Antithrombotic Properties of SSR182289A, a New, Orally Active Thrombin Inhibitor

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ABSTRACT

N-[3-[[1S]-4-(5-Amino-2-pyridinyl)-1-[[4-difluoromethylene]-1-piperidinyl]carbonyl]butyl]amino]sulfonyl][1,1′-biphenyl]-2-yl]acetamide hydrochloride (SSR182289A) is a novel, selective thrombin inhibitor. We have examined the antithrombotic properties of SSR182289A administered by i.v. and p.o. routes in several different animal thrombosis models in comparison with reference antithrombotic agents. Oral administration of SSR182289A produced dose-related antithrombotic effects in the following models: rat venous thrombosis (ED_{50} 0.9 mg/kg p.o.), rat silk thread arterio-venous (AV) shunt (ED_{50} 3.8 mg/kg p.o.), rat thromboplastin-induced AV shunt (ED_{50} 3.1 mg/kg p.o.), rat carotid artery thrombosis (ED_{200} 5.9 mg/kg p.o.), and rabbit venous thrombosis (ED_{50} 7.5 mg/kg p.o.). Administered as an i.v. bolus, SSR182289A showed antithrombotic activity in the above models with ED_{50}/ED_{200} values in the range of 0.2 to 1.9 mg/kg i.v. SSR182289A increased rat tail transection bleeding time at doses ≥10 mg/kg p.o. In the rat thromboplastin-induced AV shunt model, SSR182289A 10 mg/kg p.o. produced marked antithrombotic effects at 30, 60, 120, and 240 min after administration. Hence, SSR182289A demonstrates potent oral antithrombotic properties in animal venous, AV-shunt, and arterial thrombosis models.

Orally active antithrombotic agents represent a major therapeutic class for the treatment or prevention of cardiovascular diseases in which the underlying cause is reduced blood flow following arterial or venous thrombosis. These conditions include unstable angina, coronary angioplasty, deep vein thrombosis, myocardial infarction, peripheral vascular disease, and stroke. Several orally active inhibitors of platelet aggregation have been described that demonstrate antithrombotic properties as a result of inhibiting different platelet activation mechanisms. The best known examples include aspirin, clopidogrel (Herbert et al., 1999), which blocks adenosine diphosphate/P2Y12 receptor-dependent aggregation, and the inhibitors of platelet glycoprotein IIb/IIIa (Cannon et al., 1998). The treatment of venous thrombosis involves inhibition of the blood coagulation cascade usually by employing parentally-administered heparin or low-molecular-weight heparins. Few orally active anticoagulants are available. The vitamin K antagonists, such as warfarin, are effective in this respect, but their utility is limited by a narrow therapeutic margin, unpredictable anticoagulation, and the need for regular laboratory monitoring. More recently, intensive research into selective small-molecule inhibitors of key serine proteases in the coagulation cascade has resulted in the description of orally active anticoagulants that target either thrombin (Cook et al., 1999) or factor Xa (Kawasaki et al., 1998).

SSR182289A is a novel, selective thrombin inhibitor that inhibits the amidolytic activity of thrombin and thrombin-induced platelet aggregation in vitro and demonstrates anticoagulant properties in several animal species following i.v. or oral administration (Berry et al., 2002). In this article, we report the antithrombotic effects of SSR182289A in different animal models of thrombosis including those representative of venous, arterial, and “mixed” thrombus formation. Comparative studies have been performed with several reference antithrombotic agents and, notably, in the case of the oral route with the thrombin inhibitor ximelagatran (Gustafsson et al., 2001). Unlike ximelagatran (which is a double prodrug), SSR182289A does not require metabolism to an active form to achieve oral activity (Berry et al., 2002).

Materials and Methods

This study was performed in accordance with the European Community Standards on the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of Sanofi-Synthélabo Research.

