Restenosis Following Angioplasty in the Swine Coronary Artery Is Inhibited By an Orally Active PDGF-Receptor Tyrosine Kinase Inhibitor, RPR101511A

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**Background**—Platelet-derived growth factor (PDGF), a purported mediator of arterial response to injury, stimulates proliferation, chemotaxis, and matrix production by activation of its membrane receptor tyrosine kinase. Because these activities underlie restenosis, inhibition of the PDGF-receptor tyrosine kinase (PDGFr-TK) is postulated to decrease restenosis.

**Methods and Results**—RPR101511A is a novel compound which selectively and potently inhibits the cell-free and in situ PDGFr-TK and PDGFr-dependent proliferation and chemotaxis in vascular smooth muscle cells (VSMC). To evaluate the effect of RPR101511A (30 mg · kg⁻¹ · d⁻¹ BID for 28 days following PTCA) on coronary restenosis, PTCA was performed in hypercholesterolemic minipigs whose left anterior descending (LAD) coronary artery had been injured by overdilation and denudation, yielding a previously existing lesion. Angiographically determined prePTCA minimal lumen diameters (MLD) were similar in vehicle and RPR101511A-treated pigs (1.98 ± 0.09 versus 2.01 ± 0.08 mm) and increased to the same extent in the 2 groups following successful PTCA (2.30 ± 0.06 versus 2.52 ± 0.13). At termination, there was an average 50% loss of gain in the vehicle-treated group but no loss of gain with RPR101511A (2.16 ± 0.05 versus 2.59 ± 0.11, P < 0.001). Morphometric analysis of the LAD showed that RPR101511A caused a significant decrease in total intimal/medial ratio (0.96 ± 0.58 versus 0.67 ± 0.09, P < 0.05).

**Conclusions**—RPR101511A, which acts by inhibition of the PDGFr-TK, completely prevented angiographic loss of gain following PTCA and significantly reduced histological intimal hyperplasia. (Circulation. 1999;99:3292-3299.)

**Key Words:** angioplasty • restenosis • platelet-derived factors

A clear understanding of the phenomenon of restenosis following angioplasty remains elusive. Restenosis following angioplasty occurs as a consequence of catheter-induced SMC migration, proliferation, matrix production, vasospasm, and remodeling,1,2 events driven by inappropriate growth factor ligand and receptor expression.1,2 Platelet-derived growth factor (PDGF) is one of several growth factors implicated in the restenotic process.3 PDGF ligands, established as potent mitogens, chemotactic agents, and inducers of matrix synthesis, produce effects by dimerization and activation of PDGF-receptor (PDGFr) subunits.4 Furthermore, as an essential co-stimulator in biologic responses of other cytokines/growth factors and mediators of the coagulation cascade, PDGF has an extensive biological scope of action. In vivo, in small animal models of vascular injury, PDGF acts primarily to induce SMC migration7,8 and secondarily to promote intimal proliferation.8,9 Additionally, PDGF activity, as determined by the state of receptor autophosphorylation, increases several days postinjury and persists for several weeks,10,11 providing a functional link between expression of PDGF/ligand mRNA10 and proteins10,11 and potential biological influence on arterial stenosis. Results of immunocytochemical and in situ hybridization studies of human restenotic lesions12,13 reveal the presence of PDGF-A and -B ligands and PDGFrβ from 6 to 56 days following PTCA and the correspondent absence of proteins and mRNA in nonlesioned sites. The expression of both ligands and receptor in regions of vascular repair provides evidence that autocrine/paracrine
loops promoting PDGF-driven cellular activities, occur in human restenotic lesions.\textsuperscript{12,13} 

With evidence supporting a critical role for PDGF in arterial repair and restenosis following angioplasty, we investigated the ability of a selective inhibitor of the PDGF-tyrosine kinase (TK) to block restenosis following angioplasty. We demonstrate that RPR101511A, a novel inhibitor of PDGFr-TK, abolishes PDGF-dependent cellular activities in vitro and when administered orally to the hypercholesterolemic adult minipig significantly reduces restenosis following angioplasty.

