A Novel Synthetic Inhibitor of Factor Xa Decreases Early Reocclusion and Improves 24-h Patency after Coronary Fibrinolysis in Dogs

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ABSTRACT

Inhibition of factor Xa (FXa) attenuates thrombus progression. This study was designed to determine whether a novel, synthetic inhibitor of FXa (ZK-807834, molecular mass 527 Da, \( K_I = 0.11 \text{ nM} \)) administered during and briefly after pharmacologic coronary fibrinolysis increases 24-h patency. Either ZK-807834 (\( 1.6 \text{ mg/kg, } n = 10; 6.5 \text{ mg/kg, } n = 8 \); or 13 mg/kg, \( n = 7 \)); a peptide inhibitor of FXa, recombinant tick anticoagulant peptide (rTAP, 13.6 mg/kg, \( n = 7 \)); heparin (150 U/kg bolus and 50 U/kg/h infusion) and aspirin (5 mg/kg, \( n = 7 \)); or saline as a control (\( n = 13 \)) were administered i.v. over 135 min in conscious dogs after thrombotic occlusion induced by electrical injury to a coronary artery. Fibrinolysis was induced with recombinant human tissue-type plasminogen activator (1.0 mg/kg i.v. over 1 h), and patency was monitored continuously for 24 h with an implanted Doppler probe. Reocclusion occurred in all control and heparin/aspirin-treated dogs within 1 h after fibrinolysis. High dose ZK-807834 prevented reocclusion in five of six dogs and delayed reocclusion in the other dog (186 min after recanalization, \( p = 0.0005 \) versus heparin/aspirin). Reocclusion was delayed (406 ± 329 min), but still occurred in three of six rTAP-treated dogs (\( p = 0.003 \) versus heparin/aspirin). Patency after 24 h was 100% in ZK-807834-treated and rTAP-treated dogs compared with 67% in control and 83% in heparin/aspirin-treated dogs. PT was increased 3.7-fold, activated partial thromboplastin time 4.9-fold, and bleeding time 2.5-fold by high dose ZK-807834 compared with 1.2-fold, 11.5-fold, and 2.3-fold, respectively, for heparin/aspirin. Inhibition of FXa with ZK-807834 decreases reocclusion and improves patency of recanalized arteries without increasing bleeding compared with heparin/aspirin.

Pharmacologic fibrinolysis for treatment of acute myocardial infarction is limited by inadequate coronary recanalization in a significant minority of patients and by early thrombotic reocclusion in others despite administration of heparin (Lincoff and Topol, 1993; Verheugt et al., 1996). This reflects, in part, a procoagulant response and thrombin generation during fibrinolysis (Eisenberg et al., 1992). However, the optimal approach to inhibit procoagulant activity is still debated.

Thrombin has been the primary target to attenuate the procoagulant response associated with fibrinolysis, because it activates platelets, converts fibrinogen to fibrin, and binds to clots. Because clot-bound thrombin is resistant to inhibition by antithrombin III-dependent inhibitors like heparin (Weitz et al., 1990), considerable research was directed toward development of antithrombin III-independent thrombin inhibitors such as recombinant desulfatohirudin (hirudin) and hirulog to replace heparin. Nevertheless, although studies in experimental animals have shown that direct antithrombins given conjunctively with fibrinolytic agents improve acute patency compared with heparin (Haskel et al., 1991; Yao et al., 1992); a narrow therapeutic window and bleeding at potentially efficacious doses in patients appear to limit their application (Antman et al., 1994; Théroux et al., 1995). Moreover, persistent thrombin generation despite inhibition of thrombin activity with hirudin (Zoldhelyi et al., 1994) and recurrence of thrombosis (or “rebound”) after discontinuation of inhibitors (Théroux et al., 1992; Granger et al., 1995) have raised doubt that antithrombins will be effective to facilitate fibrinolysis and maintain the patency of recanalized arteries.