ABBREVIATIONS: SSR182289A, N-[3-[[1S]-4-(5-amino-2-pyridinyl)-1-[[4-difluoromethylene]-1-piperidinyl]carbonyl]butyl]amino]sulfonyl][1,1′-biphenyl]-2-yl]acetamide hydrochloride; AV shunt, arterio-venous shunt; SR90107, fondaparinux; DX9065a, (+)-(2S)-2-[4-[[3S]-1-acetimidoyl-3-pyrrolidinyl]oxo]phenyl]-3-[7-amino-2-naphthyl]propanoic acid hydrochloride pentahydrate; CI, 95% confidence intervals.
Rat Venous (Wessler) Thrombosis Model. Fasted male CD rats (300–410 g; Charles River, L’Arbresle, France) were anesthetized with pentobarbital sodium (60 mg/kg i.p.) and placed on a heated jacket to control body temperature. The right common carotid artery was cannulated for intravenous injection of drugs. The abdominal vena cava was exposed, and two silk sutures, 1 cm apart, were placed around the vessel to form a snare. Thrombus formation was induced by the intravenous injection of 20 μg/kg rabbit thromboplastin (La Technique Biologique, Paris, France) into the left femoral vein, followed 10 sec later by tightening the two sutures around the vena cava to induce blood stasis. Stasis was maintained for 15 min, after which time the thrombus was removed and immediately weighed. In the intravenous administration protocol, SSR182289A and reference antithrombotic agents were administered 5 min before thrombus formation. In the oral study, SSR182289A, ximelagatran, or vehicle was administered as single oral doses either 30 or 60 min before injection of thromboplastin.

Rat Arterio-Venous Shunt—Silk Thread Model. Fasted male Sprague-Dawley rats (320–430 g; Janvier, Le Genest St. Isle, France) were anesthetized with sodium pentobarbital (60 mg/kg i.p.). An arterio-venous (AV) shunt was prepared according to the technique of Umetus and Sanai (1978). Two 13 cm-long polyethylene tubes (0.85- and 1.27-mm i.d. and o.d., respectively) linked to a central part (6 cm-long; 1.14-mm i.d.) containing a 5-cm silk thread and filled with saline solution were placed between the right carotid artery and the left jugular vein. This central part of the shunt was removed after 20 min of blood circulation and the silk thread supporting the thrombus was extracted. The wet weight of the thrombus was determined. SSR182289A, ximelagatran, or vehicle was administered as single oral doses 30 min before thrombosis induction.

Rat Arterio-Venous Shunt Model (Tissue Factor Induced). Fasted male CD rats (260–410 g; Charles River) were anesthetized with sodium pentobarbital (60 mg/kg i.p.). The left jugular vein and the right common carotid artery were cannulated with an 8-cm long polyethylene cannulae (Biotrol no. 3; Paris, France) mounted on 25/8 needles. The shunt was assembled by connecting the two cannulae with a slightly curved, 6-cm long, polyvinyl chloride tube (3-mm i.d.) containing a 5-cm long cotton thread impregnated with tissue factor [threads preincubated 5 min in a 35 mg/ml thromboplastin solution (La Technique Biologique, France) and then dried]. The extracorporeal circulation was maintained for 5 min, during which time a thrombus adhered to the cotton thread. The shunt was then removed; the threads with its associated thrombus was withdrawn and immediately weighed. The thrombus weight was determined by subtracting the average weight of the long cotton thread from the total weight. All measurements were performed using the intravenous administration protocol, SSR182289A and the reference antithrombotic agents were administered 5 min before shunt assembly. In the oral study, SSR182289A, ximelagatran or vehicle was administered as single oral doses 30 min before shunt assembly. In a separate study designed to measure duration of antithrombotic effect, SSR182289A (10 mg/kg p.o.), ximelagatran (10 mg/kg p.o.), or vehicle was administered, each to four groups of animals at different times (30, 60, 120, or 240 min) before shunt assembly.

Rat Arterial Thrombosis Model. Fasted male CD rats (420–510 g; Charles River) were anesthetized with sodium pentobarbital (60 mg/kg followed by 6 mg/kg/h, i.p.) and thermoregulated by use of blankets (Harvard homeothermic blanket control unit; Lees Ulis, France). A segment (approximately 1 cm-long) of the left carotid artery was exposed and filled at the distal end with an appropriately sized Doppler flow probe. Thrombosis was induced by applying an electrical current (3 mA DC) to the external arterial surface using a DC stimulator (Sanoﬁ-Synthélabo) for 2 min. Blood flow velocity was measured using a Doppler flowmeter (system 6, model 200; Triton Technology, San Diego, CA) and recorded on a chart recorder (Graphite DMS 1000; Bioseb, Antony, France). Blood flow was recorded for 60 min postlesion. When the flow declined to zero, the time in minutes to thrombus formation was noted. The number of animals presenting no occlusion 30 min poststimulation were also noted. In the intravenous administration protocol, SSR182289A and the reference antithrombotic agents were administered 5 min before thrombus induction. In the oral study, SSR182289A, ximelagatran, or vehicle was administered as single oral doses 30 min before thrombus induction.

