SR 121787, a New Orally Active Fibrinogen Receptor Antagonist

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Summary

The aim of this study was to describe the pharmacological properties of SR 121787, a new antiaggregating drug which is metabolized in vivo into SR 121566, a potent non-peptide antagonist of Gp IIb/IIIa. In vitro, SR 121566 antagonized the binding of [125I]-fibrinogen (IC50 = 19.8 ± 6.3 nM) and of [125I]-L-692,884, an RGD-containing peptide (IC50 = 291 ± 96 nM) to activated human platelets. SR 121566 inhibited the aggregation of human platelets induced by ADP, collagen, thrombin, arachidonic acid and PAF at concentrations lower than 0.1 μM. Adhesion of human platelets to adhesive proteins was inhibited by SR 121566 (IC50 = 40.3 ± 2.5 nM) only when Gp IIb/IIIa and fibrinogen were involved. No effect was found with regard to other adhesive proteins and/or other integrins. SR 121787 demonstrated a potent and sustained antiaggregating effect when administered intravenously to baboons at a dose 50 μg/kg, and eight hours after the administration of 100 μg/kg, ADP-induced aggregation was still strongly inhibited (more than 80%). A single oral administration of 2 mg/kg of SR 121787 produced a nearly complete inhibition of platelet aggregation for up to 8 h (ED50 at 8 h = 193 ± 20 μg/kg), a significant residual antiaggregating activity being still observed 24 h after the administration. When administered orally to rabbits, SR 121787 exhibited a potent antiaggregating (ED50 = 2.3 ± 0.3 mg/kg) and antithrombotic activity in an arterio-venous shunt thrombosis model (ED50 = 10.4 ± 0.8 mg/kg). After oral and IV administration, SR 121787 was well tolerated suggesting that SR 121787, the most potent and long lasting orally active Gp IIb/IIIa antagonist described to date, is a promising antithrombotic compound.

Introduction

In several clinical trials, long term antiplatelet therapy has been shown to reduce the incidence of myocardial infarction, stroke and mortality in patients at high risk for occlusive vascular disease (1-3). Therefore, research toward the development of more effective antiaggregant and anticoagulant agents or combination of both types is intensifying. The final common pathway in the formation of platelet aggregates is the cross-linking of adjacent activated platelets by binding of fibrinogen to the platelet Gp IIb/IIIa receptor complex (referred to in the integrin nomenclature as αIIbβ3) (4, 5). Theoretically, the prevention of occlusive arterial lesions may be achieved by modulating the reactivity of platelets or by inhibiting the interaction of the activated Gp IIb/IIIa receptor with its ligand. Indeed, there is compelling evidence that adhesive proteins bind to the Gp IIb/IIIa complex and that platelet aggregates are integral components of thrombus formation in vivo (6, 7). The Gp IIb/IIIa complex provides a logical site for pharmacological interventions directed towards an inhibition of platelet aggregation and arterial thrombus formation. Several RGD containing peptides, either synthetic or derived from snake venoms (disintegrins), are known to block fibrinogen binding on platelets and to prevent the formation of platelet thrombi (7-9). Furthermore, blockade of the Gp IIb/IIIa receptor with monoclonal antibodies has been proven to be anti-aggregatory in experimental (10-12) and clinical studies (6, 13-16). However, in addition to the restriction to the parenteral use, some problems are associated with the use of peptides and antibodies as antithrombotic agents, including their short half-life, their potential antigenicity, their poor selectivity and their metabolic instability in the circulation which clearly poses a burden in terms of dosing. More recently, in an effort to achieve higher antithrombotic efficacy and better selectivity at the site of the Gp IIb/IIIa receptor, peptidomimetic and non-peptide RGD mimetics have been developed (17-19) but most of these could only be administered intravenously whereas, for chronic therapy, orally active Gp IIb/IIIa antagonists are required.

The aim of the present experimental study was to examine the antplatelet effects of the new orally active non-peptide Gp IIb/IIIa antagonist SR 121787. SR 121787 is a prodrug, which is readily absorbed after oral administration and converted to the active moiety SR 121566. SR 121566 exerted a high affinity and specificity for the Gp IIb/IIIa complex resulting in a strong inhibition of fibrinogen binding to platelets and a potent inhibition of platelet aggregation. After single oral administration to baboons, SR 121787 caused an antiplatelet effect that was more potent and lasted longer than the effects of other Gp IIb/IIIa antagonists reported to date in the literature (17-19). These data suggest that SR 121787 has a therapeutic potential as an antithrombotic agent in coronary, cerebrovascular and peripheral artery thrombotic disorders.