More recent studies in vitro have shown that activated factor X (FXa) comprises the majority of the procoagulant

ABBREVIATIONS: FXa, activated factor X; rTAP, recombinant tick anticoagulant peptide; PT, prothrombin time; aPTT, activated partial thromboplastin time; ZK-807834, N-[2-[5-[amino(methyl)iminio]-2-hydroxyphenoxo]-3,5-difluoro-6-[3-(4,5-dihydro-1-methyl-1H-imidazol-2-yl)phenoxo][pyridin-4-yl]N-methylglycine; rTFPI, recombinant tissue factor pathway inhibitor.
activity on the surface of thrombi and that specific inhibition of FXa attenuates thrombus progression (Eisenberg et al., 1993; Prager et al., 1995; McKenzie et al., 1996). Thus, FXa bound to platelets or fibrin could account for persistent generation of thrombin despite antithrombin treatment. Studies in experimental animals have shown also that antithrombin III-independent peptide inhibitors of FXa decrease coronary reocclusion acutely after successful fibrinolysis (Sitko et al., 1992; Lynch et al., 1994; Nicolini et al., 1996). Whether this approach achieves persistent coronary patency after stopping the administration of inhibitors has not been addressed.

The goal of these experiments was to determine whether inhibition of FXa during and for a brief interval after coronary fibrinolysis results in persistent patency over the first 24 h. We compared the efficacy of two FXa inhibitors: a novel 2,6-diphenoxypyridine designated ZK-807834 (also designated CI-1031), which has a $K_i$ against human free FXa of 0.11 nM (Phillips et al., 1998) and was shown to attenuate arterial and venous thrombosis in rabbits (Abendschein et al., 2000); and the prototype FXa inhibitor, recombinant tick anticoagulant peptide (rTAP), which has a $K_i$ against human free FXa of 0.18 nM and was shown to increase patency up to 180 min after coronary fibrinolysis in dogs (Neepher et al., 1990; Sitko et al., 1992; Lynch et al., 1994; Nicolini et al., 1996). The efficacy of FXa inhibition to maintain patency after fibrinolysis was compared with conventional treatment using heparin and aspirin, and with infusions of saline vehicle as controls in a well established preparation of platelet-rich thrombosis induced by electrical vascular injury in conscious dogs with continuous monitoring of coronary blood flow over 24 h (Romson et al., 1980; Haskel et al., 1991; Sitko et al., 1992; Lynch et al., 1994; Nicolini et al., 1996).

Materials and Methods

Antithrombotic Agents. The preparation of ZK-807834 has been described previously (Phillips et al., 1998). rTAP was produced from a synthetic gene made with five overlapping oligonucleotides (three 90-mers and two 60-mers; G. Rumennik, K. McLean, J.-H. Lin, and M. Wang, unpublished data), secreted in Pichia pastoris using commercial reagents (Invitrogen, Carlsbad, CA), and purified as reported (Laroché et al., 1994). SDS-polyacrylamide gel electrophoresis and N-terminal sequence analysis confirmed the purity of rTAP. Aspirin used was lysine acetylsalicylic acid (Aspegic, Laboratoires Synthelabo, Paris, France), a water-soluble analog of aspirin.

Animal Preparations. All procedures in animals were in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996) and were approved by the Animal Studies Committee at Washington University. Male, mongrel dogs weighing 18 to 25 kg were fasted for 12 h and premedicated with acepromazine maleate (0.01 mg/kg, s.c.) and atropine sulfate (0.04 mg/kg, s.c.). Anesthesia was induced with pentothal sodium (15 mg/kg, i.v.) and maintained with 2% isoflurane in oxygen-enriched room air delivered by a mechanical ventilator through a cuffed endotracheal tube.

The heart was exposed through a left thoracotomy and the anterior descending coronary artery was instrumented as described previously (Haskel et al., 1991). Briefly, a Doppler flow probe was placed on the artery proximally and a 23-gauge needle electrode was inserted transmurally into the vessel distal to the Doppler probe. The electrode lead wire and Doppler wires were tunneled through the chest wall and under the skin between the scapulae. The chest incision was closed in layers and evacuated. The dogs were given penicillin-G (30,000 U/kg s.c.) daily for 3 days to prevent infection and buprenorphine (0.05 mg/kg i.m.) as needed for pain.

