Inhibition of PDGF Receptor Binding and PDGF-Stimulated Biological Activity In Vitro and of Intimal Lesion Formation In Vivo by 2-Bromomethyl-5-Chlorobenzene Sulfonylphthalimide

Deborra E. Mullins, Fozia Hamud, Robin Reim, Harry R. Davis

Abstract The proliferation of vascular smooth muscle cells (SMCs) is a key event in the development of atherosclerotic lesions and in the restenosis of arteries after angioplasty. Polypeptide growth factors are potent SMC mitogens in vitro and are believed to be involved in SMC proliferation in vivo. Strong data exist linking platelet-derived growth factor (PDGF) activity to human atherosclerosis. However, no low-molecular-weight antagonists of this growth factor have been discovered. We identified a compound, SCH 13929 (2-bromomethyl-5-chlorobenzene sulfonylphthalimide), which inhibits binding of 125I-PDGF BB to cell surface receptors with an IC50 of 0.13 μmol/L. This compound has a lesser effect on the binding of 125I-epidermal growth factor (EGF), 125I-basic fibroblast growth factor (bFGF), or 125I-endothelin to specific cell surface receptors. Exposure of cultured SMCs to SCH 13929 inhibits PDGF BB- and PDGF AA-stimulated DNA synthesis but not EGF- or bFGF-stimulated DNA synthesis. PDGF BB-stimulated SMC division is also inhibited by exposure to SCH 13929. Chemotaxis assays indicate that SCH 13929 inhibits PDGF-stimulated directional migration and suggest that the compound interacts with PDGF rather than with the receptor. Oral administration of SCH 13929 (100 mg/kg per day) to Sprague-Dawley rats or spontaneously hypertensive rats results in significant inhibition of lesion formation in the balloon catheter-deendothelialized carotid artery. These results suggest that SCH 13929 may be a useful tool for understanding the role of PDGF in intimal lesion formation. (Arterioscler. Thromb. 1994;14:1047-1055.)

Key Words • platelet-derived growth factor • angioplasty • PDGF receptor • atherosclerosis • restenosis • cellular proliferation • vascular smooth muscle cells

Atherosclerotic lesion development is characterized by the extravasation of monocytes and lymphocytes into the artery wall, migration of smooth muscle cells (SMCs) from the media into the intima, and subsequent excessive and disorderly proliferation of intimal SMCs. These events, as well as the extensive deposition of lipid and extracellular matrix material in the artery wall, eventually result in the formation of advanced atherosclerotic lesions that compromise normal vascular function and can result in vessel occlusion and thrombosis. Polypeptide growth factors, derived from platelets, macrophages, endothelial cells, and SMCs themselves, are believed to be responsible for the SMC migration and proliferation associated with atherosclerosis and restenosis of arteries after angioplasty.1,2 Exposure of normally quiescent vascular SMCs to growth factors results in a modulation from a "contractile" to a "synthetic" phenotype that is characterized by an increased capacity for cellular replication, the loss of ability to contract, and a high level of protein synthesis.3 Several growth factors have been shown to be SMC mitogens in vitro and may be involved in this phenotypic modulation in vivo, including the platelet-derived growth factors (PDGFs),4 both acidic fibroblast growth factor (aFGF)5 and basic FGF (bFGF),6 epidermal growth factor (EGF),7 endothelin,8-10 and interleukin-1.11

Data exist linking PDGFs to human atherosclerosis. Messenger RNAs coding for both the PDGF A and B chains and the PDGF-β receptor have been identified in human endarterectomy samples by in situ hybridization.12 Also, biologically active PDGF can be produced in vitro by cells derived from human atheromatous plaques and accounts for a substantial portion of the total mitogenic activity produced by these cultures.13 Therefore, a compound that inhibits the biological activity of PDGF would be expected to block or retard lesion formation.

