4 ± 0.3[b]	6.6 ± 0.3	6.7 ± 0.3[b]	6.8 ± 0.3	6.8 ± 0.3[b]
200/100	6.8 ± 0.2	6.8 ± 0.2	6.9 ± 0.2	7.0 ± 0.3	7.2 ± 0.3	7.8 ± 0.6[a]	8.1 ± 0.7[a]	8.5 ± 1.0[a]

APTT, activated partial thromboplastin time; TT, thrombin time; ACT, activated clotting time; PT, prothrombin time; 50/25, 100/50 and 200/100 load/maintenance IU/kg/h. The loading dose was given for 1 h followed by maintenance infusion for remainder of study. Enoxaparin was dissolved in saline and administered intravenously. Baseline, baseline values prior to the administration of enoxaparin or saline; 30, 60, 90,150, 210, 270 and 330 min, time in minutes after start of drug infusion.

$N = 5$ for each group.

Values are expressed as mean ± S.E.M.

[a] $P < 0.05$ vs. baseline.

[b] $P < 0.05$ vs. control.

($P < 0.05$ vs. baseline) and 37 bpm in the three CI-1031-treated groups.

Mean arterial blood pressure and heart rate in the enoxaparin-treated animals were similar among groups prior to the administration of drug. Enoxaparin had no significant effect on mean arterial pressure or heart rate at any timepoint in the study.

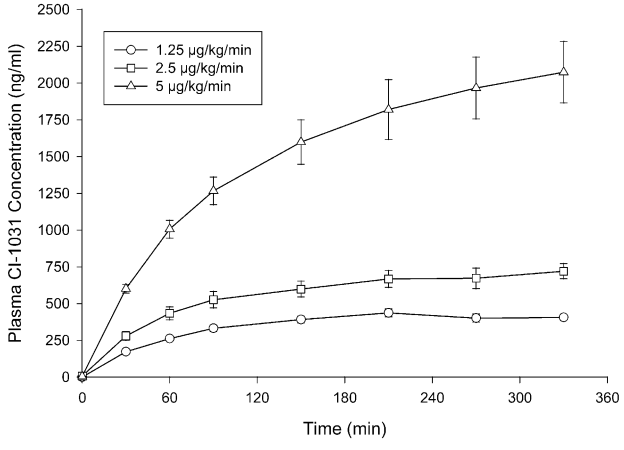

Fig. 3. Graphic illustration of the plasma concentration of CI-1031 over time.

4. Discussion

Inhibition of Factor Xa attenuates thrombin production while maintaining a level of thrombin activity necessary for primary hemostasis, thereby providing a potential advantage over other antithrombotics in regard to safety (Verstraete and Zoldhelyi, 1995; Nicolini et al., 1996). A number of experimental studies have generated support for Factor Xa inhibitors as a potential improvement in antithrombotic therapy. Vlasuk et al. (1991) demonstrated that the selective peptide Factor Xa inhibitors recombinant tick anticoagulant peptide (rTAP) and recombinant antistasin were as effective as heparin in preventing thrombus formation in a rabbit model of venous thrombosis. DX-9065a and YM-60828, non-peptide Factor Xa inhibitors, are efficacious in a variety of experimental models of arterial and venous thrombosis (Hara et al., 1995; Taniuchi et al., 1998). In addition, several studies have supported the

therapeutic potential of Factor Xa inhibitors as adjunctive treatment with tPA in models of arterial thrombolysis (Lynch et al., 1994; Lefkovits et al., 1996). Although efficacy with Xa inhibitors has been clearly demonstrated in a variety of animal models, few agents have advanced to the stage of clinical trials. The primary objective of this study was to test the efficacy of CI-1031 in an established large animal model of thrombosis.

The results of this study demonstrate that CI-1031 is an effective intravenous antithrombotic agent in a canine electrolytic injury model of thrombosis. CI-1031 dose-dependently prolonged the time to formation of an occlusive thrombus and reduced thrombus mass in both arteries and veins, with only modest changes in coagulation parameters. The maximal antithrombotic effect was observed at 5 μg/kg/min, where 7 of 10 vessels remained patent for the duration of the study (240 min). A plasma concentration of approximately 400 ng/ml was associated with a 50% reduction in thrombus weight relative to control animals.