**Methods**

**In Vitro Assays**

The effect of RPR101511A on PDGFr-TK was evaluated using the immunoprecipitated PDGFr cell-free ELISA assay\textsuperscript{14}; the PDGFr was purified from human VSMC and the final ATP concentration was 20 \textmu mol/L. Inhibition of PDGF-dependent activities, including in situ receptor autophosphorylation, mitogenesis, cell number, and viability, was as described.\textsuperscript{15} Exceptions were the use of human aortic VSMC (passage 4-6) and swine coronary VSMC. The latter were obtained by dispersal from slaughterhouse coronaries (passage 1 to 3). VSMC were growth-arrested for 24 hours, pretreated for 30 minutes with RPR101511A, and stimulated with a submaximal dose of PDGF-BB (10 ng/mL) or recombinant PDGF-AA (40 ng/mL). For western analysis, chemiluminescence was used to quantify antiphosphotyrosine and anti-PDGFr-detected proteins.

PDGFr-dependent chemotaxis was evaluated in a 96-well modified Boyden chamber (Neuroprobe, Inc) containing a collagen-coated polycarbonate membrane with cells preloaded with the fluorescent probe, calcine AM (5 \textmu mol/L). PDGF (3 ng/mL) was placed in the lower chamber, and cell migration in the presence and absence of RPR101511A after 4 hours of incubation was determined by quantitation of fluorescence associated with the pores and underside of the membrane with Cytofluor II at excitation/emission wavelengths of 485/530.

To determine the effect of RPR101511A on other kinases, published protocols for CSF-1-receptor\textsuperscript{16} and EGF-receptor\textsuperscript{17} were followed. Protein kinase C (PKC) and protein kinase A (PKA) assays were conducted using commercially available kits according to the manufacturer’s instructions (Sigma).

**Measurement of coronary artery vasoreactivity** followed published methods.\textsuperscript{18} Swine coronary artery rings were treated with RPR101511A, and steady-state tension produced by escalating concentrations of PDGF-BB, serotonin, or angiotensin II were recorded.

**Restenosis Model**

Thirty adult male Yucatan minipigs (Charles River Labs, Wilmington, Mass), weighing 16 to 30 kg, were housed individually at Rhone-Poulenc Rorer (RPR), Collegeville, Pa. Surgical and experimental procedures were performed according to a protocol approved by the Animal Care and Use Committee, RPR.

For lesion creation, pigs were anesthetized with Telazol (4.4 mg/kg, IM), intubated and ventilated with 2% isoflurane-oxygen. The medial ear vein and right external jugular vein were cannulated for administration of lactated Ringer’s solution, heparin (3000 U bolus followed by 1000 U every 20 minutes), nitroglycerin (120 \mu g/min), and lidocaine (1 mg/min). An 8F introducer sheath was placed in the right carotid artery. Using fluoroscopic-guided assistance (Stenoscan II, GE Medical Systems), the LAD was overinflated (balloon/artery diameter=1.4, 3 inflations and endothelial denudation rub) with an angioplasty catheter (Intrepid, 7 ATM, Baxter Healthcare Laboratories) in an AR2-guide catheter (Sciomed Boston Scientific Corporation). Pigs received aspirin (17 mg/kg BID) and an atherogenic diet (15% lard, 1.5% cholesterol mixed in swine minipig chow, Purina Mills, Inc) throughout the study.

One month following plaque creation, pigs underwent PTCA. Anesthesia, cannulations, and drug administrations were as described above. Plaque locus was determined from initial angiograms (first surgery). The balloon was expanded to achieve a 30% increase in lumen diameter. Following PTCA (three 20-second dilations), angiograms (postPTCA) were taken. External jugular vein access ports were implanted, subcutaneously, caudal to the scapula, to facilitate repeat blood withdrawal.

Pigs received RPR101511A (30 mg/kg) or vehicle by feeding tube twice daily for 28 days, beginning 2 hours before PTCA. A dosing suspension was prepared by mixing RPR101511A (Polytron tissumizer, Tekmar) with vehicle (0.5% methylcellulose [final]) and karo syrup, 50:50).

Twenty-eight days following PTCA, final angiograms (terminal-PTCA) were taken and pigs were euthanized with pentobarbital (78 mg/kg). The heart was removed and pressure perfusion fixed for 3 hours with 10% buffered formalin.

**Histological Evaluation**

Coronary arteries (LAD, circumflex) were cut into 4 mm sections, embedded in paraffin, and stained with Verhoeff’s van Giesen and Alcian Blue PAS (EPL Inc). A portion of each section was deparaffinized, frozen, sliced, and stained with oil red O for lipid deposition. Four 5-μm side-by-side views from 5 levels within each section were morphometrically and colorimetrically evaluated with a Nikon microscope and attached to a drawing tube with Sigma Scan software and/or an Olympus Vanox-S linked to ImagePro computer analysis system (I-Cube). The circumference of designated areas, injury index, and the number of internal elastic lamina (IEL) fragments were also quantified. Histomorphometric analyses were performed by 3 scientists blinded to treatment.