Experiment Protocol. Five to 7 days after surgery, the dogs were fasted for 12 h and given morphine sulfate (0.4 mg/kg i.v., followed by 0.2 mg/kg boluses as needed for apparent discomfort). The dogs were placed in a support sling and the Doppler and electrode wires exposed through an incision in the skin. Phasic and mean coronary flow velocities from the Doppler probe and the ECG were monitored continuously on a chart recorder (Gould 4300, Cleveland, OH). An 18-gauge catheter was inserted into a cephalic vein for blood sampling and fluid replacement (0.9% NaCl at 5 ml/kg/h). Coronary thrombosis was induced by application of 250 μA of direct, anodal current through the transmural electrode with a wire sutured to the skin to complete the electrical circuit. The current was increased by 50 μA after the 1st h and then every 30 min (up to 500 μA maximum) thereafter until complete thrombotic occlusion was achieved ($t = 0$) (Fig. 1) identified by zero flow velocity on the Doppler flow tracing. Bolus injections of lidocaine HCl (30 mg i.v.) were administered as needed to attenuate high-grade tachyarrhythmias.

Dogs were randomly assigned to receive ZK-807834, rTAP, heparin and aspirin, or saline as a control beginning 45 min after thrombotic occlusion (Fig. 1). Antithrombotics were dissolved in saline (20 ml) and administered as an i.v. bolus and constant infusion for 135 min (Table 1). The lowest dosages of ZK-807834 (0.3–1.6 mg/kg) were selected initially based on our data showing inhibition of arterial thrombosis in rabbits (Abendschein et al., 2000). However, because the potency of ZK-807834 against dog FXa was nearly 10-fold lower than for rabbit FXa (D. R. Light, unpublished data), selected higher dosages were also assessed. The dosage of rTAP was reported previously (Sitko et al., 1992; Lynch et al., 1994). The dosages of heparin (150 U/kg bolus and 50 U/kg/h infusion) and aspirin (5 mg/kg bolus) were comparable to those used clinically (Haskel et al., 1991). Fifteen minutes after the start of the infusion of antithrombotic or saline, human recombinant tissue-type plasminogen activator (rt-PA, 1 mg/kg, Genentech, South San Francisco, CA) was infused i.v. over 1 h. Recanalization was defined as a return of mean flow velocity to at least 50% of the baseline value. After recanalization, blood flow velocity was monitored continuously for 24 h to detect the occurrence of reocclusion (defined as zero flow velocity persisting for at least 1 min).

Coagulation Assays and Bleeding Time. Blood samples were collected in 3.8% sodium citrate (1 part citrate to 9 parts blood) for assay of prothrombin time (PT) and activated partial thromboplastin time (aPTT) in plasma at baseline (before application of electric current to the coronary) and 45, 60, and 180 min after complete thrombotic occlusion (Fig. 1). Bleeding time was also measured at 45, 60, and 180 min after occlusion with the 45-min measurement, before infusions of antithrombotics, serving as a baseline.

PT and aPTT were analyzed in plasma samples with use of a Coag-A-Mate XM automated coagulation timer (Organon Teknika, Durham, NC) and reagent kits (Simplastin Excel for PT and Automated aPTT reagents, Organon Teknika). The time for clot formation (seconds) in duplicate samples was averaged. The maximal value for aPTT was recorded as 150 s.