PDGF is a disulfide-linked dimer (M, 30,000 to 33,000).4 It is composed of two chains, A and B, which share approximately 50% amino acid sequence homology. Each chain contains eight cysteines, all of which are involved in either intrachain or interchain disulfide bonds.14,15 PDGF can exist as homodimers (PDGF AA or PDGF BB) or as a heterodimer (PDGF AB), all of which are biologically active, although the ability of PDGF AA to stimulate chemotaxis may vary depending on the cell type.16 Two cell surface receptors for PDGF, termed α and β, have been identified.17-19 Both are 180,000 M, transmembrane glycoproteins with intrinsic tyrosine kinase activity. Binding of PDGF to the recep-

Received July 2, 1993; revision accepted April 11, 1994.
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tor induces receptor dimerization and activation of the tyrosine kinase activity, thereby initiating a cascade of intracellular reactions that culminate in DNA synthesis and cell division. The two forms of the receptor differ in their binding specificity. The α receptor binds both PDGF A and B, but the β receptor binds only PDGF B. Therefore, the receptor dimer binds all three forms of PDGF (AA, BB, and AB), the α/β receptor dimer binds PDGF BB and AB, and the β/β receptor dimer binds only PDGF BB.

The discovery of a compound that inhibits the binding of PDGF to its cognate receptor(s) would have therapeutic use for the prevention of postangioplasty restenosis as well as other disorders associated with excessive PDGF activity. We report here the identification and characterization of a low-molecular-weight, orally active PDGF antagonist, SCH 13929 (2-bromomethyl-5-chlorobenzene sulfonylphthalimide) (Fig 1). This compound, discovered through random screening of a chemical library, inhibits the biological activity of PDGF in vitro and inhibits the formation of intimal lesions in balloon catheter–injured rat carotid arteries.

Methods

Cells

BALB/c 3T3 cells (clone A31) were obtained from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum, penicillin 50 U/mL, and streptomycin 50 μg/mL in a humidified atmosphere of 5% CO₂/95% air. The cells were passaged every 3 days using 0.25% trypsin and 1 mmol/L EDTA and were seeded at a split ratio of 1:5. Human foreskin fibroblasts were kindly provided by Dr Larry Witte (Imclone, Inc). Cells were maintained in DMEM containing 10% fetal calf serum, 2 mmol/L glucose, penicillin 50 U/mL, and streptomycin 50 μg/mL in a humidified atmosphere of 5% CO₂/95% air. The medium was replaced every 3 days. At confluence these cells displayed the "hill and valley" morphology characteristic of SMCs. Upon reaching confluency the cells were trypsinized and counted using a Coulter counter.

SCH 13929

SCH 13929 was synthesized as described by Clader et al. For in vitro experiments, SCH 13929 was dissolved in 100% dimethyl sulfoxide (DMSO) at 50 mmol/L and diluted to the desired concentration in DMEM containing 2% plasma-derived serum (PDS). The final DMSO concentration in all experiments was 0.2%. For the balloon-injury studies, SCH 13929 was suspended in 0.4% aqueous methylcellulose, which was also used as the vehicle control.

125I–Growth Factor Binding Assays

Human foreskin fibroblasts (125I-PDGF and 125I-bFGF binding) or 3T3 cells (125I-EGF binding) were grown to approximately 80% confluency and then made quiescent by incubation for 48 hours in DMEM containing 2% PDS, penicillin 50 U/mL, and streptomycin 50 μg/mL. The binding assays were performed at 4°C. Monolayer cultures were washed three times in ice-cold DMEM containing human serum albumin (1 mg/mL) (washing medium), then incubated with 0.5 mL washing medium containing either 125I-cys-PDGF BB; 1030 Ci/mmol), 125I-EGF (1264 Ci/mmol), or 125I-bFGF (825 Ci/mmol) and the DMSO vehicle (0.2%) or with SCH 13929. After 4 hours (125I-PDGF) or 2 hours (125I-EGF and 125I-bFGF) of gentle shaking, the cells were washed five times in washing medium and were solubilized by using 1.0% Triton X-100, 150 mmol/L Na₂-hydroxymethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.4, 10% glycerol, and 0.1 mg/mL bovine serum albumin. Cell-associated radioactivity was measured in a gamma counter. Non-specific binding of 125I-PDGF and 125I-EGF was determined in the presence of excess unlabeled growth factor. Specific binding was calculated by subtracting non-specific binding from total binding. In measurements of 125I-bFGF binding, low- and high-affinity binding sites were distinguished by washing the cells with 2.0 mol/L NaCl and 20 mmol/L HEPES, pH 7.5, to remove 125I-bFGF bound to low-affinity sites and 2.0 mol/L NaCl and 20 mmol/L sodium acetate, pH 4.0, to remove 125I-bFGF bound to high-affinity sites.

