Nonpeptide Factor Xa Inhibitors: I. Studies with SF303 and SK549, a New Class of Potent Antithrombotics

PANCRA S C. WONG, MIMI L. QUAN, EARL J., CRAIN, CAROL A. WATSON, RUTH R. WEXLER, and ROBERT M. KNABB

Cardiovascular Diseases Research (P.C.W., E.J.C., C.A.W., R.M.K.) and Chemical and Physical Sciences (M.L.Q., R.R.W.), DuPont Pharmaceuticals Company, Wilmington, Delaware

Accepted for publication September 23, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

A series of benzamidine isoxazoline derivatives was evaluated for their inhibitory potency against purified human factor Xa (fXa) and in a rabbit model of arteriovenous shunt thrombosis for their antithrombotic activities, expressed as K_i and IC_{50}, respectively. A highly significant correlation was found between K_i and IC_{50} (r = 0.93, P < .0001). The antithrombotic effects of SF303 [mol. wt. 536; K_i: fXa, 6.3 nM; thrombin, 3,100 nM; trypsin, 110 nM; tissue plasminogen activator >20,000 nM; plasmin, 2,500 nM] and SK549 [mol. wt. 546; K_i: fXa, 0.52 nM; thrombin, 400 nM; trypsin, 45 nM; tissue plasminogen activator >33,000 nM; plasmin, 890 nM] were compared with recombinant tick anticoagulant peptide [K_i(fXa) = 0.5 nM], DX-9065a [K_i(fXa) = 30 nM], and heparin or low molecular weight heparin (dalteparin) in a rabbit model of arteriovenous shunt thrombosis. ID_{50} values for preventing arteriovenous shunt-induced thrombosis were 0.6 μmol/kg/h for SF303, 0.035 μmol/kg/h for SK549, 0.01 μmol/kg/h for recombinant tick anticoagulant peptide, 0.4 μmol/kg/h for DX-9065a, 21 U/kg/h for heparin, and 23 U/kg/h for low molecular weight heparin. SK549 produced a concentration-dependent antithrombotic effect with an IC_{50} of 0.062 μM. To evaluate its potential oral efficacy, SK549 was given intraduodenally at a dose of 5 mg/kg; it produced a peak antithrombotic effect of 59 ± 4% with a duration of action greater than 6.7 h. Therefore, our study suggests that SF303, SK549, and their analogs represent a new class of synthetic fXa inhibitors that may be clinically useful as antithrombotic agents.

The clinical usefulness of anticoagulants such as warfarin (Coumadin) and the successful development of low molecular weight heparins (LMWHs) for the treatment and prevention of thromboembolic diseases have generated a great interest in searching for new anticoagulants (Turpie, 1998). However, warfarin is an indirect inhibitor of blood coagulation and takes several days to reach effective anticoagulant levels. LMWH requires a physiological cofactor, antithrombin III, to inactivate factor Xa (fXa). Therefore, the current drug discovery research focuses on designing small molecule inhibitors that directly act on coagulation factors such as thrombin and fXa.

The direct thrombin inhibitors have shown good antithrombotic efficacy in animal thrombosis models. However, it is not certain whether the antithrombotic effects of these inhibitors can be achieved without bleeding complications in humans (Turpie, 1998). The alternative target of new anticoagulants is inhibition of the earlier sites of the coagulation cascade such as fXa. fXa plays a major role in blood coagulation because of its central position at the convergent point of the intrinsic and extrinsic pathways of coagulation. It is believed that inhibition of fXa may reduce the production of thrombin by either the extrinsic or intrinsic pathways without interfering with a basal level of thrombin activity necessary for normal hemostasis (Harker et al., 1997).

Both peptide and nonpeptide fXa inhibitors are currently available (Kaiser, 1998; Hauptmann and Stürzebecher, 1999). Antistasin and tick anticoagulant peptide, examples of peptide fXa inhibitors, are isolated from blood-sucking animals (Kaiser, 1998). DX-9065a and YM-60828, examples of low molecular weight nonpeptide fXa inhibitors, are chemically synthesized (Hara et al., 1994; Taniuchi et al., 1998). The antithrombotic effects of these peptide and nonpeptide fXa inhibitors have been well demonstrated in various experimental models of arterial and venous thrombosis (Kaiser, 1998; Hauptmann and Stürzebecher, 1999).