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![Fig. 1. Experiment protocol](image-url)
Inhibition of Factor Xa Improves Patency after Fibrinolysis

Time and incidence of initial coronary occlusion, recanalization, and reocclusion

TABLE 2
Time and incidence of initial coronary occlusion, recanalization, and reocclusion

<table>
<thead>
<tr>
<th>Conjointive Agent</th>
<th>Occlusion</th>
<th>Recanalization</th>
<th>Reocclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time after Onset of Electrical Injury</td>
<td>n</td>
<td>Time after Start of Infusion of rt-PA</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td></td>
<td>min</td>
</tr>
<tr>
<td>Control</td>
<td>116 ± 65</td>
<td>13</td>
<td>33 ± 12</td>
</tr>
<tr>
<td>ZK-807834 ≤1.6 mg/kg</td>
<td>152 ± 80</td>
<td>10</td>
<td>26 ± 17</td>
</tr>
<tr>
<td>6.5 mg/kg</td>
<td>127 ± 98</td>
<td>8</td>
<td>36 ± 16</td>
</tr>
<tr>
<td>13.0 mg/kg</td>
<td>109 ± 32</td>
<td>7</td>
<td>17 ± 16</td>
</tr>
<tr>
<td>rTAP</td>
<td>79 ± 87</td>
<td>7</td>
<td>21 ± 14</td>
</tr>
<tr>
<td>Hep/ASA</td>
<td>156 ± 56</td>
<td>7</td>
<td>28 ± 27</td>
</tr>
</tbody>
</table>

rTAP, recombinant tick anticoagulant peptide (13.6 mg/kg); Hep/ASA, heparin (150 U/kg bolus, 50 U/kg/h/aspirin (5 mg/kg).

p = 0.0005 compared with heparin/aspirin and 0.0004 compared with control dogs by survival analysis using a logrank test considering both the incidence and time of reocclusion.

p = 0.003 compared with heparin/aspirin and 0.002 compared with control.

**Results**

Among the 56 animals subjected to electrical vascular injury, 52 exhibited thrombotic occlusion approximately 2 h after the onset of electrical injury and were randomized to the conjunctive treatment groups (Table 2).

**Effects of Conjointive Antithrombotic Agents on Recanalization and Reocclusion.** Fibrinolysis and coronary recanalization occurred in all but three control dogs and three low dose ZK-807834-treated dogs approximately 30 min after the start of the infusion of rt-PA (Table 2). Recanalization trended to be accelerated in dogs given the 13 mg/kg dose of ZK-807834 together with rt-PA, but the difference compared with controls was not significant (p = 0.06).

Coronary reocclusion occurred in all control dogs within the 1st h after recanalization (Table 2). Recocclusion also occurred universally and rapidly despite administration of the lowest dosages (≤1.6 mg/kg) of ZK-807834 or heparin/aspirin, although the time to reocclusion was prolonged slightly by heparin/aspirin. In contrast, 13 mg/kg ZK-807834 prevented reocclusion in five of six dogs and prolonged the time to reocclusion in the other dog (p = 0.0005 compared with heparin/aspirin for incidence and time of reocclusion) (Table 2). The 6.5 mg/kg dose of ZK-807834 reduced the incidence of reocclusion and increased the delay to the onset of reocclusion more modestly (p = 0.05 compared with heparin/aspirin). Reocclusion occurred in three of six dogs given rTAP, but was delayed to 406 ± 329 min after recanalization (p = 0.003 compared with heparin/aspirin for incidence and time of reocclusion).

**Coronary Patency over 24 h.** Patency assessed by continuous recording of the Doppler flow profiles was restored and maintained after 15 h in the one dog given the highest dosage of ZK-807834 that exhibited earlier reocclusion (Fig. 2). Patency was also restored within 15 h in the one surviving rTAP-treated animal exhibiting reocclusion and in several of the dogs given heparin/aspirin or saline conjunctively with rt-PA. Nevertheless, the percentage of time when coronary vessels were patent trended to be higher in dogs given the highest dosage of ZK-807834 (91 ± 21% of the first 24 h) compared with saline-treated controls (60 ± 39% of 24 h, p = 0.1). In addition, maximal coronary flow velocity after 24 h trended to be higher in dogs given inhibitors of factor Xa conjunctively with rt-PA (88 ± 29% of baseline velocity for ZK-807834 and 94 ± 13% of baseline velocity for rTAP) compared with those given either heparin/aspirin (72 ± 45% of baseline velocity) or saline (67 ± 52% of baseline velocity).