Preliminary results of this study have been presented at the 14th International Congress on Thrombosis in Montpellier, France, October 14-19, 1996.

Methods

Reagents

SR 121787 (3-[[4-[(amino-ethoxycarbonylimino-methyl)-phenyl]-1,3-thiazol-2-yl]-(1 ethoxycarbonylmethyl-piperidin-4-yl)-amino])-propionic acid ethyl ester) and SR 121566 (3-[[4-[(amino-imino-methyl)-phenyl]-thiazol-2-yl]-(1-carboxymethyl-piperidin-4-yl)-amino])-propionic acid (Fig. 1) were from Sanofi Recherche (Toulouse, France). Peptides RGDS (Arg-Gly-
Asp-Ser) and cyclic GPenGRGDSPCA (Gly-Penicillamine-Gly-Arg-Gly-Asp-Ser-Pro-Cys-Ala) were synthesised by Neosystem (Strasbourg, France). 35S-methionine (>1000 Ci/mmol) and 125I-fibrinogen (200 Ci/mg) were from Amersham (Amersham, UK). 125I-PIB labeled L-692,884 (cyclo-4-125I-henzoyl-(Cys-Asn-Pro-Arg-Gly-Asp-Cys)-OH) (2200 Ci/mmol) was from DuPont (Boston, MA, USA). Arachidonic acid was purchased from Nu Check Prep (Interchim, Montluçon, France). Human a-thrombin was from the Centre de Transfusion Sanguine (Strasbourg, France) and platelet activating factor (C16-PAF) was from Bachem (Bubendorf, Switzerland). Adenosine 5'-diphosphate (ADP), human collagen and human fibrinogen were from Stago (Sanguine, Toulouse, France). Human umbilical vein endothelial cells (HUVEC) used in this study were purchased from Clonetics (Tebu, Le Perray en Yvelines, France). They were routinely cultured in 75 cm² flasks coated with human fibronectin (5 µg/cm²) in RPMI 1640 medium containing 10% fetal calf serum (FCS), antibiotics (penicillin 100 IU/ml, streptomycin 100 µg/ml), heparin (100 µg/ml) and endothelial cell growth supplement (30 µg/ml). The human erythro-leukemic cells (HEL) AD1 and 5J20 were kindly provided by Dr N. Kieffer (CRP-Santé, Luxembourg). They expressed Gp IIb/IIa and αIβI complexes and cultured in RPMI 1640 medium containing 10% FCS as described by Kieffer et al. (20). Platelet concentrates were obtained from the Centre Régional de Transfusion Sanguine (Toulouse, France).

Cells

Human umbilical vein endothelial cells (HUVEC) used in this study were purchased from Clonetics (Tebu, Le Perray en Yvelines, France). They were routinely cultured in 75 cm² flasks coated with human fibronectin (5 µg/cm²) in RPMI 1640 medium containing 10% fetal calf serum (FCS), antibiotics (penicillin 100 IU/ml, streptomycin 100 µg/ml), heparin (100 µg/ml) and endothelial cell growth supplement (30 µg/ml). The human erythro-leukemic cells (HEL) AD1 and 5J20 were kindly provided by Dr N. Kieffer (CRP-Santé, Luxembourg). They expressed Gp IIb/IIa and αIβI complexes and cultured in RPMI 1640 medium containing 10% FCS as described by Kieffer et al. (20). Platelet concentrates were obtained from the Centre Régional de Transfusion Sanguine (Toulouse, France).

Animals

Male New Zealand rabbits (Lago, Vonnas, France) weighing between 3.5 and 4.0 kg and male baboons (Papio Ursinus) weighing between 7 and 9 kg provided by CAPE (Capetown, South Africa) were used in this study. Animals had free access to water but were fasted from 16 h before to 8 h after the administration of compounds.

In Vitro Effects on Human Platelet Fibrinogen Receptor

The binding of 125I-fibrinogen on gel filtered human platelets, was studied according to the method of Foster et al. (21). Fresh platelet concentrate was centrifuged (900 × g, 15 min) and the platelet pellet was resuspended (2 × 10⁹ cells/µl) in modified Tyrode’s (MT) solution (NaCl 103 mM, KCl 2.4 mM, NaHCO³ 25.2 mM, dextrose 22 mM, MgCl₂ 0.9 mM, L-histidine 21.9 mM, citric acid 1.9 mM, trisodium citrate 11 mM). Platelet suspension was incubated for 30 min with hirudin (10 U/ml), fructose (1.75 mM), CaCl₂ (2 mM) and PGI₂ (10 µM) at room temperature under gentle stirring, washed once in the same volume of MT (+ 10 U/ml hirudin and 1.75 mM fructose) and gel filtered on sepharose. Two ml of the platelet suspension were injected at the top of a plastic eoncoclum (Bio-Rad, Ivory sur Seine, France) containing 1 ml of sepharose gel CL-2B (Pharmacia, Uppsala, Sweden) equilibrated in MT buffer.