Because GluGlyArg (EGR), the substrate sequence of prothrombin for fXa, is similar to the sequence of ArgGlyAsp (RGD) of the platelet glycoprotein (GP) IIb/IIIa receptor, we reasoned that compounds designed originally as GP IIb/IIIa...
receptor antagonists might have affinity for fXa (Quan et al., 1997). Accordingly, we tested our library of GP IIb/IIIa receptor antagonists and identified several weak benzamidine isoxazoline compounds with $K_I$ values of less than 50 μM for fXa (Fig. 1; Quan et al., 1997). Based on these prototype molecules, we initiated structure-activity studies that led to the synthesis of a potent bisbenzamide fXa inhibitor with a $K_I$ of 94 nM (Quan et al., 1997). Because of poor pharmacokinetic profiles of benzamidine compounds, we focused on designing monobasic fXa inhibitors (Quan et al., 1999a,b). The replacement of the benzamidine group with a biphenyl group resulted in the synthesis of SF303, which has a $K_I$ of 6.3 nM for fXa (Quan et al., 1999a,b). Subsequently, we replaced the ester side chain of SF303 with a tetrazole side chain and made SK549, which represented our first breakthrough into orally active fXa inhibitors (Quan et al., 1999a,b). In this report, a series of benzamidine isoxazoline derivatives of fXa inhibitors was evaluated in vitro for their inhibitory effects on human fXa activity and on antithrombotic activities in a rabbit model of arteriovenous shunt thrombosis (AVST). Furthermore, SF303 ([(+)-5-isoxazoleacetonic acid, 3-[3-(aminoiminomethyl)phenyl]-5-[[2′-(aminosulfonyl)-1,1′-biphenyl]-4-yl]-amino[carbonyl]-4,5-methyl ester]-trifluoroacetic acid salt) and SK549 ((-)-5-isoxazoleamide, 3-[3-(aminoiminomethyl)phenyl]-N-5-[2′-(aminosulfonanyl)-1,1′-biphenyl]-4-yl]-4,5-dihydro-5(1H-tetrazol-1-ylmethyl)-trifluoroacetic acid salt) were selected for additional characterization.

Materials and Methods

Reagents. The following drugs and chemicals were used in this study: chromogenic substrates, S-2765, S-2366, S-2222, S-2251, and S-2765 (Chromogenix AB products distributed by DiaPharma Group, Inc., West Chester, OH); Spectrozyme-tPA (tissue plasminogen activator; American Diagnostica Inc., Greenwich, CT); human α-thrombin, trypsin, and fXa (Enzyme Research Laboratories, Inc., South Bend, IN); human tissue plasminogen activator (tPA) and plasmin (American Diagnostica); human γ-thrombin (ICN Biomedicals, Inc., Costa Mesa, CA); activated partial thromboplastin time (APTT) reagent, ADP, and thromboplastin with calcium (Sigma Chemical Co., St. Louis, MO); heparin (Upjohn, Kalamazoo, MI); and dalteparin (fragmin; Pharmacia AB, Stockholm, Sweden). Nonpeptide fXa inhibitors listed in Fig. 2 and DX-9065a were synthesized at DuPont Pharmaceuticals Co. (Wilmington, DE). Purified recombinant tick anticoagulant peptide (rTAP) was prepared from culture medium of Saccharomyces cerevisiae as described by Neeper et al. (1990) with modifications.

Inhibitory Effects on Serine Proteases. The affinity of test compounds for human fXa, thrombin, trypsin, tPA, and plasmin was determined by using the chromogenic substrates S-2765, S-2366, S-2222, Spectrozyme-tPA, and S-2251, respectively, as reported previously by Kettner et al. (1990). Assays were performed in a Hewlett-Packard (Palo Alto, CA) model 8452A spectrophotometer with a temperature-controlled multiecell transport system. The hydrolysis rates of chromogenic substrates were assayed by continuously measuring absorbance at 405 nm at 25°C. The $K_I$ values were determined by the Lineweaver-Burk method.