**Coagulation and Bleeding.** A dose-dependent increase in PT was observed with ZK-807834 (Table 3). At the 13 mg/kg dose, PT was increased to 3.7 ± 0.6-fold baseline by 15 min after the start of the infusion and to 4.3 ± 0.3-fold baseline at the end of the infusion. PT was increased more modestly with rTAP achieving levels 1.6 ± 0.3-fold baseline by the end of the infusion (Table 3). aPTT was also increased with ZK-807834, but the differences from baseline were significant only at the end of the infusion (6.1 ± 2.5-fold baseline, Table 3). As expected, heparin increased aPTT markedly early after the bolus (11.5 ± 6.2-fold baseline after 15 min).

Bleeding time increased dose dependently with ZK-807834, but was different from baseline only 15 min after the bolus and start of infusion of the 13 mg/kg dose (2.5 ± 1.5-fold baseline, Table 3). Bleeding time for rTAP was also increased significantly only 15 min after the start of the infusion (2.9 ± 1.0-fold baseline), although it trended toward a more marked, albeit slight, increase compared with baseline.

**Statistical Analysis.** Results are expressed as the mean ± S.D. Recanalization times, coronary patency, and flow velocity were compared among groups with analysis of variance and an unpaired Student’s t test for specific contrasts. The incidence and time of onset of reocclusion was compared with use of a logrank test. Multiple analysis of variance was used for comparisons of hemalogic variables over time in the various treatment groups. A value of p ≤ 0.05 was considered significant.

Bleeding time was measured with use of a spring-loaded blade (Simpplate II, Organon Teknika) applied to the dog’s inner lip. The interval until the bleeding stopped was recorded as the bleeding time.

Dissolved in 20 ml of saline and delivered over 135 min.

* Dissolved in 20 ml of saline and delivered over 135 min.

a Other animals with recanalization were excluded because of ventricular fibrillation.
taries; closed bars, occluded arteries; crosshatched bars, animals that died.

Fig. 2. Coronary patency after recanalization (reperfusion) assessed by Doppler flow velocity profiles in individual dogs. Open bars, patent arteries; closed bars, occluded arteries; crosshatched bars, animals that died.

Discussion

Our results show that direct inhibition of FXa during coronary thrombolysis in dogs decreases the incidence of early reocclusion of the recanalized arteries and improves patency over the first 24 h compared with results in dogs given either heparin/aspirin or saline as controls (Fig. 2 and Table 2). Thrombotic reocclusion occurred in all control dogs within the 1st h after recanalization. Although much higher than the average rate of 11% reported for clinical trials (Verheugt et al., 1996), universal reocclusion is typical for this very robust prothrombotic canine preparation facilitating studies of inhibitors (Haskel et al., 1991; Sitko et al., 1992; Lynch et al., 1994; Nicolini et al., 1996). Arterial injury induced electrically has been shown to result in tissue factor-mediated coagulation (Speidel et al., 1996), and deposition of platelet-rich occlusive thrombi analogous to those observed clinically during acute myocardial infarction (Haskel et al., 1991). In contrast to universal reocclusion in controls, however, conjunctive administration of ZK-807834 delayed the onset of reocclusion, if it occurred, until after the plasma clearance of inhibitor (\(t_\text{1/2}\) of 1.5 to 2 h for ZK-807834 (Phillips et al., 1998), and 30 to 60 min for rTAP (Lynch et al., 1994)). These results suggest that thrombogenicity of the residual thrombus/arterial wall was attenuated by short-term administration of FXa inhibitors leading to pharmacologic passivation of the injury site accounting for increased patency. A similar response has been reported on artificial grafts treated with rTAP for 2 h resulting in decreased thrombus accumulation over the next 3.5 days (Kotze et al., 1997).