Platelets were then diluted in MT, and samples containing 50 × 10⁶ cells were incubated at 37°C, with 125I-human fibrinogen (2 µg/ml), CaCl₂ (2 mM), ADP (20 µM), epinephrine (20 µM), in the presence of the tested compounds for 15 min (final volume 250 µl). Bound ligand was separated from free ligand by centrifuging 200 µl of the incubate (15,000 × g, 2 min) onto 150 µl of dioctyl phthalate/dibutyl phthalate (6/4, v/v) (Aldrich, L’Isle d’Abeau, France), at 4°C, in a microsedimentation tube (Sarstedt, Nümbrecht, Germany). The tube bottom, containing platelet pellet, was cut by a razor blade and radioactivity measured in a gamma counter (1261 Multigamma, Wallace, Turku, Finland). Non specific binding was measured in the presence of 1 mM RGDS (Neosystem, Strasbourg, France).

Binding of the radiolabeled RGD-containing peptide (125I-L-692,884) was studied under similar conditions. Gel filtered platelets in MT buffer were incubated with 0.2 nM of the ligand, CaCl₂ (2 mM), ADP (0.2 µM, epinephrine (0.2 µM)) in the presence of the tested compounds at room temperature for 15 min Separation of bound from free ligand was done by centrifugation on phthalate mixture as described above.

In Vitro Antiaggregating Activity

Platelet rich plasma (PRP) was obtained by centrifugation (1000 × g, 10 min) of citrated (0.38% final concentration) blood from human volunteers who had not taken any medication for at least 2 weeks, or from hirudinized (10 U/ml final concentration) blood from male baboons. Platelet aggregation was measured on PRP (diluted 2 fold in saliné for thrombin activation) in an aggregometer (ChronoLog, Havertown, PA, USA), after activation by various agonists (for human platelets: ADP 2.5 µM, collagen 12.5 µg/ml, thrombin 0.1 U/ml, arachidonic acid 500 µM, PAF 0.5 µM; for baboon platelets: 2.5 µM ADP) according to the method of Born (22). The extent of aggregation was quantified by measuring the maximum height of the curve, except for collagen where aggregation was evaluated by the measurement of the slope factor. Drugs were added 1 min. before activation, in a DMSO solution (0.01% final concentration). The antiaggregating activity was expressed as a percent inhibition by comparison with aggregation measured in the presence of the vehicle alone.

Cell Adhesion Assays

HUVEC as well as HEL AD1 and SJ20 cells were cultured in RPMI 1640 medium (Seromed, Poly Labo, Strasbourg, France) supplemented with 10% FCS. Prior to the adhesion assay, cells were rinsed twice and labeled in methionine-free RPMI 1640 medium (Seromed, Poly Labo, Strasbourg, France) in the presence of 35S-methionine (20 µCi per 10⁶ cells for AD1 and SJ20 cells, and 150 µCi per 10⁶ cells for HUVEC) for 24 h at 37°C.

Adhesion assays were performed in 96 microwell polyethylene cluster plates for 35S-methionine-labeled cells or in polysytrene flat bottom microplates for platelets. Plates were coated for 5 h at 37°C with the various adhesive proteins in solution in RPMI 1640 (200 µl/well). The following concentrations were used: fibrinogen and collagen 10 µg/ml, vitronectin 5 µg/ml. Plates were then rinsed with RPMI 1640 and incubated overnight with HSA (1 mg/ml) at 4°C. Control plates were prepared by incubating with HSA only. Plates were rinsed with phosphate buffered saline (PBS) and stored at 4°C until use.

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Adherent radiolabeled HEL cells or HUVEC were incubated for 5 min. in PBS, 1 mM EDTA, rinsed twice and resuspended in RPMI 1640 at a concentration of $5 \times 10^5$ cell/ml. Cells were incubated for 30 min at 4°C, in the presence or absence of the tested compounds, and distributed in the coated plates (100 μl/well). After 2 h at 37°C, non-adherent cells were removed and wells were washed twice with 100 μl of RPMI 1640. Radioactivity in the microplates was read in a scintillation counter (Microbeta Wallac, Turku, Finland).