**Angiographic Analysis**

Lumen diameters on angiograms were determined by densitometric analysis (Pharmacia LKB Biotechnology Inc). Eight measurements were made spanning the lesion, and 3 measurements were made along the dye-filled catheter. The location of the MLD, identified on the prePTCA angiogram, was used to obtain lumen diameters at the corresponding locus on post- and terminal-angiograms. The circumflex artery was evaluated as the reference artery in a similar manner. Angiograms were analyzed by 2 researchers blinded to treatment.

**Plasma and Pharmacokinetic Analyses**

Venous blood samples were obtained during PTCA surgery (day 0) and on days 7, 14, 21, and 27 following angioplasty, 2 hours after the morning dose. Plasma was analyzed for cholesterol by the Hitachi 717 Chemistry Analyzer (Boehringer Mannheim Corp). For quantitation of RPR101511A concentration, plasma was diluted with acetonitrile and analyzed by HPLC fluorescence assay using a LC/Chromer RP18 with a linear range of detection of 0.01 to 5 \mu g/mL.

**Reagents**

Cell culture media and human aortic VSMC were purchased from Clonetics. Porcine PDGF-BB and recombinant human PDGF-AA were products of R&D; RPR101511A was synthesized by Medicinal Chemistry, RPR (Collegeville, Pa), according to patented synthesis.\textsuperscript{19} Antibodies were purchased from Transduction Laboratories and Genzyme.

**Statistics**

Results are presented as the mean±SEM. Statistical significance was calculated using the paired t test for means and the Student’s t test assuming equal variance. IC\textsubscript{50} values were determined from linear regression analysis of the percent inhibition from a minimum of 4 concentrations of RPR101511A.
Results

In Vitro Effects of RPR101511A

RPR101511A (6,7-dimethoxy-2-thiophen-3-yl-quinoxaline hydrochloride) (Figure 1), is a potent inhibitor of the human cell-free PDGFβr-TK with an IC₅₀ value of 106 nmol/L (Figure 2) and is selective for the PDGFβr-TK compared with other tyrosine kinases (EGFr, CSF-1r) and serine/threonine kinases (PKC, PKA) (Table 1). In a dose-related manner, RPR101511A inhibited in situ PDGFβr-TK autophosphorylation stimulated by PDGF (Figure 3A) and PDGF-dependent mitogenesis in swine coronary SMC (SCSMC), the relevant cell type in our in vivo restenosis model (Figure 3B). IC₅₀ values (nmol/L) were 220 ± 120 (n=3) and 254 ± 90 (n=7) for the 2 activities, respectively. At concentrations slightly higher than those required to inhibit SCSMC mitogenesis, RPR101511A inhibited PDGFr autophosphorylation, and PDGF-dependent chemotaxis and mitogenesis in human aortic SMC (Table 2). In a 6-day cell growth assay, a single application of RPR101511A retarded PDGF-dependent growth (Table 2). RPR101511A inhibited PDGF-AA-stimulated mitogenesis suggesting that RPR101511A also blocked the PDGFαr-TK (Table 2).

Initial signs of cytotoxicity were not evident until RPR101511A was added at a concentration which exceeded IC₅₀ values by 100-fold (ie, 100 μmol/L), demonstrating that the inhibition of PDGF-dependent activities was not mediated by nonspecific VSMC cytotoxicity.

Inhibition of Angiographic Restenosis With RPR101511A

To evaluate the effects of a selective PDGFr-TKI on coronary artery restenosis, RPR101511A was administered BID, beginning 2 hours before PTCA and continuing for 28 days. PrePTCA MLD of the vehicle and RPR101511A-treated pigs were similar (Figure 4). Following successful dilation, the MLD increased significantly and to the same extent in both groups. Twenty-eight days following PTCA and oral treatment with vehicle or RPR101511A, there was a significant decrease in the MLD of vehicle-treated pigs but no angiographic change in the MLD of RPR101511A-treated pigs. Thus, 54% (7 of 13) of the vehicle-treated pigs exhibited >50% loss of gain compared with 23% (3 of 13) of RPR101511A-treated pigs. This represents a 57% decrease in restenotic rate. As shown by the equivalency of gain in the