Rabbit Venous (Wessler) Thrombosis Model. Male, New Zealand White rabbits (Lago, France) weighing 2.7 to 3.2 kg were used in this study. The animals had free access to standard diet and tap water. Rabbits were anesthetized by intravenous injection of 60 mg/kg sodium pentobarbital. According to Buchanan et al. (1985), each jugular vein was isolated, and two loose sutures were placed 2.5 cm apart. Recombinant human tissue thromboplastin (1 ng/kg) (DADE, Baxter, Germany) was injected slowly during 30 s into the carotid artery in a total volume of 1 ml. Thirty seconds later, both jugular vein segments were closed by the distal and proximal sutures. Stasis was maintained for 15 min. The segments were then opened longitudinally, and any existing thrombus was removed. After blotting on filter paper, the thrombi were weighed. The wet weights of the thrombi were averaged for the left and the right jugular vein. The antithrombotic effect was expressed as percentage of inhibition of mean thrombus weight compared with control animals. In the intravenous administration protocol, SSR182289A and the reference antithrombotic agents were administered 5 min before thrombus induction. For the oral administration protocol, prior pharmacokinetic studies (data not shown) had demonstrated that in the rabbit plasma Cmax levels of SSR182289A and ximelagatran occurred at 60 and 120 min after oral dosing, respectively. These time intervals were therefore adopted for the present oral study.

Determination of Bleeding Time in the Rat. Fasted, male CD rats (350–450 g; Charles River) were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and thermoregulated by use of blankets (Harvard homeothermic blanket control unit). Bleeding was induced by section of the extremity of the tail 3 mm from the tip. The tails were maintained in contact with air and gently blotted with filter paper each minute during a 10-min period and then every 2 min; the time in minutes to cessation of bleeding (no rebleeding for 1 min) was noted. The observation time was limited to 90 min. Care was taken that no pressure was exerted on the tail tips that could affect hemostasis. SSR182289A, ximelagatran, or vehicle was administered as single oral doses either 30 or 60 min before tail transection.

Data Analysis. In venous and AV shunt thrombosis models mean thrombus weight ± S.E.M. was determined for each experimental group, and tests for statistical significance between the treatment and control groups were performed by one-way analysis of variance followed by a Dunnett’s test using Everstat SAS (SAS Institute, Cary, NC) or R (R development Core Team, 2005). The percent inhibition of thrombus formation was determined for each treatment group. ED50 values (the dose that produced a 50% inhibition of thrombus formation) were determined from dose-inhibition curves (by regression analysis using Everstat SAS or R software). Groups were considered significantly different if p < 0.05.

In the arterial thrombosis model, the mean time to occlusion ± S.E.M. was determined for each group, and tests for statistical significance between the treatment and control groups were performed by one-way analysis of variance followed by a Kruskall Wallis test using Everstat SAS software (SAS Institute). The percent increase in the time to occlusive thrombus formation was determined for each treatment group. If the vessels were still patent at the end of the observation period, a value of 60 min was ascribed for the sake of statistical analysis. Differences between the incidences of occlusions in treated groups versus the control group were assessed using the Fisher test. Groups were considered significantly different if p < 0.05. An ED50 value (the dose that produced a 200% increase in time to occlusion) was calculated for each compound using RS1 software. The percent increase of arterial blood flow in normal and treated animals was determined using R software (R Development Core Team, 2005). Percentage changes were calculated for each individual/time point compared with controls. The percentage of inhibition of mean arterial blood flow compared with control animals was determined for each compound using RS1 software.
bleeding time was determined for each treatment group. Groups were considered significantly different if \( p < 0.05 \).