Gel filtered human platelets were prepared from fresh human citrated blood, as described above. Washed platelet suspension ($35 \times 10^9$ platelets/ml) was incubated with the tested compounds in coated microplates for 60 min at room temperature. Non-adherent cells were removed and the plates were washed twice with PBS. Acid phosphatase activity was measured in each well, according to the method of Bellavite et al. (1994). Briefly, 150 μl of 0.1 M citrate buffer (pH 5.4) containing 5 mM p-nitrophenyl phosphate and 0.1% Triton X-100 were added into the micro wells. After 1 h of incubation at 25°C, the reaction was stopped by adding 100 μl of 2 N NaOH. Optical density was measured at 405 nm in a Tweenreader microplate reader (Flow Laboratories, Lugano, Switzerland). The number of adhered platelets was calculated from a standard curve and the specific adhesion was obtained by subtracting the number of platelets detected in HSA-coated plates from the number obtained in the adhesive protein-coated plates.

Ex Vivo Antiaggregating Effect

Platelet aggregation was measured in PRP from baboons or rabbits treated by the various compounds administered either IV (drugs dissolved in saline, 1 ml/kg) or by oral route (drugs dissolved in 1% ethanol, 5 ml/kg). Blood samples (4.5 ml) were taken from the femoral veins (alternatively from the brachial vein) using hirudin as the anticoagulant (100 U/ml). PRP was obtained by centrifugation (800 g, 15 min.) and ADP (2.5 μM)-induced platelet aggregation was performed in duplicate in a Coultronics aggregometer according to Born (22). The maximum extent of aggregation was measured for 3 min and the percent inhibition was calculated by comparison with the aggregation of PRP from each animal withdrawn just prior to the treatment. This protocol has been approved by the Comité d’Expérimentation Animale (Animal Care and Use Committee) of Sanofi Recherche.

Pharmacokinetic Studies

Pharmacokinetic studies were performed with blood samples from baboons treated with SR 121566 or SR 121787 by IV or oral route. Concentration of circulating active metabolite(s) was estimated in a bioassay by measuring the antiaggregating activity of plasma from treated animals on hirudinized PRP from untreated baboons, and expressed as nM equivalent of SR 121566. Briefly, 200 μl of control PRP was diluted with 200 μl of the tested plasma and incubated for 2 min. at 37°C. Then, aggregation was measured after induction by 2.5 μM ADP as described above. When inhibition exceeded 80%, the tested plasma was diluted in its respective control plasma (obtained before the treatment). SR 121566 concentration in the tested plasma was determined as a concentration of SR 121566, calculated from the in vitro concentration/effect relationship and corrected by the dilution factor.

In Vivo Arterial-type Thrombosis Model (Arteriovenous Shunt)

The oral antithrombotic activity of SR 121787, clopidogrel, ASA and heparin was determined in an arteriovenous shunt model in the rabbit as previously described (23). In brief, two hours after the oral administration of the indicated doses of SR 121787 two 12-ml polyethylene tubings (0.6/0.9 mm inner/outer diameter) were placed between the right carotid artery and the left jugular vein of pentobarbital-anesthetized (30 mg/kg iv) animals. The tubes were linked to a central part (6 cm long, 0.9 mm inner diameter) containing a 5 cm silk thread and filled with a heparin saline solution (50 IU/ml). Continuous blood flow was restored during a 20-min observation period. After 20 min, the central part of the shunt was removed, the silk thread carrying the thrombus was pulled out, and the wet weight of the thrombus was determined.

Data Analysis

Results are presented as mean ± S.D. Concentrations and doses inhibiting 50% of the effects ($IC_{50}$ and $ED_{50}$) were calculated according to Ratkovsky and Reedy (24) using the 4-parameter logistic model. The adjustment was obtained by non-linear regression using the Levenberg-Marquardt algorithm (25). Areas under the curve (AUCO-$\infty$) were calculated by extrapolation from the 8 first hours pharmacokinetic data with the trapezoidal method. Statistical evaluation of ex vivo data was performed by the T test of Wilcoxon taking p < 0.05 to indicate a significant difference. All calculations were performed using validated in house programs on the RSI/1 software (BBN, Cambridge, MA, USA).