In previous studies, CI-1031 was compared to a variety of other anticoagulants in experimental models of thrombosis. For example, Sullivan et al. (1998) found that CI-1031 had a better therapeutic ratio than warfarin in a rat venous stasis thrombosis model. Abendschein et al. (2000) demonstrated that CI-1031 (ZK-807834) also inhibited electrolytic injury-induced thrombus formation in the rabbit carotid artery at molar doses approximately 10- and 2-fold less than DX-9065a and rTAP, respectively. The relatively greater potency in vivo likely reflects the high affinity of CI-1031 for Xa ($K_i = 0.11$ nM), which compares very favorably with other small molecule Factor Xa inhibitors that have been tested in vivo (Hara et al., 1995; Taniuchi et al., 1998; Leadley et al., 1999; Heran et al., 2000; Wong et al., 2000). Interestingly, Abendschein et al. (2000) demonstrated that antithrombotic efficacy in a rabbit model of venous thrombosis can be achieved with CI-1031 at doses that do not prolong PT and APTT, unlike similar efficacious doses of ardeparin (a low molecular weight heparin) which prolonged APTT approximately 2-fold over baseline. The results of the current study indicate that significant antithrombotic effects can be achieved at doses of CI-1031 that prolong APTT 1.3- to 1.4-fold and PT 1.7- to 1.9-fold. Collectively, these data suggest that antithrombotic efficacy can be achieved with CI-1031 without producing marked systemic hypocoagulability. The higher levels of anticoagulation required in this study are likely due to the severity of the injury compared to the venous stasis model used by Abendschein et al. (2000).

Much greater concentrations are required for inhibition of thrombus formation in vivo as compared to concentrations required to inhibit amidolytic activity of Factor Xa in a plasma-free system. Similar large discrepancies have been observed for other Factor Xa inhibitors (Heran et al., 2000; Wong et al., 2000). Unlike in vitro buffer systems, plasma contains all of the components of the enzymatic pathway that lead to the local activation of Factors Xa and IIa. In addition, the severity of the injury and exposure of subendothelial matrix provide further hurdles for the drug to overcome in vivo. Also, CI-1031 does exhibit a species-dependent effect on purified Factor Xa and coagulation parameters in vitro. For example, the K_i's for inhibition of dog and human Factor Xa are 2.6 and 0.11 nM, respectively, and PT doubling concentrations in dog and human plasma are 1.0 and 0.23 μM, respectively (J.R. Morser, unpublished data). These species-dependent differences also contribute to the relatively high plasma concentrations required to inhibit thrombus formation in the dog electrolytic injury model.

A greater potential benefit/risk ratio of inhibiting Factor Xa, as opposed to other antithrombotic approaches, has been demonstrated in several studies of potent small molecule inhibitors of Factor Xa (Abendschein et al., 2000; Sato et al., 1998a,b; Wong et al., 2000; Heran et al., 2000). Accordingly, the secondary objective of this study was to compare CI-1031 with enoxaparin, a low molecular weight heparin that is the current leading clinical injectable anticoagulant for use in acute thrombotic indications. The doses of CI-1031 selected provided the same degree of antithrombotic efficacy as the selected doses of enoxaparin. However, bleeding in the CI-1031-treated animals was generally less compared to the enoxaparin groups. Enoxaparin doses were selected based on effective doses used in preclinical studies of this drug in similar animal models of thrombosis (Leadley et al., 1997). The maximally effective dose in this study was 100/50 IU/kg/h, which prolonged APTT approximately 1.7-fold over baseline. Other canine experiments used maximally effective doses that yielded 1.5- and 1.9-fold increases in APTT (Leadley et al., 1998). These doses also produced plasma anti-Xa levels of 2.2 and 2.6 IU/ml, much higher than the maximally tolerated plasma anti-Xa levels (approximately 1.4 IU/ml) determined in clinical studies with enoxaparin (TIMI 11A, 1997). Consequently, the doses of enoxaparin required for maximal efficacy in the current study were relatively high compared to the accepted clinical dosing regimen. It is difficult to make appropriate comparisons between CI-1031 and enoxaparin because the maximally tolerated dose of CI-1031 is not known and the appropriate pharmacodynamic markers for monitoring safety and efficacy of direct Factor Xa inhibitors have yet to be determined. Regardless, the present data indicate that a maximally effective antithrombotic dose of CI-1031 can be administered without markedly altering routine measurements of systemic coagulation (APTT, TT, ACT and PT) or markers of primary hemostasis (blood loss and template bleeding time).