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TABLE 1. Effect of RPR101511A on Kinase Activity

<table>
<thead>
<tr>
<th>Kinase</th>
<th>IC₅₀, nmol/L</th>
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<tbody>
<tr>
<td>PDGFβr</td>
<td>106 ± 6 (15)</td>
</tr>
<tr>
<td>EGFr</td>
<td>&gt;50 000 (3)</td>
</tr>
<tr>
<td>CSF-1r</td>
<td>&gt;30 000 (3)</td>
</tr>
<tr>
<td>PKC</td>
<td>&gt;100 000 (3)</td>
</tr>
<tr>
<td>PKA</td>
<td>&gt;100 000 (3)</td>
</tr>
</tbody>
</table>

Kinase activity was measured as described in Methods. IC₅₀ values given as mean ± SEM. Values in parentheses represent number of separate experiments. EGFr indicates epidermal growth factor receptor and CSF-1r, colony-stimulating factor-1 receptor.

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Figure 2. Effect of RPR101511A on cell-free PDGFβr-TK activity. PDGF-stimulated autophosphorylation of the PDGFβr immunoprecipitated from human VSMC was evaluated by an ELISA assay, described in Methods. n=15 separate experiments.

Figure 3. Effect of RPR101511A on inhibition of PDGF-stimulated in situ autophosphorylation and mitogenesis in cultured swine coronary VSMC. A, PDGFβr from lysates (70 μg protein per lane) of coronary VSMC treated with RPR101511A and PDGF as described in Methods. Molecular weight marker shown to right identifies the PDGFr at 180 kDa. Confirmation of the receptor was determined by western blotting with anti-PDGFβr antibodies (data not shown). B, Cells were growth-arrested, pretreated with RPR101511A at doses shown, and stimulated with PDGF (10 ng/mL). Proliferation was assayed by [3H]-thymidine incorporation. n=9 separate experiments. VSMC indicates vascular smooth muscle cell.
two groups, by the similar angiographically determined balloon to artery ratio in the 2 groups (Table 3) and by the lack of change in the reference diameter of the 2 groups over time (Table 3), the beneficial effect of RPR101511A on the MLD following angioplasty was unlikely attributable to variable degrees of injury or angiographic artifacts.

### Inhibition of Histological Intimal Hyperplasia by RPR101511A

Figure 5 shows representative examples of histologically stained arterial lesions from vehicle-treated (left) and RPR101511A-treated (right) pigs. Total neointimal area, defined as the region between the lumen and the IEL, is the complex lesion generated by the initial injury plus the atherogenic diet albeit altered by PTCA compression and the intimal growth which occurred during the 28 days following PTCA. Total intimal area and total intimal/medial area (I/M) ratios of sections displaying the minimal luminal area were significantly reduced in tissues removed from pigs treated with RPR101511A compared with vehicle (Figure 6). The average histomorphometic minimal lumen area, lumen circumference, adventitial area, artery area, and medial area were not significantly different between RPR101511A-treated and control preparations.

Total intimal area and I/M ratios of the entire lesion were reduced with RPR101511A treatment (Table 4). However, statistical significance was not achieved. Lumen area, medial area, and artery areas were similar in the 2 groups (data not shown). The extent of injury as estimated by the injury index and the number of IEL fragments throughout the lesion were comparable in vehicle and RPR101511A-treated pigs suggesting that the applied injury was similar in the 2 groups. This is consistent with the angiographic data. In addition, the EEL and IEL areas of the reference circumflex artery untouched by the balloon catheter were comparable in the 2 groups (data not shown).

### Vasoreactivity

Maximal isometric tension induced by three vasoconstrictor agonists in porcine coronary artery rings and the effect of RPR101511A on this vasoreactivity are given in Table 5. RPR101511A significantly inhibited PDGF and angiotensin-mediated vasoconstriction but had no effect on serotonin-induced vasoconstriction.

### RPR101511A Plasma Concentration

Pharmacokinetic studies conducted with RPR101511A in the Yucatan minipig demonstrate a T_{max} of 2 hours and a T_{1/2} of 8 hours following oral administration. On the basis of these

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**TABLE 2. Effect of RPR101511A on PDGFr-Dependent Activities of Human VSMC**

<table>
<thead>
<tr>
<th>Cellular Activity</th>
<th>IC_{50}, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>In situ autophosphorylation</td>
<td>631±239 (4)</td>
</tr>
<tr>
<td>Mitogenesis</td>
<td></td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>605±81 (22)</td>
</tr>
<tr>
<td>PDGF-AA</td>
<td>559±89 (7)*</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td>492±86 (4)</td>
</tr>
<tr>
<td>Cell Growth</td>
<td>4400±1300 (4)</td>
</tr>
</tbody>
</table>

*Cellular activities were measured as described in Methods. The concentration of PDGF used for IC_{50} determination produced 80% of maximal response. IC_{50} values given as mean±SEM. Values in parentheses represent number of separate experiments.*

*Cultures stimulated with PDGF-AA.