Results

In Vitro Experiments

Binding of $^{125}$I-fibrinogen and $^{125}$I-L 692,884 to Activated Human Platelets

As shown in Table 1, SR 121566 inhibited the binding of $^{125}$I-fibrinogen to activated human gel filtered platelets in a dose-dependent manner. The concentration which inhibited 50% of specific binding ($IC_{50}$) was 19.8 ± 6.3 nM. SR 121566 also inhibited the binding of $^{125}$I-L 692,884, a cyclic RGD-containing peptide, to activated platelets ($IC_{50}$ = 291 ± 96 nM). When calculated from the Cheng and Prusoff equation, the $K_i$ values of SR 121566 for the specific binding of $^{125}$I-fibrinogen and $^{125}$I-L 692,884 to activated human platelets were 19.5 ± 6.2 and 94.8 ± 31 nM respectively. The Schild analysis of SR 121566 inhibition of $^{125}$I-fibrinogen and $^{125}$I-L 692,884 binding to human platelets was linear and of unit one slope (slope = 1.04 and 0.96), indicative of competitive binding at a single high affinity binding site. SR 121566 not only prevented $^{125}$I-fibrinogen and $^{125}$I-L 692,884 binding but also displaced the radiolabelled ligands bound to activated platelets (not shown).

Platelet Aggregation

As shown in Table 1, SR 121566 inhibited human platelet aggregation in a concentration-dependent manner whatever the agonist used. The $IC_{50}$ values for the various agonists ranged between 23 and 68 nM. This effect was reversible, a normal level of aggregation being restored when platelets were washed and resuspended for 1 h at 20°C in control plasma (not shown). With regard to ADP (2.5 μM)-induced aggregation of PRP from baboons, SR 121566 exhibited almost the same activ-

Table 1 Effect of SR 121566 on human platelet aggregation

<table>
<thead>
<tr>
<th>Agonists</th>
<th>concentration (μM)</th>
<th>$IC_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>2.5 μM</td>
<td>46 ± 11</td>
</tr>
<tr>
<td>Collagen</td>
<td>12.5 μg/ml</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>Thrombin</td>
<td>0.1 μU/ml</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>Anachidonic acid</td>
<td>500 μM</td>
<td>56 ± 6</td>
</tr>
<tr>
<td>PAF</td>
<td>0.5 μM</td>
<td>68 ± 7</td>
</tr>
</tbody>
</table>

Aggregation of human platelets was induced by various agonists at the indicated concentrations. Results are expressed as mean ± SD calculated from 3 different experiments.
Adhesion of HEL cells expressing the Gp IIb/IIIa (5J20) or the αvβ3 complexes (AD1) was measured as described in Materials and Methods. Results are expressed as mean ± SD calculated from 3 different experiments.

The specificity of SR 121566 for the Gp IIb/IIIa-fibrinogen interaction was further confirmed in a cellular model, using HEL cells lineage. 5J20 cells are HEL cell variants expressing the α2b-β3 but lacking αv-β3 complex, whereas AD1 are HEL cells expressing both of them. These cells were used for they adhesive properties, either on fibrinogen or vitronectin coated plastic, which have been demonstrated by Kieffer et al (20) to be relevant of the expression of Gp IIb-IIIa and αv-β3 respectively. AD1 cell adhesion to vitronectin was not inhibited by SR 121566 at concentrations up to 100 μM, whereas 5J20 cell adhesion to coated fibrinogen was strongly antagonized by co-incubation with SR 121566 (IC50 = 19.2 ± 1.3 nM) (Table 2). The peptide RGDS affected the adhesion of both cell types with the same potency whereas the cyclic synthetic peptide GPenGRGDSPCA was more active in inhibiting the adhesion of AD1 cells to vitronectin (Table 2). These results indicate that RGD integrins may be involved in HEL cell-substrate interactions.

The adhesion of HUVEC (cells which do not express the Gp IIb/IIIa complex) to various RGD-containing adhesive proteins such as fibrinogen, vitronectin, von Willebrand factor, fibronectin, and laminin was not affected by SR 121566 at concentrations up to 100 μM. SR 121566 did not affect the attachment of subconfluent HUVECs to all the tested matrixes when compared with cells treated with buffer and, after a 24 h incubation with SR 121566, no differences in adhesion were observed between the exposed and control cells, whereas RGDS inhibited the interactions of HUVEC with vitronectin, von Willebrand factor and fibronectin (IC50 = 11 μM, 34 μM and 1 mM respectively). These observations show that SR 121566 is a selective inhibitor of the Gp IIb/IIIa-fibrinogen interaction and does not affect the other adhesive systems.

**Table 2** Effect of SR 121566 on the adhesion of transfected HEL cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 5J20 / fibrinogen (nM)</th>
<th>IC50 AD1 / vitronectin (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR 121566</td>
<td>10.2 ± 1.3</td>
<td>&gt;10¹⁰</td>
</tr>
<tr>
<td>RGDS</td>
<td>16 ± 3</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>GPenGRGDSPCA</td>
<td>17 ± 0.3</td>
<td>0.1 ± 0.02</td>
</tr>
</tbody>
</table>

Adhesion of HEL cells expressing the Gp IIb/IIIa (5J20) or the αvβ3 complexes (AD1) was measured as described in Materials and Methods. Results are expressed as mean ± SD calculated from 3 different experiments.