Antithrombotic Studies. Experiments, using a modification of the methods of Wong et al. (1996), were conducted on male New
 Zealand White rabbits that were anesthetized with ketamine (50 mg/kg i.m.) and xylazine (10 mg/kg i.m.). These anesthetics were supplemented as needed. The femoral artery, jugular vein, and femoral vein were isolated and catheterized. A saline-filled arteriovenous (AV) shunt device was connected between the femoral arterial and femoral venous cannuulas. The AV shunt device consisted of an outer piece of Tygon tubing (length, 8 cm; i.d., 7.9 mm) and an inner piece of 2-0 silk thread (Ethicon, Somerville, NJ). Blood flowed from the femoral artery via the AV shunt into the femoral vein. The exposure of flowing blood to a silk thread induced the formation of a significant thrombus. After 40 min, the shunt was disconnected and the silk thread, which was covered with thrombus, was weighed.

The compounds or saline vehicle was given as continuous i.v. infusion via the jugular vein, starting 1 h before blood was circulated. APTT and PT were measured on a fibrometer (BBL Fibrosystem, Becton Dickinson, Cockeysville, MD) (Kettner et al., 1990). APTT was measured by incubating 100 μl of plasma with 100 μl of APTT reagent for 3 min, followed by addition of 100 μl 25 mM CaCl₂. PT was measured by incubating 100 μl of plasma for 2 min at 37°C, followed by addition of 200 μl of prewarmed thromboplastin with calcium. Data points were the means of duplicate measurements and were expressed as a ratio of treated samples versus baseline control.

Platelet Aggregation. Platelet-rich plasma was prepared by centrifugation of fresh citrated blood collected from rabbits. Platelet aggregation was measured with a platelet aggregometer (model PAP-4D, BioData, Horsham, PA). Platelet-rich plasma (200 μl) was mixed with 20 μl of either saline or 1 μM SK549 in saline and incubated for 3 min at 37°C. Percentages of platelet aggregation were determined 4 min after the addition of 20 μl of the agonist (10 μM ADP and 35 nM γ-thrombin, final concentrations). Statistical Analysis. The statistical analyses that we used were correlation, linear regression, analysis of variance, and Duncan’s new multiple-range test (Cody and Smith, 1991). A value of P < .05 was considered statistically significant. All data are means ± S.E.
In Vitro Characterization of SF303 and SK549

SF303 (mol. wt. 536) and SK549 (mol. wt. 546) were evaluated for inhibitory potency against several human proteases. Inhibitory constants ($K_i$) for SF303 ($f$Xa, 6.3 nM; thrombin, 3100 nM; trypsin, 110 nM; TP A > 20000 nM; plasmin, 2500 nM) and SK549 ($f$Xa, 0.52 nM; thrombin, 400 nM; trypsin, 45 nM; TP A > 33000 nM; plasmin, 890 nM) show them to be potent and selective small molecule inhibitors of $f$Xa. rTAP and DX-9065a were included for comparison. rTAP inhibited human $f$Xa with a $K_i$ value of 0.5 nM. DX-9065a was less potent than SF303 and SK549 and inhibited human $f$Xa with a $K_i$ value of 30 nM.

AV Shunt Model in Rabbits

Antithrombotic Effects of Heparin, rTAP, DX-9065a, and Dalteparin. Allowing blood to flow through the AV shunt for 40 min led to the formation of a thrombus weighing approximately 350 mg. In this model, heparin, rTAP, DX-9065a, and dalteparin, given as an i.v. infusion 1 h before blood was circulated in the shunt, inhibited formation of the thrombus in a dose-dependent fashion with ID50 values of 21 U/kg/h i.v.; rTAP at 0.01 (Fig. 3 and 4). Dalteparin, given s.c. at 100, 200, and 400 U/kg, caused a dose-dependent antithrombotic effect; the maximum antithrombotic effects for these doses occurred at 200 min postdose (data not shown). The ID50 for s.c. dalteparin was 182 U/kg (Fig. 4).