**TABLE 3. Angiographic Analysis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle</th>
<th>RPR101511A</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD/AD*</td>
<td>1.57±0.11</td>
<td>1.47±0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Circumflex artery diameter, mm†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PostPTCA</td>
<td>1.96±0.05</td>
<td>1.82±0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>Termination</td>
<td>1.84±0.05</td>
<td>1.78±0.05</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Angiographically determined balloon and artery diameters at site of MLD.
†Locus of diameter measurement for circumflex coronary artery was selected on postPTCA angiogram and compared with corresponding locus on terminal angiogram. Circumflex angiograms were available for 13 vehicle-treated pigs and 10 RPR101511A-treated pigs.

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Figure 4. Effect of RPR101511A on angiographically determined MLD of the swine LAD artery. A, MLD from prePTCA, postPTCA, and terminal angiograms of control (n=13) and RPR101511A-treated pigs (n=13) were expressed as mean±SEM. * P<0.05 compared with prePTCA within the same group. **P<0.05 compared with vehicle. B, Gain=change in MLD produced by PTCA and loss=change in MLD from postPTCA to termination. ***P<0.05 compared with vehicle. LAD indicates left anterior descending; MLD, mean lumen diameter.
results, plasma samples were collected 2 hours postdosing every 7 days throughout the study. The mean plasma concentration in RPR101511A-treated pigs ranged from 200 to 400 ng/mL (0.65 to 1.29 μmol/L) during the study (Figure 7), concentrations in excess of those used to inhibit PDGF-dependent mitogenesis of SCSMC in vitro (Figure 3).

**Plasma Cholesterol**

Because pigs were fed an atherogenic diet throughout the study, plasma cholesterol increased from time of PTCA surgery to termination. The increase in the control group was not significantly different from the RPR101511A-treated group (mean change in total cholesterol from day of PTCA to termination, control, 63±58 mg/dL; RPR101511A-treated, 110±86 mg/dL). Additionally, the relative density of oil red O staining for lipid (vehicle:RPR101511A, mean±SEM) in the artery, (7781±990; 6988±764) plaque (9167±1166; 8213±1004), and the section with the MLD (9841±1864; 8704±1298) was similar in the 2 groups.

**Discussion**

The oral efficacy of the selective PDGFr-TKI, RPR101511A, was evaluated in a porcine model of coronary artery restenosis following PTCA. This model shares similarities with human restenosis in several ways: PTCA was performed on a preestablished coronary lesion in the presence of elevated plasma cholesterol, surgical protocols were similar to those used clinically, lumen diameters were measured before and after PTCA and again 1 month later by angiography (Figure 4) and at termination, the restenotic lesion contained smooth

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Figure 5. Effect of RPR101511A on histology 28 days after PTCA. Representative views of LAD from vehicle-treated pigs (A–D) and RPR101511A-treated pigs (E–H). LAD was stained with Verhoeff’s van Giesen stain. L indicates lumen; I, intima; and M, media. Original magnification ×40.
muscle cells, matrix, and lipids (Figure 5) comparable to human restenotic lesions.20,21 This model differs significantly from previously described models in which naïve coronary arteries of normocholesterolemic pigs were subjected to a single catheter-induced injury.22–24

RPR101511A is a novel, low molecular weight inhibitor (Figure 1) of PDGF-rTK autophosphorylation (Figures 2 and 3A) and an inhibitor of multiple PDGF-dependent activities of VSMC (Figure 3B, Table 2). Although PDGF has long been implicated in the development of restenosis,1 the concordant expression of ligands and the PDGF\(\beta\)r in human restenotic lesions has only recently been demonstrated12,13; it reinforces the role of PDGF as a critical player in the restenotic reparative process. In this study, RPR101511A was used as a prototype selective inhibitor of the PDGFr-TK to evaluate the role of PDGF-dependent responses in a porcine model of coronary restenosis. Our data demonstrate that oral administration of RPR101511A prevented angiographically defined restenosis (Figure 4). Pigs treated with RPR101511A exhibited no angiographic loss of gain following PTCA whereas vehicle-treated controls exhibited significant loss of gain (50%). Because both groups received the same degree of angiographic dilation (Table 3, Figure 4), the effect of RPR101511A was unlikely a consequence of differences in applied injury.