DNA Synthesis Assay

SMCs or 3T3 cells were grown to approximately 80% confluency in 24-well dishes, washed with Dulbecco's phosphate-buffered saline (PBS), then made quiescent by incubation for 5 days (SMCs) or 2 days (3T3 cells) in DMEM supplemented with 2% PDS, penicillin 50 U/mL, and streptomycin 50 μg/mL. The SMC culture medium was replaced every 2 days. Quiescent cultures were exposed for 16 hours to growth factor and DMSO (0.2%) or to growth factor and SCH 13929 in DMSO (0.2%). The medium was then replaced with DMEM, 2% PDS, penicillin 50 U/mL, and streptomycin 50 μg/mL containing 2.5 μCi/mL methyl[3H]thymidine (73.7 Ci/mmol) for 4 hours. The cells were then washed twice in PBS, extracted with ice-cold 5% trichloroacetic acid (TCA) for 5 minutes, and washed once with ice-cold water. TCA-precipitable material was solubilized in 0.5 mL 0.2N NaOH, and the radiolabeled material contained therein was quantified by liquid scintillation spectrometry.

Cell Division Assay

SMCs were plated into 24-well culture dishes at a density of 1.5 x 10⁴ cells/cm². Twenty-four hours after plating, the medium was replaced with DMEM, 2% PDS, penicillin 50 U/mL, and streptomycin 50 μg/mL to induce quiescence as described above. Upon reaching quiescence the medium was replaced with fresh medium with or without PDGF (2 ng/mL) and the indicated concentrations of SCH 13929. The medium was not changed after the addition of the test agents. On days 1, 3, and 5 the cells were removed from the culture wells with trypsin/EDTA and counted using a Coulter counter.

Chemotaxis Assay

The ability of PDGF to stimulate the directional migration of 3T3 cells was measured by using blind-well chambers. PDGF (1 ng/mL) alone or with the indicated concentration of SCH 13929 was placed in the lower well of the chamber, a
Viability Assays

The ability of cells to exclude trypan blue was used as an indicator of viability. Cultures were incubated overnight in SCH 13929 or 0.2% DMSO, washed three times in PBS, removed from the culture dish with trypsin/EDTA, and incubated with 0.08% trypan blue in PBS for 5 minutes at room temperature. Stained cells were visualized and counted by light microscopy using a hemacytometer. 30 The release of lactate dehydrogenase (LDH) activity into the culture medium was also used as a measure of toxicity. Cultures were incubated for 96 hours with SCH 13929, and the conditioned medium was removed and clarified by centrifugation at 1500g for 5 minutes. LDH activity in the supernatant fraction was assayed by measuring the reduction of pyruvate by NADH. The assay was quantified by measuring the disappearance of NADH, monitored by a decrease in absorbance at 340 nm.

Balloon-Injury Model

All animals were treated and cared for in accordance with the recommendations set forth in the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” and the Animal Welfare Act in a program accredited by the American Association for the Accreditation of Laboratory Animal Care under an approved institutional animal protocol.

The left carotid artery of male Sprague-Dawley rats (400 to 450 g) or male spontaneously hypertensive rats (SHRs; 300 to 350 g) were balloon catheter–injured by the method of Baumgartner31 as modified by Clowes et al.32 The rats were anesthetized with methoxyflurane vapors (Metofane), and a 2F Fogarty embolectomy catheter was inserted into the left external carotid artery. The catheter was advanced 5 cm and inflated and retracted three times, turning the catheter 90° each time. We have confirmed by scanning electron microscopy and Evan’s blue staining that this procedure causes complete deendothelialization of the common carotid artery. Groups of rats were given SCH 13929 100 mg/kg per day PO or vehicle for 2 days before and 14 days after injury. Animals were necropsied after exanguination under anesthesia with ketamine 50 mg/kg and xylazine 10 mg/kg IM. The DNA content of 5-mm samples of the right and left carotid arteries taken 5 mm from their aortic origin was determined by the method of LaBarca and Paigen.33 The DNA measurements were expressed as the ratio of the left balloon catheter–injured carotid to the right control carotid. Three carotid artery samples were taken for histology at 2.5-mm intervals cephalad from the DNA sample and fixed in 4% paraformaldehyde containing 10% sucrose (wt/vol). Paraffin-embedded sections were stained with the Gomori trichrome–aldehyde fuchsin stain, and computer-assisted morphometric analyses were performed using the Bioquant System IV image analyzer.