**Fig. 2** Effect of an IV administration of SR 121566 on ADP-induced platelet aggregation in baboons. Male baboons received IV SR 121566 at 10 (▲), 30 (▼) or 100 μg/kg (●). Inhibition was calculated by comparison with aggregation measured with PRP obtained from the same animal before the treatment. Results represent mean ± S.D. of 4 different experiments

**Fig. 3** Effect of an IV administration of SR 121787 on ADP-induced platelet aggregation in baboons. Male baboons received intravenously SR 121787 at 50 (▲), 100 (▼) and 200 μg/kg IV (●). Inhibition was calculated by comparison with aggregation measured with PRP obtained from the same animal before the treatment. Results are mean ± SD of 3 to 6 different experiments

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In Vivo Experiments

Antiplatelet Effect of SR 121566 in Baboons

The \textit{ex vivo} antiaggregating activity of SR 121566 was evaluated in baboons, following a bolus IV administration of 10, 30, or 100 \(\mu\)g/kg. ADP-induced aggregation was measured at different time points and compared with the aggregation measured before the treatment. Under these experimental conditions, no significant changes of platelet aggregation were seen in vehicle-treated animals during the experimental period (not shown). SR 121566 exhibited a dose-dependent antiaggregating effect (Fig. 2). At the lowest dose (10 \(\mu\)g/kg, IV), SR 121566 inhibited platelet aggregation almost completely immediately after administration for 8 hours. The ID\(_{50}\) (dose which inhibited 50\% of platelet aggregation) at 8 h was 24 ± 3.3 \(\mu\)g/kg. The duration of the anti-platelet effect of SR 121566 was dose-dependent. After the administration of 100 \(\mu\)g/kg, a significant antiaggregating effect (56\% inhibition, \(p < 0.05\)) was still observed after 24 h. Following single oral administration of 500 \(\mu\)g/kg SR 121566 to baboons, a significant but low antiaggregating effect (35\% inhibition, \(p < 0.05\)) was observed 4 h after the administration (data not shown). However, the antiaggregating activity obtained after oral administration of SR 121566 was much lower than that observed following IV administration.

Antiplatelet Effect of SR 121787 in Baboons

Following bolus IV administration of 50, 100 or 200 \(\mu\)g/kg of SR 121787, platelet aggregation was significantly inhibited at 30 min. (Fig. 3). For the lowest dose, the maximum effect (60\% inhibition) was reached within 2 h whereas doses of 100 and 200 \(\mu\)g/kg produced a potent antiaggregating effect exceeding 75\% inhibition between 1 and 4 h and the inhibitory activities remained higher than 60\% up to 8 hours after the administration. At this time point, the ID\(_{50}\) value for the aggregation inhibition was 51 ± 19 \(\mu\)g/kg. A significant antiaggregating activity remained 24 h after a single IV administration of 100 or 200 \(\mu\)g/kg of SR 121787 (47\% and 59\% inhibition, respectively, \(p < 0.05\)). Following an oral administration of a single dose of 125 \(\mu\)g/kg of SR 121787, a significant antiaggregating activity (26\% inhibition, \(p < 0.05\)) was observed as soon as 15 min. after treatment (Fig. 4A). The maximum effect (68\% inhibition) with this dose was reached at 4 h. At this time point, a nearly maximum inhibition (96\%) was observed with 500 \(\mu\)g/kg, and a significant inhibition (28\%, \(p < 0.05\)) was still detected 16 h after SR 121787 treatment. Higher doses produced a more prolonged effect which persisted for up to 24 h following the treatment (43\% inhibition with 2 mg/kg, \(p < 0.05\)) (Fig. 4A). The ID\(_{50}\) value at 8 h after oral SR 121787 administration was 193 ± 20 \(\mu\)g/kg (Fig. 4B).

Pharmacokinetic Studies

In order to evaluate the pharmacokinetic parameters of SR 121566 and SR 121787 after IV or oral administration, the plasma concentration of the active moiety (SR 121566) was evaluated in a bioassay and expressed as SR 121566 equivalent. After IV bolus administration of SR 121566 (25, 50 or 100 \(\mu\)g/kg) to baboons, the plasma concentration decreased gradually within 8 h (see for instance 50 \(\mu\)g/kg in Fig 5A). When expressed as estimated plasmatic concentration of SR 121566 equivalent, calculated half-life (\(T_{1/2}\)) was 2.35 ± 0.57 h, volume of distribution was 0.581 ± 0.105 l/kg and clearance was 0.178 ± 0.031 l/kg h\(^{-1}\) (mean of the 3 doses ± SD, \(n = 2\) for each dose).