**Drugs.** Sources of drugs used were as follows: sodium pentobarbitone and nadroparin (Praxiparine) (Sanofi-Synthélabo, Toulouse, France), SSR182289A, melagatran, and ximelagatran (Sanofi-Synthélabo Recherche, Chilly-Mazarin, France), SR90107 (fondaparinux-Arixtra) (Sanofi-Synthélabo, Toulouse, France/Organon, Oss, Netherlands), heparin calcium salt (Sigma-Aldrich, St. Quentin Fallavier, France), hirudin (Lepirudin, Hoechst, Germany), DX9065a (Daichi Pharmaceuticals Co., Ltd., Tokyo, Japan). Drugs were administered in 0.9% saline for intravenous studies and in water or 0.2 or 1% Tween 80 in water for oral studies. Oral dosing was performed by gavage. Doses refer to the free bases.

**Results**

**Rat Venous (Wessler) Thrombosis Model.** The antithrombotic effects of SSR182289A following i.v. administration compared with reference compounds in three thrombosis models are shown in Table 1. In the rat Wessler model, SSR182289A had an ED\(_{50}\) of 0.22 mg/kg. The potency order of the compounds tested was melagatran \( \geq \) fondaparinux \( \geq \) heparin \( \geq \) hirudin > SSR182289A > DX9065a.

Oral administration of SSR182289A or ximelagatran 30 min before thromboplastin injection leads to a dose-dependent decrease in thrombus weight. The ED\(_{50}\) values obtained were 0.9 mg/kg (CI 0.7–1.3) for SSR182289A and 0.4 mg/kg (CI 0.3–0.6) for ximelagatran. When administered 60 min before thrombus induction, the ED\(_{50}\) values obtained were, 1.1 mg/kg (CI 0.8–1.4) for SSR182289A and 0.7 mg/kg (CI 0.6–0.9) for ximelagatran. Thus, the different pretreatment times did not significantly affect antithrombotic potency for both compounds. Data from the study performed with a 60 min pretreatment time are shown in Fig. 1. Mean thrombus weight recorded in the control group was 27.5 \( \pm \) 1.4 mg (\( n = 7 \)). Statistically significant reductions in thrombus weight were seen at doses equal to or greater than 0.3 mg/kg for both compounds.

**Rat Arterio-Venous Shunt (Silk Thread Model).** Mean thrombus wet weights recorded in the control groups were 38.5 \( \pm \) 0.6 mg (ximelagatran) and 37.1 \( \pm \) 0.7 mg (SSR182289A) (\( n = 6–8 \)).

The results, expressed as a percent reduction in thrombus weight after oral administration of different doses of SSR182289A and ximelagatran, are shown in Fig. 2. Both compounds demonstrated a dose-dependent antithrombotic effect. ED\(_{50}\) values of ximelagatran and SSR182289A were 0.34 mg/kg (CI 0.26–0.43) and 3.8 mg/kg (CI 3.3–4.4), respectively.

**Rat Arterio-Venous Shunt Model (Tissue Factor Induced).** Following i.v. administration, SSR182289A showed dose-related antithrombotic effects, with an ED\(_{50}\) value of 0.85 mg/kg (Table 1). The relative potency order of compounds tested was melagatran > hirudin > heparin > nadroparin > SSR182289A > DX9065a \( \geq \) fondaparinux.

In the oral administration study, the insertion between the carotid artery and the jugular vein led to the formation of a thrombus weighing 191 \( \pm \) 4 or 180 \( \pm \) 15 mg in the SSR182289A and ximelagatran control groups, respectively (\( n = 6–8 \)). The antithrombotic effects of different doses of SSR182289A and ximelagatran administered orally 30 min before shunt assembly are shown in Fig. 3. SSR182289A produced a dose-dependent reduction in thrombus weight with an ED\(_{50}\) value of 3.1 mg/kg (CI 2.2–4.6). The ED\(_{50}\) value for ximelagatran in this model was 5.3 mg/kg (CI 2.9–15.6). Statistically, significant reductions in thrombus weight were observed starting from a dose of 0.3 mg/kg p.o. SSR182289A and 3 mg/kg p.o. ximelagatran.