In conclusion, the results of this study demonstrate that CI-1031, a potent and selective inhibitor of Factor Xa, is an effective antithrombotic agent in a canine model of thrombosis. CI-1031 compared favorably with enoxaparin, a safe

and effective clinical anticoagulant which has been utilized extensively. These results add further support to the theory that Xa inhibitors will afford a greater therapeutic index over conventional anticoagulants, a hypothesis that will be tested as compounds such as CI-1031 advance through clinical trials.

References

Abendschein, D.R., Baum, P.K., Martin, D.J., Vergona, R., Post, J., Rumennik, G., Sullivan, M.E., Eisenberg, P.R., Light, D.R., 2000. Effects of ZK-807834, a novel inhibitor of factor Xa, on arterial and venous thrombosis in rabbits. J. Cardiovasc. Pharmacol. 35, 796–805.

Al-Obeidi, F., Ostrem, J.A., 1999. Factor Xa inhibitors. Exp. Opin. Ther. Patients 9, 931–953.

Brener, S.J., 2000. Unfractionated and low-molecular-weight heparins in acute coronary syndromes: current recommendations. Cleveland Clin. J. Med. 67, 59–65.

Derendorf, H., Hochaus, G. (Eds.), 1995. Handbook of Pharmacokinetic/Pharmacodynamic Correlation. CRC Press, Boca Raton, FL, pp. 10–14.

Gardell, S.J., Sanderson, E.J., 1998. Novel anticoagulants based on direct inhibition of thrombin and factor Xa. Coron. Artery Dis. 9, 75–81.

Hara, T., Yokoyama, A., Morishima, Y., Kunitada, S., 1995. Species differences in anticoagulant and anti-Xa activity of DX-9065a, a highly selective factor Xa inhibitor. Thromb. Res. 80, 99–104.

Heran, C., Morgan, S., Kasiewski, C., Bostwick, J., Bentley, R., Klein, S., Chu, V., Brown, K., Colussi, D., Czekaj, M., Perrone, M., Leadley, R., 2000. Antithrombotic efficacy of RPR208566, a novel factor Xa inhibitor, in a rat model of carotid artery thrombosis. Eur. J. Pharmacol. 389, 201–207.

Leadley, R.J., Kasiewski, C.J., Bostwick, J.S., Bentley, R., McVey, M.J., White, F.J., Perrone, M.H., Dunwiddie, C.T., 1997. Comparison of enoxaparin, hirulog, and heparin as adjunctive therapy during thrombolysis with rtPA in the stenosed canine coronary artery. Thromb. Haemost. 78, 1278–1285.

Leadley, R.J., Kasiewski, C.J., Bostwick, J.S., Bentley, R., Dunwiddie, C.T., Perrone, M.H., 1998. Inhibition of repetitive thrombus formation in the stenosed canine coronary artery by enoxaparin, but not by unfractionated heparin. Arterioscler. Thromb. Vasc. Biol. 18, 908–914.

Leadley, R.J., Morgan, S.R., Bentley, R., Bostwick, J.S., Kasiewski, C.J., Heran, C., Chu, V., Brown, K., Moxey, P., Ewing, W.R., Pauls, H., Spada, A.P., Perrone, M.H., Dunwiddie, C.T., 1999. Pharmacodynamic activity and antithrombotic efficacy of RPR120844, a novel inhibitor of coagulation factor Xa. J. Cardiovasc. Pharmacol. 34, 791–799.

Lefkovits, J., Malycky, J.L., Rao, J.S., Hart, C.E., Plow, E.F., Topol, E.J., Nicolini, F.A., 1996. Selective inhibition of factor Xa is more efficient than factor VIIa-tissue factor complex blockade at facilitating coronary thrombolysis in the canine model. J. Am. Coll. Cardiol. 28, 1858–1865.

Lynch, J.L., Sitko, G.R., Mellott, M.J., Nutt, E.M., Lehman, E.D., Friedman, P.A., Dunwiddie, C.T., Vlasuk, G.P., 1994. Maintenance of canine coronary artery patency following thrombolysis with front loaded plus low dose maintenance conjunctive therapy. A comparison of factor Xa versus thrombin inhibition. Cardiovasc. Res. 28, 78–85.