Relationship Between $K_i$($f$Xa) and In Vivo Potency IC50. A series of benzamidine isoxazoline derivatives of $f$Xa inhibitors was evaluated against purified human $f$Xa for their inhibitory effects on $f$Xa activity in a rabbit model of AVST for their antithrombotic activities, expressed as $K_i$ and IC50, respectively (Fig. 2). Although there was a significant correlation between $K_i$ and ID50 ($r = 0.85, P < .0003$), we found a better and highly significant correlation between $K_i$ and IC50 ($r = 0.93, P < .0001$) as shown in Fig. 5.

![Antithrombotic effects of heparin, rTAP, DX-9065a, and dalteparin in AV shunt model.](image)

Fig. 3. Antithrombotic effects (expressed as % inhibition of thrombus formation) of heparin at 8 (n = 5), 16 (n = 5), 32 (n = 5), and 64 (n = 5) U/kg/h i.v.; rTAP at 0.01 (n = 5), 0.07 (n = 6), and 0.36 mg/kg/h i.v. (n = 5); and DX-9065a at 0.0058 (n = 5), 0.04 (n = 5), 0.2 (n = 5), and 1 mg/kg/h i.v. (n = 5) in rabbit AV shunt model. Control clot weight for heparin-treated group = 328 ± 14 mg. Overall effects of heparin were significant (ANOVA, $F_{2,14} = 213.54, P < .0001$). Control clot weight for rTAP-treated group = 353 ± 10 mg. Overall effects of rTAP were significant (ANOVA, $F_{2,13} = 138.29, P < .0001$). Control clot weight for DX-9065a-treated group = 357 ± 15 mg. Overall effects of DX-9065a were significant (ANOVA, $F_{2,16} = 218.93, P < .0001$). Means ± S.E.

![Scatterplot showing relationship between in vivo antithrombotic potency (IC50) in AV shunt rabbits and inhibitory constants of fXa ($K_i$) of a series of nonpeptide fXa inhibitors listed in Fig. 2.](image)

Fig. 5. Scatterplot showing relationship between in vivo antithrombotic potency (IC50) in AV shunt rabbits and inhibitory constants of fXa ($K_i$) of a series of nonpeptide $f$Xa inhibitors listed in Fig. 2.

Antithrombotic Effects of SF303 and Its Enantiomers. As shown in Fig. 6, SF303 produced a dose-dependent antithrombotic effect with an ID50 of 0.6 μmol/kg/h. SG007, the positive enantiomer of SF303, inhibited human $f$Xa with a $K_i$ of 7 nM and an ID50 of 2 μmol/kg/h i.v. (Fig. 6). SG008, the negative enantiomer of SF303, had a $K_i$($f$Xa) of 4 nM and an ID50 of 0.2 μmol/kg/h i.v. (Fig. 6).

Effects on APTT. Effects of SF303 on APTT were compared with heparin, rTAP, and DX-9065a (Fig. 7). Heparin, given i.v. at 64 U/kg/h, significantly increased APTT ($P < .05$). rTAP, given i.v. at 0.01 to 0.36 mg/kg/h, and SF303, given i.v. at 0.04 to 1 mg/kg/h, did not alter APTT levels significantly. DX-9065a, given i.v. at 0.2 and 1 mg/kg/h, significantly prolonged APTT ($P < .05$).