An additional study was performed to evaluate the kinetics and duration of action of the antithrombotic effects of SSR182289A and ximelagatran in this model. The results are shown in Fig. 4. The administration of vehicle 30, 60, 120, or 240 min before shunt assembly induced no significant change in thrombus weight. SSR182289A (10 mg/kg p.o.) and ximelagatran (10 mg/kg p.o.) produce statistically significant reductions in thrombus weight at each of the administration times tested. The antithrombotic effect of SSR182289A was well maintained with time (51 and 39% reductions in thrombus weight 30 and 240 min after drug administration, respectively). Ximelagatran was slightly more potent than SSR182289A when administered 30 min before shunt assembly (76% decrease in thrombus weight); however, its antithrombotic effect appeared to decline more rapidly leaving a residual 28% reduction of thrombus weight at 240 min.

**Rat Arterial Thrombosis Model.** Application of an electrical current to the adventitial surface of the carotid artery led to the formation of a stable thrombus. Zero blood flow in the artery occurred 11.4 \( \pm \) 0.8 and 9.3 \( \pm \) 1.1 min after the end of electrical stimulation in control groups for SSR182289A

### TABLE 1

<table>
<thead>
<tr>
<th>Rat Venous Thrombosis (ED(_{50}) mg/kg i.v.)</th>
<th>Rat Arteriovenous Shunt (Tissue Factor Induced) (ED(_{50}) mg/kg i.v.)</th>
<th>Rat Carotid Arterial Thrombosis (ED(_{50}) mg/kg i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSR182289A</td>
<td>0.22 (0.07–1.04)</td>
<td>0.85 (0.57–1.57)</td>
</tr>
<tr>
<td>Melagatran</td>
<td>0.01 (0.007–0.013)</td>
<td>0.06 (0.035–0.11)</td>
</tr>
<tr>
<td>Heparin</td>
<td>0.015 (0.011–0.020)</td>
<td>0.23 (0.19–0.28)</td>
</tr>
<tr>
<td>Nadroparin</td>
<td>0.63 (0.52–0.81)</td>
<td>1.60 (1.16–1.99)</td>
</tr>
<tr>
<td>Fondaparinux</td>
<td>0.012 (0.009–0.013)</td>
<td>2.6 (1.92–3.42)</td>
</tr>
<tr>
<td>DX9065a</td>
<td>0.63 (0.41–0.98)</td>
<td>1.6 (1.27–2.16)</td>
</tr>
<tr>
<td>Hirudin</td>
<td>0.023 (0.017–0.031)</td>
<td>0.16 (0.115–0.210)</td>
</tr>
</tbody>
</table>

Values shown are mean values with 95% confidence limits (\( n = 5–8 \)).
and ximelagatran, respectively \((n = 6–8)\). The results of the oral administration protocol are shown in Fig. 5. When administered by the oral route 30 min before electrical stimulation, SSR182289A led to a dose-dependent increase in the time to occlusion. Statistically significant increases were observed at 5 and 10 mg/kg. At 10 mg/kg, there was a 340\% increase in the time to occlusion with six of eight animals having patent arteries. The effects of ximelagatran were more marked at the same dose (540\% increase in the occlusion delay with six of six animals not occluding). Corresponding ED\(_{200}\) values were 5.9 mg/kg p.o. for SSR182289A and 1.8 mg/kg p.o. for melagatran, respectively. Administered by the i.v. route, SSR182289A showed an ED\(_{200}\) value of 1.9 mg/kg in this model (Table 1). The relative order of i.v. potencies was melagatran > hirudin \(\approx\) DX9065 > heparin \(\approx\) SSR182289 \(\approx\) fondaparinux.

**Rabbit Venous Thrombosis Model.** In the oral administration study, thrombus wet weights of 165 \(\pm\) 18 and 183 \(\pm\) 15 mg, respectively, were obtained in the control groups for ximelagatran and SSR182289A. The results, expressed as wet thrombus weight in each of the treatment groups, are shown in Table 2. Oral administration of SSR182289A and ximelagatran reduced the thrombus weight in a dose-dependent manner. For both compounds, a maximal inhibitory effect was obtained at the dose of 30 mg/kg (100\%). ED\(_{50}\) values of ximelagatran and SSR182289A were 5.3 mg/kg (CI 3.2–8.3) and 7.5 mg/kg (CI 3.6–12.3), respectively. Administered by the i.v. route, SSR182289A (ED\(_{50}\) 0.89 mg/kg; CI 0.45–1.78) and melagatran (ED\(_{50}\) 0.026 mg/kg; CI 0.020–0.031) produced dose-related antithrombotic effects.