Morphometrically, the total amount (ie, resulting from both the initial injury and restenosis) of intimal hyperplasia in coronaries of pigs receiving RPR101511A was significantly smaller (30%) than in vehicle-treated pigs (Figure 6). Because we chose to evaluate the effect of RPR101511A on PTCA-induced restenosis, ie, the vascular response in the

Figure 6. Effect of RPR101511A on histomorphometrics of arteries removed 28 days after PTCA. Measurements were obtained from the section with minimal lumen area (MLA). * \(P<0.05\).
Coronary Restenosis Is Inhibited by RPR101511A

**TABLE 4. Morphometric Parameters Averaged Across Lesion**

<table>
<thead>
<tr>
<th>Histological Region/Parameter</th>
<th>Vehicle (n=13)</th>
<th>RPR101511A (n=13)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total intimal area, mm²</td>
<td>0.82±0.06</td>
<td>0.64±0.08</td>
<td>0.053</td>
</tr>
<tr>
<td>Total I/M</td>
<td>0.80±0.05</td>
<td>0.66±0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Injury index</td>
<td>0.25±0.01</td>
<td>0.22±0.03</td>
<td>NS</td>
</tr>
<tr>
<td>IEL fragments (average)</td>
<td>1.00±0.20</td>
<td>2.00±0.23</td>
<td>NS</td>
</tr>
<tr>
<td>IEL fragments (total)</td>
<td>6.00±0.81</td>
<td>7.00±0.92</td>
<td>NS</td>
</tr>
</tbody>
</table>

Morphometric measurements were averaged from all sections within the lesion. Intimal area indicates region from lumen to IEL; I/M, intimal area to medial area; injury index, distance between IEL fragments/IEL circumference. IEL fragments measured in 4 contiguous sections. Values given as mean±SEM.

presence of a preexisting lesion, morphometric quantitation of the intimal growth post PTCA was not possible and hence only total intimal lesion data are presented. Thus, one explanation for the modest inhibitory effect of RPR101511A on intimal hyperplasia may relate to our inability to measure selectively PTCA-induced restenotic intimal growth. Although attempts were made to detect restenotic growth by differential staining (eg, proteoaminoglycan sulfation with Alcian blue PAS), this will be more meaningfully accomplished by the use of intravascular ultrasound and will be included in subsequent reports.

From in vitro characterization of RPR101511A as a potent inhibitor of PDGF-dependent migration and proliferation in VSMC (Table 2, Figure 3B) and from plasma levels of RPR101511A (Figure 7) achieved in this study which exceeded the in vitro IC₅₀ values (Table 2), it is reasonable to propose that inhibition of restenosis may occur by inhibition of cellular activities initiated by PDGF activation. Although RPR101511A is without effect on EGFr-TK, CSF-1r-TK, and PKC and PKA (Table 1), an effect on the TK receptors for insulin–like growth factor-1 and fibroblast growth factor, growth factors also implicated in restenosis, cannot be excluded. In addition, it is possible that RPR101511A exerted a favorable vasodilatory effect on coronary arteries by inhibition of vasoconstriction induced by PDGF or angiotensin II (Table 5). However, the role of vasoconstrictors in restenosis is unknown. RPR101511A reduced restenosis independent of an effect on plasma cholesterol and arterial wall accumulation of lipid.

**TABLE 5. Effect of RPR101511A on Vasoreactivity to Select Agonists in Porcine Coronary Artery Rings**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Maximal Isometric Tension, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>RPR101511A</td>
</tr>
<tr>
<td>Serotonin</td>
<td>7.0±1.3 (4)</td>
</tr>
<tr>
<td>Angiotensin</td>
<td>15.1±1.0 (4)</td>
</tr>
<tr>
<td>PDGF</td>
<td>5.4±1.8 (6)</td>
</tr>
</tbody>
</table>

Porcine coronary artery rings were preincubated with RPR101511A (1 μmol/L) and stimulated with increasing concentrations of select vasoconstrictors. Values given as mean±SEM. Number of separate experiments given in parentheses.

*P<0.05.

Acknowledgments

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