Antithrombotic Effects of SK549. As shown in Fig. 8, SK549 produced a dose-dependent antithrombotic effect with an ID50 of 0.035 μmol/kg/h. Effects of the saline vehicle and SK549 on PT were evaluated in a separate group of experiments. Compared with the vehicle (n = 3), SK549 at 0.008…
at 0.04 (n = 5), 0.2 (n = 5), and 5 mg/kg/h i.v. (n = 5); and SG008 (the negative enantiomer of SF303) at 0.04 (n = 5), 0.2 (n = 5), and 1 mg/kg/h i.v. (n = 5) in rabbit AV shunt model. Control clot weight for SF303-treated group = 335 ± 15 mg. Overall effects of SF303 were significant (ANOVA, F$_{3,15}$ = 82.07, P < .0001). Control clot weight for SG007-treated group = 317 ± 8 mg. Overall effects of SG007 were significant (ANOVA, F$_{3,12}$ = 163.63, P < .0001). Control clot weight for SG008-treated group = 357 ± 15 mg. Overall effects of SG008 were significant (ANOVA, F$_{3,12}$ = 119.65, P < .0001). Means ± S.E.

Fig. 6. Antithrombotic effects (expressed as % inhibition of thrombus formation) of SF303 at 0.04 (n = 6), 0.2 (n = 6), and 1 mg/kg/h i.v. (n = 6); SG007 (the positive enantiomer of SF303) at 0.2 (n = 5), 1 (n = 5), and 5 mg/kg/h i.v. (n = 5); and SG008 (the negative enantiomer of SF303) at 0.04 (n = 5), 0.2 (n = 5), and 1 mg/kg/h i.v. (n = 5) in rabbit AV shunt model. Control clot weight for SF303-treated group = 335 ± 15 mg. Overall effects of SF303 were significant (ANOVA, F$_{3,15}$ = 82.07, P < .0001). Control clot weight for SG007-treated group = 317 ± 8 mg. Overall effects of SG007 were significant (ANOVA, F$_{3,12}$ = 163.63, P < .0001). Control clot weight for SG008-treated group = 357 ± 15 mg. Overall effects of SG008 were significant (ANOVA, F$_{3,12}$ = 119.65, P < .0001). Means ± S.E.

Fig. 7. Effects of vehicle (V), heparin (8, 16, 32, and 64 U/kg/h i.v.), rTAP (0.01, 0.07, and 0.36 mg/kg/h i.v.), DX-9065a (0.008, 0.04, 0.2, and 1 mg/kg/h i.v.) and SF303 (0.04, 0.2, and 1 mg/kg/h i.v.) on APTT in rabbit AV shunt model. n = 5 to 6 per group. Means ± S.E.

Fig. 8. Antithrombotic effects (expressed as % inhibition of thrombus formation) of SK549 at 0.008 (n = 3), 0.04 (n = 6), 0.2 (n = 6), and 1 mg/kg/h i.v. (n = 6) in rabbit AV shunt model. Overall effects of SK549 were significant (ANOVA, F$_{3,17}$ = 63.32, P < .0001). Means ± S.E.

Fig. 9. Relationship between plasma concentrations of SK549 and antithrombotic effects (expressed as % inhibition of thrombus formation) in rabbit AV shunt model. Antithrombotic effect of SK549 was found to be greater than 6.7 h (Fig. 10).

Discussion

With the emphasis on structure-based design, a series of potent benzamidine isoxazoline FXa inhibitors was synthesized (Quan et al., 1999a,b). Unlike DX-9065a and YM-60828 (Hara et al., 1994; Taniuchi et al., 1998), which are dibasic compounds, these benzamidine isoxazoline FXa inhibitors are monobasic. Our data suggest that the structural requirement for potent FXa inhibitors does not necessarily require two basic moieties. Moreover, replacing the strongly basic para-benzamidine group of the bisbenzamidine FXa inhibitor (Fig. 1) with a substituted biphenyl moiety improves inhibitory potency against FXa. For instance, the monobasic FXa inhib-
shunt-induced thrombosis has also been reported in rats with an ID_{50} of 182 U/kg. Because the clinical effective dose thrombus formation dose dependently in the AVST model are effective in inhibiting thrombus formation in the rabbit thrombus formation (Peters et al., 1991).

platelets and the coagulation cascade contribute to the silk thread anchored in the shunt and both the activation of characterized as a 'mixed' thrombosis model. In this model, the inhibitors in this model. The AVST model has been charac-

unpublished observation). Because rTAP and nonpeptide fXa inhibitors such as DX-9065a and YM-60828 have been well reported by Taniuchi et al., 1998), respectively. Our results also suggest that monobasic isoxazoline fXa inhibitors still retain selectivity. When compared with the other serine proteases, SF303 and SK549 were at least 397 to 1712 times more potent in inhibiting fXa than thrombin, tPA, and plasmin, and 17 and 87 times more potent than blocking trypsin, respectively.