**Determination of Bleeding Time in the Rat.** Bleeding times measured in the control groups following tail transection were 10.4 \(\pm\) 1.3 (a 30-min pretreatment) and 10.6 \(\pm\) 0.9 min (a 60-min pretreatment). The effects of oral administration of SSR182289A and ximelagatran on bleeding times are shown in Table 3. Both compounds prolonged bleeding time in a dose-dependent fashion at doses greater than or equal to 10 mg/kg p.o.
Discussion

In this article, we describe the antithrombotic properties of SSR182289A administered by i.v. and p.o. routes in several animal thrombosis models. We have previously reported (Berry et al., 2002) that SSR182289A is a potent and highly selective thrombin inhibitor ($K_i$ 31 nM). In particular, SSR182289A has little or no affinity for other serine proteases involved in the blood coagulation cascade (i.e., factor Xa, factor VIIa/tissue factor, factor IXa). Similarly, SSR182289A potently inhibits thrombin-induced aggregation in human, rat, and rabbit platelets in vitro with IC$_{50}$ values between 10–32 nM but had no significant effect against platelet aggregation mediated by other mechanisms (collagen, arachidonic acid, thrombin receptor activator protein, and ADP). These in vitro studies clearly establish the thrombin-inhibitory activity of SSR182289A and, in addition, exclude several other potential antithrombotic mechanisms related to blood coagulation and platelet function.

The antithrombotic activity of SSR182289A is also consistent with its anticoagulant properties measured ex vivo. We have previously shown that i.v. or oral administration of SSR182289A produces dose-related increases in coagulation parameters (thrombin time, ecarin clotting time, and activated partial thromboplastin time) in several animal species (rat, rabbit, dog, and macaque) (Berry et al., 2002). In this context, the ecarin clotting time has been reported to be a good predictor of antithrombotic effects for direct thrombin inhibitors in rat venous and arterial thrombosis models (Berry et al., 1998).

Using an intravenous administration protocol in different thrombosis models, we have compared the antithrombotic activity of SSR182289A with reference antithrombotic agents.

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**Fig. 3.** Effects of different oral doses of SSR182289A (0.3–10 mg/kg p.o.; black histograms), ximelagatran (0.3–10 mg/kg p.o.; hatched histograms), and vehicle (white histograms) on thrombus weight in the rat thromboplastin-dependent arterial shunt model. Compounds were administered 30 min before shunt assembly. Thrombus weights shown are means ± S.E.M. ($n = 6–8$). *, $p < 0.05$; **, $p < 0.01$ versus vehicle group.

**Fig. 4.** Study of the time course of the antithrombotic effects of 10 mg/kg p.o. SSR182289A (■) and 10 mg/kg p.o. ximelagatran (□) in the rat thromboplastin-dependent arterial shunt model versus the vehicle-treated group, which for clarity were pooled in the figure (●). A separate group of animals was used for each time interval. Values are means ± S.E.M. ($n = 6–12$). **, $p < 0.01$ versus vehicle group.
including several (heparin, nadroparin, fondaparinux, and hirudin) for which the clinical efficacy is well documented. In addition, the reference agents chosen show different mechanistic profiles in terms of their site of inhibition of the coagulation cascade, i.e., melagatran (Gustafsson et al., 1998) and hirudin are selective thrombin inhibitors, fondaparinux (Herbert et al., 1997) and DX9065a (Herbert et al., 1996) selectively inhibit factor Xa, whereas heparin and nadroparin possess both thrombin-inhibitory and factor Xa-inhibitory properties. A further distinguishing factor is that the antithrombotic effects of heparin, nadroparin, and fondaparinux are antithrombin III-dependent. SSR182289A showed dose-dependent antithrombotic activity in all four thrombosis models following i.v. administration. SSR182289A appeared to be slightly more potent in the two stasis-dependent venous thrombosis models where the thrombus has been characterized as fibrin-rich than in the AV shunt (Peters et al., 1991), in which, in addition to fibrin and erythrocytes, platelets make a significant contribution. The arterial thrombosis model is characterized by arterial damage and platelet deposition in the context of elevated shear stress. The direct thrombin inhibitor melagatran was always among the most potent agents tested, irrespective of the model used. Heparin and hirudin also showed overall good potency. The two factor Xa inhibitors were moderately active in the “platelet-rich” models; however, in addition, fondaparinux demonstrated potent antithrombotic effects (>DX9065a) in the rat venous thrombosis model. This result is in accordance with a previous study showing superiority of fondaparinux versus DX9065a in a factor Xa-dependent rat venous thrombosis model (Herbert et al., 1996). Variations in the relative antithrombotic activities of these two factor Xa inhibitors may be due to their differential reliance on endogenous antithrombin III. In addition to mechanism of anticoagulant action, it is