Statistics

Statistical differences between groups were analyzed by using the unpaired Student’s t test, and significance was assumed if P <.05.
sures to 100 μmol/L SCH 13929 resulted in 56.3% inhibition of high-affinity binding. Less than 1 μmol/L SCH 13929 was required to achieve this level of inhibition of $^{125}$I-PDGF BB receptor binding.

SCH 13929 was also tested for its ability to inhibit the binding of $^{125}$I-endothelin to a rat liver homogenate endothelin receptor preparation. SCH 13929 inhibited endothelin binding with an IC$_50$ of 138 μmol/L (data not shown). Taken together, these data indicate that SCH 13929 is at least 100-fold selective for inhibition of PDGF receptor binding.

**Cellular Growth**

A compound that inhibits the binding of PDGF to its receptor should also block PDGF-stimulated cellular growth. This was ascertained by measuring the inhibition of growth factor-stimulated DNA synthesis and cell division by SCH 13929.

Exposure of quiescent SMCs to PDGF BB (0.5 ng/mL) caused a 13.1-fold increase in DNA synthesis (Fig 4A). SCH 13929 resulted in 56.3% inhibition of high-affinity binding. Less than 1 μmol/L SCH 13929 was required to achieve this level of inhibition of $^{125}$I-PDGF BB receptor binding.

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Exposure of quiescent SMCs to PDGF BB (0.5 ng/mL) caused a 13.1-fold increase in DNA synthesis (Fig 4A). The addition of SCH 13929 to the culture medium resulted in a dose-dependent inhibition of DNA synthesis over a concentration range of 0.1 to 3 μmol/L. Fifty percent inhibition of DNA synthesis was achieved with 0.6 μmol/L SCH 13929. In contrast, SCH 13929 was only moderately effective in reducing the level of DNA synthesis stimulated by EGF or bFGF (Fig 4A). SCH 13929 had little effect on EGF-stimulated $[^3H]$thymidine incorporation, resulting in only 19.5% inhibition at 10 μmol/L. Exposure of bFGF-treated cells to SCH 13929 resulted in modest decreases in DNA synthesis, with 10 μmol/L SCH 13929 producing 31.2% inhibition. Similar results were obtained with PDGF BB-, EGF-, and bFGF-stimulated DNA synthesis in 3T3 cells (data not shown).

Because SMCs express relatively few PDGF-α receptors, the effect of SCH 13929 on PDGF AA-stimulated mitogenesis was measured in BALB/c 3T3 cells (Fig 4B). Exposure of PDGF AA-stimulated cells to SCH 13929 resulted in a dose-dependent inhibition of DNA synthesis, with an IC$_50$ of 0.2 μmol/L.

To ascertain that the inhibition of PDGF-stimulated DNA synthesis in SCH 13929-treated cells was reflected in an inhibition in cellular proliferation, the effect of the compound on SMC replication was measured. PDGF BB and SCH 13929 were added to the medium of sparse cultures of quiescent cells, and the subsequent increases in cell number were measured over a 5-day incubation period. Incubation of cells with PDGF BB (2 ng/mL) resulted in a 3.4-fold increase in cell number in 5 days. The addition of SCH 13929 dramatically reduced cellular proliferation, with 0.1, 1, and 10 μmol/L SCH 13929 resulting in 57.6%, 82.4%, and 92.7% inhibition, respectively, after 5 days (Fig 5).