The finding that inhibition of FXa alone was sufficient to maintain the patency of recanalized coronary arteries is consistent with observations that FXa, and not thrombin, is primarily responsible for the procoagulant activity on the surface of thrombi and accelerated thrombogenesis during fibrinolysis (Eisenberg et al., 1993; Prager et al., 1995; McKenzie et al., 1996). Thus, although the thrombin generated from FXa converts fibrinogen to fibrin, activates platelets, and synergizes its own activation through activation of factors V and VIII (Pieters et al., 1989), attenuation of the procoagulant response to fibrinolysis by inhibiting thrombin will not be as efficient as inhibiting FXa and will require higher dosages of inhibitors [approximately 10-fold higher judging from inhibition of the same thrombogenic effect in vitro (Gitel et al., 1977)], and maintenance of inhibition until FXa is no longer accessible to circulating thrombomycin. Our own results with heparin as well as clinical trials showing rebound thrombosis after discontinuation of heparin administration and high dosage requirements for direct inhibitors of thrombin used conjunctively during fibrinolysis, confirm this hypothesis (Théroux et al., 1992, 1995; Antman et al., 1994; Granger et al., 1995). In addition, several previous preclinical studies have shown that inhibition of FXa is superior to direct inhibition of thrombin for increasing short-term patency after thrombolysis (Sitko et al., 1992; Lynch et al., 1994; Nicolini et al., 1996).

Our results with direct inhibition of FXa appear superior as well to those reported with inhibitors of the complex of tissue factor and FVIIa that activates factor X to FXa (Abendschein et al., 1995; Lefkovits et al., 1996). Lefkovits et al. (1996) have shown that conjunctive administration of either recombinant tissue factor pathway inhibitor (rTFPI), the mimic of the physiological inhibitor of the complex of tissue factor and factor VIIa that also inhibits FXa, or an inactivated form of FVIIa that binds tissue factor but cannot catalyze activation of factor X, resulted in similar reocclusion rates over 2 h compared with controls (67, 78, and 70%, respectively), whereas rTAP universally prevented reoclu-
tion. In contrast, we reported that rTFPI administered during fibrinolysis in dogs increased 24-h patency (four of six dogs exhibited continuous patency over 24 h) (Abendschein et al., 1995) but required high plasma concentrations of rTFPI in the range of 4 to 5 μg/ml, suggesting that the effect may have resulted primarily from inhibition of FXa (Broze et al., 1988). Compared with direct inhibition of FXa in the present study, inhibition of FXa generation does not appear as effective for maintenance of coronary patency possibly, because the main source of FXa is already bound to fibrin and re-exposed during fibrinolysis.

It is interesting that all animals given a FXa inhibitor conjunctively during fibrinolysis exhibited patent arteries after 24 h despite earlier reocclusion in some (Fig. 2). In contrast, patency was 83% in heparin/aspirin-treated animals and only 67% in saline control animals. The restoration of patency among animals initially exhibiting reocclusion is probably a manifestation of increased intrinsic fibrinolytic activity, which is known to be well developed in dogs and is induced by platelet-rich thrombosis (Lang et al., 1993). Thus, inhibition of FXa appears to decrease thrombogenesis facilitating intrinsic recanalization.

Another noteworthy finding in our study was that the high dose of ZK-807834 trended to accelerate the time of onset of recanalization (from 33 ± 12 min in controls to 17 ± 16 min in ZK-807834-treated dogs, p < 0.06). Accelerated recanalization has been reported previously with rTAP when rt-PA was administered over shorter intervals analogous to the front-loaded regimen employed currently in many centers (Abendschein, 1996; Nicolini et al., 1996). This suggests that ZK-807834 may more markedly accelerate recanalization when given with front-loaded rt-PA.

Importantly, the highest dosage of ZK-807834 shown to optimize patency did not potentiate bleeding compared with conventional treatment with heparin and aspirin (Table 3). Bleeding time for ZK-807834 was increased 2.5-fold compared with 2.3-fold for heparin/aspirin.