TABLE 2
Antithrombotic effects of SSR182289A and ximelagatran following oral administration in the rabbit venous (Wessler) thrombosis model

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dose</th>
<th>n</th>
<th>Wet Thrombus Weight (Mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg p.o.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>6</td>
<td>164.7 ± 18.4</td>
</tr>
<tr>
<td>Ximelagatran</td>
<td>10</td>
<td>6</td>
<td>227.7 ± 23.1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5</td>
<td>41.0 ± 21.5</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>6</td>
<td>183.0 ± 14.8</td>
</tr>
<tr>
<td>SSR182289A</td>
<td>6.7</td>
<td>6</td>
<td>162.6 ± 29.5</td>
</tr>
<tr>
<td></td>
<td>8.9</td>
<td>6</td>
<td>57.1 ± 36.2*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5</td>
<td>0.0 ± 0.0*</td>
</tr>
</tbody>
</table>

* P < 0.01 versus control group.

Table 3

**TABLE 3**
Effects of SSR182289A and ximelagatran on the rat tail transection bleeding time

The compounds were administered orally 30 and 60 min prior to tail transection.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Bleeding Time (Mean ± S.E.M.)</th>
<th>% Increase</th>
<th>Bleeding Time (Mean ± S.E.M.)</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>min</td>
<td></td>
<td>min</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>10.4 ± 1.3</td>
<td>0</td>
<td>10.6 ± 0.9</td>
<td>24</td>
</tr>
<tr>
<td>SSR182289A</td>
<td>10</td>
<td>10.4 ± 1.6</td>
<td>0</td>
<td>13.1 ± 1.5</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>23.4 ± 6.1</td>
<td>125</td>
<td>22.3 ± 3.9</td>
<td>168**</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>69.9 ± 10.3</td>
<td>573**</td>
<td>66.3 ± 11.4</td>
<td>528**</td>
</tr>
<tr>
<td>Ximelagatran</td>
<td>5</td>
<td>18.3 ± 3.7</td>
<td>76</td>
<td>13.9 ± 1.7</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>16.0 ± 1.9</td>
<td>54</td>
<td>31.0 ± 6.2</td>
<td>194**</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>74.8 ± 6.2</td>
<td>620**</td>
<td>56.1 ± 12.2</td>
<td>432**</td>
</tr>
</tbody>
</table>

* P < 0.01 versus vehicle, Kruskall Wallis test (n = 7–9/group).
likely that the relative i.v. antithrombotic potency order in these studies was also influenced in part by the affinities of the different agents for their molecular targets and by pharmacokinetic factors. For example, comparison of the direct thrombin inhibitors SSR182289A and melagatran shows that melagatran possesses higher i.v. antithrombotic potency than SSR182289A but is also a more potent thrombin inhibitor in vitro (Berry et al., 2002), although this factor alone appears not to fully explain the difference in antithrombotic potency. Argatroban, which has a $K_i$ value against thrombin (Bush, 1991) similar to that reported for SSR182289A (Berry et al., 2002), shows comparable i.v. antithrombotic potency to SSR182289A in rat venous, AV shunt, and arterial thrombosis models (Berry et al., 1994). Another factor that appears to influence the relative antithrombotic properties of thrombin inhibitors is the association rate constant for the enzyme (Elg et al., 1997).