To rule out the possibility that the growth inhibition was due to cytotoxicity, several methods were used to assess cell viability. First, incubation of cells with SCH 13929 (100 μmol/L) for 1 through 7 days did not result in morphological changes obvious by microscopic examination at ×400 magnification. We also evaluated the effect of SCH 13929 on membrane permeability by measuring the ability of SCH 13929-treated cells to exclude trypan blue and by determining whether SCH 13929 caused the release of the cytoplasmic enzyme LDH into the culture medium. Incubation of 3T3 cells with SCH 13929 (100 μmol/L) for 4 days did not result in greater uptake of trypan blue than that of untreated cells (data not shown). Also, LDH activity in the culture medium of SCH 13929-treated 3T3 cells was not greater than that assayed in untreated cultures (data not shown). Therefore, the inhibition of PDGF receptor binding by SCH 13929 appears to result in inhibition of PDGF-stimulated growth without affecting cell viability.
Chemotaxis
PDGF is a potent chemotactic agent for mesenchymal cells in vitro.33-35 PDGF released by endothelial cells, macrophages, platelets, or intimal SMCs may stimulate the migration of medial SMCs into the intima, thus initiating a myointimal lesion.1 A PDGF antagonist would be expected to inhibit this activity. SCH 13929 was evaluated for its ability to inhibit PDGF-stimulated chemotaxis in vitro by using blind-well chambers (Table 1). When SCH 13929 (1.0 μmol/L) was present in the upper well of a blind-well chamber along with 7500 3T3 cells, PDGF-directed chemotaxis was not inhibited. However, when SCH 13929 was present in the lower well, chemotaxis was inhibited by 87%. The presence of the compound in both chambers did not result in significantly greater inhibition. Because chemotaxis was inhibited only when SCH 13929 was incubated with PDGF and not when the compound was incubated with cells, the results suggested that the inhibitory activity of the compound may be due to its interaction with the ligand rather than with the receptor.

Mode of Inhibition
Two additional lines of evidence suggest that the inhibitory activity of SCH 13929 is due to ligand antagonism. The first is based on the results shown in Table 2. In this experiment, PDGF was incubated with SCH 13929 in the absence of cells and then separated from the compound by dialysis before being assayed for residual SMC mitogenic activity. This exposure to SCH 13929 reduced its activity, with [3H]thymidine incorporation being inhibited 88% relative to an untreated sample of PDGF. This magnitude of inhibition was similar to that which was obtained when both PDGF and SCH 13929 were included in the DNA synthesis assay. A control experiment indicated that the dialysis conditions used in the experiment removed all the inhibitory activity of SCH 13929 (ie, dialysis of the compound alone before the addition of PDGF), indicating that inhibition of [3H]thymidine incorporation was not due to a carryover of free compound into the assay (Table 2). Because cell surface PDGF receptors were never exposed to free SCH 13929, the mode of inhibition of the compound was judged to be inactivation of the ligand.

In another experiment (data not shown), 3T3 cells were exposed to SCH 13929 (1, 10, or 100 μmol/L) overnight in the absence of PDGF. The compound was then removed, and the cells were washed thoroughly
TABLE 1. Effect of SCH 13929 on PDGF-Stimulated Chemotaxis in 3T3 Cells

<table>
<thead>
<tr>
<th>Lower Chamber</th>
<th>Upper Chamber</th>
<th>Cells/5 ×400 Fields±SEM (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>7±1</td>
</tr>
<tr>
<td>PDGF BB</td>
<td>0</td>
<td>194±43</td>
</tr>
<tr>
<td>PDGF BB</td>
<td>1.0 μmol/L SCH 13929</td>
<td>202±26</td>
</tr>
<tr>
<td>PDGF BB+1.0 μmol/L SCH 13929</td>
<td>0</td>
<td>25±3</td>
</tr>
<tr>
<td>PDGF BB+1.0 μmol/L SCH 13929</td>
<td>1.0 μmol/L SCH 13929</td>
<td>16±8</td>
</tr>
</tbody>
</table>

PDGF indicates platelet-derived growth factor. Chemotaxis was measured in blind-well chambers using polycarbonate filters with 8-μm pores. The indicated test agents were added to the appropriate chambers, and 7500 3T3 cells were added. Chemotaxis was allowed to proceed for 2 hours at 37°C. The filters were then fixed and stained, and cells on the lower side of the filter were counted.