To examine the efficacies of these benzamidine isoxazoline fXa inhibitors, we evaluated these agents in a rabbit model of AVST. We selected the rabbit as our animal model because rabbit and human fXa have similar binding affinity to enzyme substrate and small molecule inhibitors of fXa (Hara et al., 1995; RMK, unpublished observation). In contrast, rat and dog fXa are much less sensitive to small molecule inhibitors of fXa (Hara et al., 1995; Taniuchi et al., 1998; R.M.K., unpublished observation). Because rTAP and nonpeptide fXa inhibitors such as DX-9065a and YM-60828 have been well characterized in the AVST model (Hara et al., 1994; Wong et al., 1996; Sato et al., 1998), we evaluated our isoxazoline fXa inhibitors in this model. The AVST model has been characterized as a 'mixed' thrombosis model. In this model, the thrombosis is probably initiated by platelet adherence to the silk thread anchored in the shunt and both the activation of platelets and the coagulation cascade contribute to the thrombus formation (Peters et al., 1991).

This study shows that heparin and the LMWH dalteparin are effective in inhibiting thrombus formation in the rabbit AVST model. When given s.c., dalteparin inhibited the thrombus formation dose dependently in the AVST model with an ID_{50} of 182 U/kg. Because the clinical effective dose of dalteparin for the treatment of deep vein thrombosis is 100 to 200 U/kg s.c. (Dunn and Sorkin, 1996), our results suggest that the degree of severity of thrombosis in the AVST model is similar to that in deep vein thrombosis. In this model, rTAP is a very potent antithrombotic agent with an ID_{50} of 0.01 μmol/kg/h. A similar efficacy of rTAP in inhibiting AV shunt-induced thrombosis has also been reported in rats (Wong et al., 1996). We also confirmed that DX-9065a is an effective antithrombotic agent in the rabbit thrombosis model with an ID_{50} of 0.4 μmol/kg/h. The observed potency of DX-9065a in this study is consistent with the previously reported potency by Nagahara et al. (1995). Because rTAP and DX-9065a are potent and selective fXa inhibitors, the observed antithrombotic efficacies of both agents support the inhibition of fXa as an attractive anticoagulant target in thrombotic diseases.

To substantiate that the antithrombotic effect of benzamidine isoxazoline fXa inhibitors is due to the inhibition of fXa, the correlation between the inhibitory constants for the fXa, K_{I}, and the in vivo antithrombotic potencies, IC_{50}, of a series of benzamidine isoxazoline fXa inhibitors was determined. It should be noted that plasma concentrations of all these fXa inhibitors except SK549 were determined by the anti-fXa assay. This functional assay did not take the contribution of potentially active metabolites into account. However, our study shows an excellent correlation between K_{I} and IC_{50}-suggesting that the contribution of potentially active metabolites of these fXa inhibitors to the readout of the anti-fXa assay may be minimal. Furthermore, the excellent correlation supports that the inhibition of fXa is the primary mechanism of the antithrombotic effects of nonpeptide fXa inhibitors. It is interesting that the IC_{50} of this series of fXa inhibitors averaged approximately 150-fold greater than the K_{I}. Reasons for this difference between K_{I} and IC_{50} are not clear, but may be due to differences between in vitro and in vivo experimental conditions, e.g., protein binding and inhibition kinetics.