Following IV (200 \(\mu\)g/kg, IV) or oral administration (1 mg/kg, PO) of SR 121787, the concentration of SR 121566 equivalent in the plasma increased gradually during the first 2 to 4 h (Fig. 5A). The concentration of SR 121566 equivalent in the plasma from orally or IV SR 121787-treated animals increased in a dose-dependent manner proportional to the IV or oral doses. The AUC of the circulating concentration of SR 121787 was evaluated to be 47\%. By comparing the AUC of SR 121787 after oral and IV administration, an oral bioavailability of 27\% was determined.
Antiplatelet Effect and Antithrombotic Efficacy of SR 121787 in Rabbits

Ex-vivo platelet aggregation induced by ADP was inhibited in a dose-dependent manner 2 h after oral SR 121787 treatment of rabbits (Table 3). An approximately 90% inhibition of platelet aggregation was observed at the dose of 20 mg/kg. The $ED_{50}$ values for ADP-induced aggregation and amounted to 2.3 ± 0.3 mg/kg.

The antithrombotic effect of SR 121787 in an arteriovenous shunt model is demonstrated in Table 3. Oral SR 121787 pretreatment 2 h prior to shunt insertion resulted in a dose-dependent inhibition of thrombus growth. A maximum antithrombotic efficacy of 60% was observed at SR 121787 doses of 20 mg/kg; the $ED_{50}$ value was 10.4 ± 0.8 mg/kg.

Discussion

In this report, we compared the biochemical and pharmacological effects of SR 121787, with those of two other reference compounds recognized as the most potent non peptide Gp IIb/IIIa antagonists (18). SR 121787 is a produg whose active metabolite (SR 121566) is a potent and selective antagonist of the major fibrinogen receptor, the activated Gp IIb/IIIa complex. In vitro, SR 121566 competitively antagonized the binding of $^{125}$I-fibrinogen and of an RGD-containing peptide, $^{125}$I-L 652,884 on activated human platelets which is in accordance with an inhibition of the fibrinogen binding through the occupation of an RGD-binding site on the Gp IIb/IIIa complex. SR 121566 also demonstrated a high activity to inhibit human platelet adhesion to fibrinogen in vitro. SR 121566 was equally effective in inhibiting human platelet aggregation induced by different stimuli in vitro. The comparable platelet aggregation inhibition with different inducers was expected considering the mechanism of action of SR 121566. Since fibrinogen binding is essential for platelet aggregation regardless of the stimulus and since SR 121566 inhibits this step, the inhibition of aggregation is expected to be similar for all agonists. This provides an advantage for SR 121566 over inhibition with other antagonists such as aspirin, ticlopidine or thromboxane antagonists which are agonist-dependent.

Since SR 121566 inhibited fibrinogen binding to platelet Gp IIb/IIIa through an RGD binding site and because several integrins other than Gp IIb/IIIa bind to their extracellular matrix protein ligands through an RGD domain, it was important to determine the specificity of SR 121566 for Gp IIb/IIIa relative to other RGD-dependent integrin-mediated events. In particular, the vitronectin receptor ($\alpha_v\beta_3$) and Gp IIb/IIIa share the same $\beta_3$ subunit. Nevertheless, SR 121566 demonstrated a high degree of selectivity toward the platelet Gp IIb/IIIa receptors compared with other closely related receptors on platelets, HEL cells or on endothelial cells whereas RGDbinding peptides affected cell adhesion in several other models. The inhibition of platelet aggregation in vivo was confirmed ex vivo following single IV or oral administration to baboons. The antiplatelet effects of SR 121787 were observed at extremely low dose levels and it was shown that the antagonism of the platelet Gp IIb/IIIa receptors in baboons by SR 121787 had no effect on hemostasis parameters and blood cell counts over the wide range of doses administered. No spontaneous bleeding was observed at any sites. These data suggest that SR 121787 is a potent and orally active antiplatelet agent. Additionally, the high affinity and specificity of SR 121566 for platelet Gp IIb/IIIa complex might explain the observed optimal efficacy/safety ratios. The plasma concentrations of estimated SR 121566 equivalent at which ex vivo platelet aggregation was...
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Single doses of SR 121787 were administered orally. Platelet aggregation induced by 2.5 μM ADP and thrombus formation in an arterio-venous shunt were measured 2 h later as described in "Methods". Results are expressed as percent inhibition of platelet aggregation or thrombus growth in a simultaneously investigated vehicle-treated control group. The average thrombus wet weight in this group amounted to 36.8 ± 2.8 mg (n = 11). Each point represents the mean ± SEM of 5 animals (* p < 0.05 compared with control animals).