and then exposed to PDGF BB (200 pmol/L) alone or to PDGF BB and SCH 13929 (1, 10, or 100 μmol/L) for 30 minutes. The removal of PDGF BB from the medium after only 30 minutes was intended to increase the likelihood that any mitogenic activity would be mediated by SCH 13929–exposed receptors and not by nascent receptors synthesized after removal of the compound. Cells that had been exposed overnight to SCH 13929 showed no significant loss of ability to undergo DNA synthesis when subsequently exposed to PDGF BB compared with cells that had not been exposed to SCH 13929 (2.0±2.1, and 1.9-fold stimulation of DNA synthesis in cells exposed overnight to 1, 10, or 100 μmol/L SCH 13929, respectively, versus 2.2-fold stimulation of DNA synthesis in cells not exposed to SCH 13929). No increase in DNA synthesis relative to vehicle-exposed control cells was measured in cells that were exposed to SCH 13929 both overnight and in the presence of PDGF.

In Vivo Assessment

The unilateral balloon catheter deendothelialization of the carotid artery in rats was used to evaluate inhibition of smooth muscle cell proliferation. The use of the balloon catheterization model to denude vessels of endothelium leading to the development of an intimal lesion is the classic method to study vascular SMC migration and proliferation in vivo.

SMCs isolated from SHRs have been reported to be more sensitive to the mitogenic activity of PDGF than SMCs obtained from normotensive rats.36–38 We therefore evaluated the effect of SCH 13929 on balloon catheter–induced injury in SHRs. SCH 13929, 50 mg/kg PO, was administered twice daily beginning 2 days before balloon injury and for 14 days thereafter (Table 3). Administration of SCH 13929 significantly reduced intimal lesion thickness (30% inhibition), intimal lesion cross-sectional area (36% inhibition), and the intimal/medial area (44% inhibition) relative to vehicle-treated balloon-injured rats. The DNA content of the carotid arteries was determined as a secondary end point in this model and was found to be reduced 45% relative to vehicle-treated controls. Similar results were obtained with Sprague-Dawley rats (data not shown). SCH 13929 was evaluated in five separate experiments in SHRs, which received 50 mg/kg twice daily; the intimal/medial area reduction ranged from 36% to 44% (P<.05). SCH 13929 at doses equal to or less than 30 mg/kg per day was found to give variable, nonsignificant levels of inhibition in the SHR model. Although intimal lesion formation was not completely inhibited in either SHRs or Sprague-Dawley rats, these results did indicate that oral administration of SCH 13929 results in statistically significant inhibition of lesion formation, inhibiting vascular SMC proliferation in vivo.

Administration of SCH 13929 50 mg/kg twice daily for 16 days in SHRs did not affect body weight, hematocrit, white or red blood cell counts, activated partial thromboplastin times, or other routine clinical chemistry values (data not shown). Therefore, the effect on intimal hyperplasia could not be accounted for by any general toxicologic effect of SCH 13929.

Discussion

These studies provide evidence that SCH 13929 is a potent and relatively specific inhibitor of PDGF receptor binding and PDGF–stimulated biological activities. Inhibition of receptor binding, rather than inhibition of

TABLE 2. Inhibition of PDGF Mitogenic Activity by Ligand–SCH 13929 Interaction

<table>
<thead>
<tr>
<th>Reagents Added to DNA Synthesis Assay</th>
<th>[3H]Thymidine Incorporation, dpm x 10–4 ±SD (n=3)</th>
<th>Inhibition, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialyzed</td>
<td>Not Dialyzed</td>
<td></td>
</tr>
<tr>
<td>PDGF</td>
<td>88.1±2.2</td>
<td>...</td>
</tr>
<tr>
<td>PDGF+SCH 13929</td>
<td>23.5±4.3</td>
<td>88.0</td>
</tr>
<tr>
<td>PDGF+SCH 13929</td>
<td>18.7±2.7</td>
<td>94.5</td>
</tr>
<tr>
<td>SCH 13929</td>
<td>PDGF</td>
<td>0</td>
</tr>
<tr>
<td>No additions</td>
<td>14.7±1.0</td>
<td>...</td>
</tr>
</tbody>
</table>