The principal finding of this article is the demonstration that SSR182289A produces dose-dependent antithrombotic activity following oral administration in five different thrombosis models. SSR182289A was most potent in the rat venous thrombosis model (ED50 $\approx$ 1 mg/kg p.o.), showed intermediate potency in the two rat AV shunt models (ED50 value between 3 and 4 mg/kg p.o.), and was least potent in the rat arterial thrombosis model (minimum active dose 5 mg/kg p.o.). This profile of antithrombotic activity corresponds globally with a more important role of thrombin in a venous thrombosis model, which is strongly dependent on fibrin deposition following coagulation activation compared with AV shunt and arterial thrombosis models for which the contribution of platelet aggregation becomes progressively more important. In clinical studies, direct thrombin inhibitors have demonstrated efficacy in both venous thrombosis (Eriksson et al., 1997) and acute coronary artery thrombosis (The Direct Thrombin Inhibitor Trialists Collaborative Group, 2002).

Ximelagatran was also orally active in the thrombosis models used in our study. To the best of our knowledge, these data represent the most detailed characterization of ximelagatran in animal thrombosis models to date. Gustafsson et al. (2001) demonstrated a significant reduction in thrombus weight in a rat model of venous thrombosis induced by stenosis plus topical application of ferric chloride following oral administration of ximelagatran (10 $\mu$mol/kg). Our study complements this report by showing the full dose-response curve for the antithrombotic effect of ximelagatran in five different thrombosis models. Conversion of the active dose values for ximelagatran in our thrombosis models (0.5–5.3 mg/kg p.o.) into molar equivalents (0.7–11 $\mu$mol/kg p.o.) demonstrates a similar potency of ximelagatran to that reported by Gustafsson et al. (2001). Overall, in our thrombosis models, ximelagatran shows a slightly higher oral potency than SSR182289A for which the active doses varied from 0.9 to 7.5 mg/kg p.o. in the different models (molar equivalents, 1.5–13 $\mu$mol/kg p.o.). The major difference between SSR182289A and ximelagatran is that SSR182289A is a thrombin inhibitor that is orally active in its own right, whereas ximelagatran is an orally bioavailable double-prodrug form of the direct thrombin inhibitor melagatran (Gustafsson et al., 1998, 2001). The starting point for the design of SSR182289A was argatroban, a selective small-molecule thrombin inhibitor devoid of significant oral bioavailability. We set ourselves the objective of introducing oral activity into this chemical series without resorting to the “prodrug” approach. Prodrugs require one or more metabolic conversions to liberate the active molecule, which, in theory, could provide additional sources of pharmacokinetic variability due to, for example, interindividual differences in metabolism or drug interactions. Our demonstration that the oral antithrombotic potency of SSR182289A approaches that of ximelagatran despite that fact that the active form of the latter, melagatran, shows significantly greater i.v. potency than SSR182289A indicates that we have achieved an antithrombotic molecule with good oral activity.

We have studied the time course of the antithrombotic effect of SSR182289A administered at 10 mg/kg p.o. in the rat AV shunt model. The effect was maximal 30 min after administration and well maintained because it was little changed at 60 and 120 min. A marked antithrombotic effect was still observed 4 h after administration of SSR182289A. This long duration of action is in agreement with the prolonged anticoagulant effects observed after oral administration of SSR182289A in rats, rabbits, macaques ($\geq$6 h), and dogs ($\geq$8 h) (Berry et al., 2002). In the rat AV-shunt thrombosis model, the same dose of ximelagatran also gave a significant antithrombotic effect at 4 h, although the rate of decline of this activity with time appeared more rapid than with SSR182289A.

Increased risk of hemorrhage is a recognized potential side effect of antithrombotic treatments. To evaluate this aspect of the profile of SSR182289A, we have used the rat tail transection model, which is well documented in the literature and has been used to evaluate antithrombotic agents of different mechanistic classes including thrombin inhibitors (Berry et al., 1994, 2001; Herbert et al., 1996). Despite its widespread use, however, extrapolation of this model to the clinical setting should be performed with caution. At relatively high oral doses ($\geq$10 mg/kg), both SSR182289A and ximelagatran produced significant increases in tail transection bleeding time. This dose range is significantly higher than that necessary for antithrombotic efficacy in the rat venous thrombosis model.

In conclusion, we have demonstrated that SSR182289A, a new small molecule thrombin inhibitor, possesses potent oral antithrombotic properties in animal models of venous, “mixed”, and arterial thrombosis.

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