Table 3 Effect of SR 121787 on rabbit platelet aggregation ex vivo and on thrombus formation in an arteriovenous shunt

<table>
<thead>
<tr>
<th>Doses (mg/kg, po)</th>
<th>% inhibition of platelet aggregation</th>
<th>% inhibition of thrombus formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>22 ± 3</td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>54 ± 9*</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>5.0</td>
<td>62 ± 13*</td>
<td>36 ± 9*</td>
</tr>
<tr>
<td>10.0</td>
<td>78 ± 4*</td>
<td>51 ± 8*</td>
</tr>
<tr>
<td>15.0</td>
<td>81 ± 2*</td>
<td>-</td>
</tr>
<tr>
<td>20.0</td>
<td>92 ± 5*</td>
<td>62 ± 10*</td>
</tr>
</tbody>
</table>

Inhibited were comparable to those observed for SR 121566 to inhibit platelet aggregation in vitro. The plasma clearance of SR 121566 (0.178 l/kg.h⁻¹) was low demonstrating that SR 121566 is not metabolized rapidly by the liver. This confirms data obtained in vitro on hepatic microsomes (not shown). However, despite a low clearance, a low volume of distribution and a long T½, the oral absorption of SR 121566 was too low and precluded the use of SR 121566 as a potent orally active inhibitor of platelet aggregation.

Since, for purposes of safety, it is desirable that a large fraction of the administered compound be absorbed, SR 121787 was made as a pro-drug of SR 121566. Oral administration of SR 121787 produced inhibition of ex vivo platelet aggregation with a relatively high onset and with an elimination time course similar to that seen after IV administration of SR 121566. Pharmacokinetic analysis demonstrated that the onset of action of a single oral and IV administration of SR 121787 were similar, therefore suggesting that oral absorption is not the limiting step of SR 121787’s activity but rather its metabolism into SR 121566.

It is generally assumed that the rabbit is an inappropriate species for investigations with GP IIb/IIIa antagonists (27, 28). Present results show, however, that SR 121566, contrary to other GP IIb/IIIa antagonists, exhibited a potent in vitro antiaggregatory activity in rabbit platelets (this study and 29). Though, the potency of SR 121566 to inhibit ADP-induced platelet aggregation in vitro was more than one order of magnitude lower for rabbit platelets than for platelets of baboons and humans. At present, we cannot explain the variance in the inhibitory activity between SR 121566 and the other two GP IIb/IIIa antagonists on rabbit platelets but a possible explanation is that variability in the molecular mechanism of GP IIb/IIIa activation among species may result in a different sensitivity to GP IIb/IIIa antagonists with diverse chemical structure.

SR 121787 potently inhibited ex vivo ADP-induced platelet aggregation but was equally effective against the three stimuli ADP, AA and collagen (29). The arteriovenous shunt model was used to examine the effects of SR 121787 on arterial-type thrombus formation. Developing thrombi in this type of model contain large platelet agglomerates, all originating on the thrombogenic silk thread, surrounded by red blood cells and fibrin (30, 31). In this study, it was shown that SR 121787 inhibited thrombus formation on the silk thread in a dose-dependent manner. A linear correlation between the ex vivo antiaggregatory activity and the antithrombotic efficacy of SR 121787 was observed. This relationship confirms the platelet dependency of the employed rabbit arteriovenous shunt model and is in accordance with the view that the mechanism by which SR 121787 exerts its antithrombotic effect in the arteriovenous shunt model is the inhibition of the interaction between the platelet GP IIb/IIIa complex and fibrinogen.

The maximum antithrombotic effect of SR 121787 was about 60%, although platelet aggregation was inhibited by about 90% at this dose. This restricted antithrombotic efficacy of SR 121787 may be due to the contribution of the clotting system in the formation of the thrombus on the silk thread. The relative importance of the coagulation system in the present model was demonstrated by the antithrombotic activity of heparin (not shown). Therefore, the present study provides for the first time a comparative in vivo evaluation between a GP IIb/IIIa antagonist and other antiplatelet drugs in rabbits.

Thus, the present data suggest that SR 121787, due to a combination of a long T½ and a high bio-availability and safety will favor its use as an orally active GP IIb/IIIa antagonist for chronic use as an effective antithrombotic agent in coronary and peripheral artery thromboembolic disorders.

References

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