PDGF indicates platelet-derived growth factor. Samples were dialyzed or incubated (not dialyzed) for 48 hours at 4°C in Dulbecco's modified Eagle's medium and plasma-derived serum. Amounts used were PDGF BB, 0.5 ng/mL, and SCH 13929, 100 μmol/L.
TABLE 3. Effect of Oral Administration of SCH 13929 on Intimal Lesion Development In the Deendothelialized Spontaneously Hypertensive Rat Carotid Artery

<table>
<thead>
<tr>
<th></th>
<th>Maximal Intimal Thickness, mm</th>
<th>Intimal Area, mm²</th>
<th>Intimal Area/Medial Area</th>
<th>Carotid DNA, Balloon Injury/Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-Day control</td>
<td>0.114±0.008</td>
<td>0.106±0.008</td>
<td>0.799±0.053</td>
<td>5.57±0.89</td>
</tr>
<tr>
<td>14-Day SCH 13929, 100 mg/kg per day</td>
<td>0.080±0.012*</td>
<td>0.068±0.009†</td>
<td>0.449±0.060†</td>
<td>3.08±0.43*</td>
</tr>
</tbody>
</table>

*P<.05, †P<.01 vs control.

Each experimental group had 10 spontaneously hypertensive rats. Three histology sections and one DNA sample were taken from each carotid artery. Values are mean±SEM.

Sm CMC proliferation in the balloon catheter–injured rat carotid artery but only within 2 days after injury.44 The SMC proliferation that occurs later in lesion development is not blocked by the infusion of antibodies against bFGF.50 Thrombin stimulates the proliferation of SMCs in vitro,51 and its receptor has been identified in human endarterectomy specimens and fatty streaks.52 Likewise, insulin-like growth factor I mRNA has been localized in situ hybridization to rat carotid artery myointimal lesions following balloon catheterization.53 However, proof of an in vivo role for thrombin or insulin-like growth factor I has not yet been provided.

Interleukin-1α and interleukin-1β,11 transforming growth factor–β,54,55 and angiotensin II56 stimulate SMC proliferation or hypertrophy in vitro under certain conditions. These activities are due to the fact that each can induce the expression of PDGF AA activity by SMCs. The PDGF AA then stimulates cellular proliferation or hypertrophy in an autocrine manner. SCH 13929 inhibits both PDGF BB– and PDGF AA–stimulated cell growth and, therefore, should be an effective antagonist in situations in which PDGF-stimulated growth occurs as a secondary response to another growth factor or cytokine whose activity is not directly blocked by the compound.

The inhibitory effect of SCH 13929 appears to be due to the interaction of the compound with the ligand rather than with the receptor. This conclusion is based on experiments described above that demonstrate that when receptors (ie, intact cells) or ligand are exposed separately to SCH 13929, the compound is efficacious only when allowed to interact with the ligand. The use of a radiolabeled analogue of SCH 13929 revealed that the compound binds nonspecifically to many proteins, including those grow factor whose biological activity was not blocked (data not shown). The high doses of SCH 13929 needed to inhibit intimal lesion formation in vivo may be due to the compound’s propensity to bind to protein.

An estimated 250,000 patients in the United States undergo coronary angioplasty each year,57 and similar numbers undergo angioplasty for peripheral vascular disease. Restenosis occurs in 30% to 50% of cases within 6 months. All current treatments for prevention of postangioplasty restenosis have proven to be ineffective in clinical trials.58 A therapeutic agent with an inhibitory profile similar to SCH 13929 may be effective in preventing postangioplasty restenosis. However, although data from in vivo studies and animal models of restenosis suggest that PDGF plays an important role in intimal lesion development, the importance of PDGF activity in human
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restenosis has not yet been established. It is also important to bear in mind that the efficacy of a PDGF antagonist may be affected by the cellular environment and biochemical milieu of the affected artery. Therefore, a compound that inhibits lesion development in a normal vessel injured by a balloon catheter, such as a rat carotid artery, may not show the same activity in a complex human atherosclerotic artery.

Based on its low-molecular-weight nature and growth factor inhibitory specificity, SCH 13929 may be a useful tool with which to probe further the role of PDGF in postangioplasty restenosis and other diseases.

Acknowledgments

We thank Dr Stuart Rosenblum for synthesizing SCH 13929 and Lizbeth Hoos and Dan McGregor for expert technical assistance.

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doi: 10.1161/01.ATV.14.7.1047

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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