Nicolini, F., Lee, P., Malycky, L., Lefkovits, J., Kottke-Marchant, K., Plow, E., Topol, E., 1996. Selective inhibition of factor Xa during thrombolytic therapy markedly improves coronary artery patency in a canine model of coronary thrombosis. Blood Coagulation Fibrinolysis 7, 39–48.

Phillips, G.B., Buckman, B.O., Davey, D.D., Eagen, K.A., Guilford, W.J., Hinchman, J., Ho, E., Koovakkat, S., Liang, A., Light, D.R., Mohan, R., Ng, H.P., Post, J.M., Shaw, K.J., Subramanyam, B., Sullivan, M.E., Trinh, L., Vergona, R., Walters, J., White, K., Whitlow, M., Wu, S., Xu, W., Morrissey, M.M., 1998. Discovery of *N*-[2-[5-[Amino (imino)-methyl]-2-hydroxyphenoxy]-3,5-difluoro-6-[3-(4,5-dihydro-1-methyl-1*H*-imidazol-2-yl)phenoxy]pyridin-4-yl]-*N*-methylglycine (ZK-807834): a potent selective, and orally active inhibitor of the blood coagulation factor Xa. J. Med. Chem. 41, 3557–3562.

Sato, K., Kaku, S., Hirayama, F., Koshio, H., Matsumoto, Y., Kawasaki, T., Iizumi, Y., 1998a. Antithrombotic effect of YM-75466 is seperated from its effect on bleeding tiome and coagulation time. Eur. J. Pharmacol. 352, 59–63.

Sato, K., Taniuchi, Y., Kawasaki, T., Hirayama, F., Koshio, H., Matsumoto, Y., 1998b. Relationship between the antithrombotic effect of YM-75466, a novel factor Xa inhibitor, and coagulation parameters in rats. Eur. J. Pharmacol. 347, 231–236.

Subramanyam, B., Ho, E., Walters, J., Cheesman, S., Guilford, W., Vergona, R., White, K., Philips, G., Davey, D., Morrissey, M., Light, D., Morset, J., Sullivan, M., 1998. The pharmacokinetics of BX-807834, a potent, selective factor Xa inhibitor in animals. FASEB J. 12, A719 (abstract no. 4169).

Sullivan, M., Morser, J., Light, D., Guilford, W., Vergona, R., Herrman, M., White, K., Philips, G., Davey, D., Morrissey, M., Fredrich, M., Baldus, B., 1998. The efficacy and safety of BX-807834, a potent, selective factor Xa inhibitor, in animal models. FASEB J. 12, 719 (abstract no. 4171).

Taniuchi, Y., Sakai, Y., Hisamichi, N., Kayama, M., Mano, Y., Sato, K., Koshio, H., Matsumoto, Y., Kawasaki, T., 1998. Biochemical and pharmacological characterization of YM-60828, a newly synthesized and orally active inhibitor of human factor Xa. Thromb. Haemost. 79, 543–548.

Thrombolysis in Myocardial Infarction (TIMI) 11A Trial Investigators, 1997. Dose-ranging trial of enoxaparin for unstable angina: results of TIMI 11A. J. Am. Coll. Cardiol. 29, 1474–1482.

Verstraete, M., Zoldhelyi, P., 1995. Novel antithrombotic drugs in development. Drugs 49, 856–884.

Vlasuk, G.P., Ramjit, D., Fujita, T., Dunwiddie, C.T., Nutt, E.M., Smith, D.E., Shebuski, R.J., 1991. Comparison of the in vivo anticoagulant properties of standard heparin and the highly elective factor Xa inhibitors antistasin and tick anticoagulant peptide (TAP) in a rabbit model of venous thrombosis. Thromb. Haemost. 65, 257–262.

Weitz, J.I., 1997. Low-molecular weight heparins. N. Engl. J. Med. 337, 688–698.

Weitz, J.I., Hirsh, J., 1998. New antithrombotic agents. Chest 114, 715S–727S.

Wong, P.C., Quan, M.L., Crain, E.J., Watson, C.A., Wexler, R.R., Knobb, R.M., 2000. Nonpeptide factor Xa inhibitors: 1. Studies with SF303 and SK549, a new class of potent antithrombotics. J. Pharmacol. Exp. Ther. 292, 351–357.

Zed, P., 1999. Low-molecular weight heparin should replace unfractionated heparin in the management of acute coronary syndromes. J. Thromb. Thrombolysis 8, 79–87.