Although preprocedural measurements of template bleeding time as well as PT and aPPT cannot predict the risk of peri-procedural bleeding in the clinic (Gewirtz et al., 1996; Peterson et al., 1998), these measurements have been useful to monitor the likelihood of bleeding during anticoagulation therapy (Suchman and Griner, 1986; Charney et al., 1988).

Thus, absence of marked bleeding in the well controlled preclinical model reported here suggests that anticoagulation with ZK-807834 may be well tolerated in place of heparin during coronary thrombolysis in patients.

In summary, we have shown that brief administration of inhibitors of FXa during fibrinolysis appears to result in pharmacologic passivation of the residual thrombus and injured artery leading to persistence of arterial patency. Conjunctive administration of the synthetic FXa inhibitor ZK-807834 was particularly effective compared with rTAP or conventional heparin/aspirin without markedly increasing the bleeding time. Accordingly, based on the efficacy and safety observed in this animal preparation, clinical testing of ZK-807834 appears warranted.

Acknowledgments

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References


Granger CB, Miller JM, Bovill EG, Gruber A, Tracy RP, Krucow MF, Green C, Berrios E, Harrington RA, Ohman EM and Califf RM (1996) Rebound increase in

TABLE 3

Prothrombin time (PT), activated partial thromboplastin time (aPTT), and bleeding time (BT) in control and antithrombotic-treated animals

<table>
<thead>
<tr>
<th>Conjunctive Agent</th>
<th>Time after Onset of Infusion (Time after Coronary Occlusion)</th>
<th>PT (s)</th>
<th>aPTT (s)</th>
<th>BT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 (45)</td>
<td>7.9 ± 0.6</td>
<td>9.6 ± 0.9</td>
<td>116 ± 22</td>
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<tr>
<td></td>
<td>15 (60)</td>
<td>7.7 ± 0.8</td>
<td>10.2 ± 1.9</td>
<td>92 ± 37</td>
</tr>
<tr>
<td></td>
<td>135 (180)</td>
<td>8.2 ± 0.8</td>
<td>10.5 ± 1.4</td>
<td>109 ± 13</td>
</tr>
<tr>
<td>ZK-807834 (13 mg/kg)</td>
<td>0 (45)</td>
<td>8.1 ± 0.4</td>
<td>11.1 ± 5.6</td>
<td>111 ± 36</td>
</tr>
<tr>
<td></td>
<td>15 (60)</td>
<td>30.0 ± 4.6*</td>
<td>46.9 ± 9.2</td>
<td>264 ± 189*</td>
</tr>
<tr>
<td></td>
<td>135 (180)</td>
<td>34.7 ± 3.4*</td>
<td>58.6 ± 13.0*</td>
<td>213 ± 99</td>
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<tr>
<td>rTAP</td>
<td>0 (45)</td>
<td>7.5 ± 0.9</td>
<td>9.8 ± 2.1</td>
<td>87 ± 23</td>
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<td>15 (60)</td>
<td>10.3 ± 2.2</td>
<td>14.6 ± 2.5</td>
<td>246 ± 79*</td>
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<td></td>
<td>135 (180)</td>
<td>11.8 ± 2.7*</td>
<td>23.9 ± 19.4</td>
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<td>Hep/ASA</td>
<td>0 (45)</td>
<td>8.6 ± 2.8</td>
<td>9.3 ± 2.8</td>
<td>71 ± 13</td>
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<td>15 (60)</td>
<td>9.6 ± 1.6</td>
<td>103.7 ± 52.5*</td>
<td>158 ± 73</td>
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<td>135 (180)</td>
<td>9.2 ± 1.7</td>
<td>24.3 ± 14.0</td>
<td>132 ± 16</td>
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</table>

rTAP, recombinant tick anticoagulant peptide (13.6 mg/kg); Hep/ASA, heparin (150 U/kg bolus, 50 U/kg/h/aspirin (5 mg/kg).

* p < 0.05 compared with t = 0.


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