To illustrate the characteristics of benzamidine isoxazoline derivatives of fXa inhibitors, SF303 and SK549 were selected for additional study. SF303 is a selective and competitive fXa inhibitor with a K_{I} of 6.3 nM. It is also a potent antithrombotic agent in the AVST rabbits with an ID_{50} of 0.6 μmol/kg/h. Because SF303 is a racemic mixture, we studied its enantiomers in the anti-fXa assay and in the AVST rabbits. Our results indicate that the negative enantiomer of SF303, SG008, is more potent than the positive enantiomer, SG007, both in terms of inhibitory constants for fXa (K_{I}) and in vivo antithrombotic potency (ID_{50}). These results suggest that the binding of SF303 to the active site of fXa is stereospecific and depends on the configuration of SF303 presenting its chiral center to the active site.

APTT is used universally to monitor the therapeutic level of heparin-induced anticoagulation (Kher et al., 1997). The dose of heparin that doubles the APTT is often taken as a measure of adequate heparin administration. In this study, although heparin, at its antithrombotic ID_{50}, only minimally prolonged APTT, it produced a greater than 5-fold increase in APTT at its maximum antithrombotic dose. By contrast, maximum inhibition of thrombus formation was achieved with selective fXa inhibitors, including rTAP, DX-9065a, and SF303, that only minimally prolonged APTT. In addition, the antithrombotic efficacy of SK549 was not correlated with any change in PT. Other studies have also shown that fXa inhibitors such as rTAP, DX-9065a, and YM60828 produced anti-thrombotic efficacy without the prolongation of APTT seen in animal experiments (Wong et al., 1996; Hauptmann and Stürzebecher, 1999). It is still not known whether a lower level of anticoagulation induced by fXa inhibitors may ac-
count for the reduced bleeding time observed in animals (Harker et al., 1997; Hauptmann and Stürzebecher, 1999).

Our study shows, for the first time in the rabbit AVST model, that monobasic isoxazoline compounds, such as SF303, SK549, and their analogs, are potent antithrombotic agents. SK549 is as potent as rTAP in inhibiting Xa activity in vitro (K_i for both compounds is approximately 0.5 nM). In the AVST rabbits, SK549 given i.v. was only 3.5 times less potent than rTAP given i.v., which may be related to enhanced inhibitory potency of rTAP in the prothrombinase complex (for review see Kaiser, 1998). However, rTAP lacks oral activity because of its pepticid nature. In this regard, SK549 is better than rTAP. When given intraduodenally, SK549 exerted a long-acting antithrombotic effect in the rabbit AV shunt model of thrombosis, suggesting that SK549 may be active after oral administration. It should be noted that the antithrombotic effect of SK549 may not be due to the inhibition of platelet aggregation because 1 μM SK549 (17-fold greater than its IC_{50} of 0.064 μM) did not affect platelet aggregation in vitro.

In summary, our study shows that SF303 and SK549 are effective antithrombotic agents in a model of thrombosis in rabbits. Unlike heparin, the antithrombotic actions of Xa inhibitors at the maximal doses studied were associated with a minimum degree of anticoagulation. Because our study shows an excellent correlation between K_i and IC_{50} of a series of benzamidine isoxazoline Xa inhibitors, it suggests that the inhibition of Xa is the primary mechanism of the antithrombotic effects of these inhibitors. Because most of the reported small molecule Xa inhibitors, such as DX-9065a and YM-60828, are dibasic compounds whose therapeutic applications may be limited by poor pharmacokinetics, we believe this new series of monobasic isoxazoline Xa inhibitors may eventually lead to compounds with better pharmacokinetics. Thus, this series of small nonpeptide inhibitors represents a new class of selective Xa inhibitors. Furthermore, these new agents prevent the thrombus formation induced by the AV shunt in rabbits and may, therefore, be clinically useful as antithrombotic agents.

Acknowledgments

We thank Drs. M. Thoolen and A. Slee for helpful comments; R. Carney for plasma levels determination; A. Liauw, C. Ellis, and J. Luettgen for technical assistance; Drs. J. Duke and S. Rosenfeld for providing rTAP; and Dr. Q. Han for providing DX-9065a.

References


Send reprint requests to: Dr. Pancras C. Wong, DuPont Pharmaceuticals Company, P.O. Box 80400, Wilmington, DE 19880-0400. E-mail: pancras.c.wong@dupontpharma.com