The Role of Alpha and Beta Platelet-Derived Growth Factor Receptor in the Vascular Response to Injury in Nonhuman Primates

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Abstract—Restenosis remains a significant clinical problem associated with mechanical interventional procedures for arterial revascularization or repair, including coronary angioplasty and stenting. Studies with rodents have established that platelet-derived growth factor (PDGF), a potent chemotactic and mitogenic agent for vascular smooth muscle cells, is a key mediator of lesion formation after vascular injury. To further explore this hypothesis in a more clinically relevant model, neutralizing monoclonal antibodies (mAbs) were used to examine the effect of selective inhibition of alpha or beta PDGF receptor (PDGFR) on neointima formation in nonhuman primates. Carotid arteries were injured by surgical endarterectomy and femoral arteries by balloon catheter dilatation. Immunostaining revealed that both injuries induced cell proliferation and the upregulation of beta PDGFR but not alpha PDGFR. By 7 days after injury, beta PDGFR staining was limited to the luminal region of the media, the small areas of neointima, and the adventitia. Nearly all bromodeoxyuridine-positive cells were found in these regions as well. After 30 days, a concentric neointima that stained strongly for beta PDGFR had formed in the carotid and femoral arteries. Treatment of baboons with anti-beta PDGFR mAb 2A1E2 for 6 days after injury reduced the carotid artery and femoral artery lesion sizes by 37% (P<0.05) and 48% (P<0.005), respectively, when measured at 30 days. Under the same conditions, treatment with anti-alpha PDGFR mAb 2H7C5 had no effect. These findings suggest that PDGF mediates neointima formation through the beta PDGFR, and that antagonism of this pathway may be a promising therapeutic strategy for reducing clinical restenosis. (Arterioscler Thromb Vasc Biol. 1999;19:900-909.)

Key Words: angioplasty ■ endarterectomy ■ tyrosine kinase ■ smooth muscle cell ■ monoclonal antibody
homeostatic mechanisms similar to those found in humans.20,21 Experimental arterial injury was produced by 2 methods that simulate therapeutic procedures used in humans, namely, surgical carotid artery endarterectomy and balloon catheter dilatation of the femoral artery. Using these models, the effects of alpha and beta PDGFR antagonism on vascular lesion formation was evaluated.

Methods

Monoclonal Antibody Inhibition of Baboon PDGFR Phosphorylation In Vitro

To compare the relative potency of monoclonal antibody (mAb) 2H7C5 against human vascular fibroblasts and anti-PDGFR human BCE63 cells (American Type Tissue Collection, CRL 1427) and lung primary baboon fibroblasts were used. A similar characterization of anti-beta mAb 2A1E2 was done using HR5 cells that overexpress recombinant human beta PDGFR and primary baboon SMCs, which express endogenous beta PDGFR.22 Confluent monolayers of cells that had been serum-starved for 18 hours were stimulated with 50 ng/mL PDGF AA or PDGF BB for 10 minutes at 37°C in the absence or presence of increasing concentrations of mAb. The cells were solubilized in lysis buffer (20 mmol/L Tris HCl, pH 7.4, 150 mmol/L NaCl, 1% Triton X-100, 10 mmol/L tetrasodium pyrophosphate, 50 mmol/L NaF, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 1 mmol/L sodium orthovanadate, 1 mmol/L PMSF), and lysates were separated by SDS-polyacrylamide gel electrophoresis and subjected to Western blot analysis with PY20 mouse anti-phosphotyrosine mAb (Transduction Labs) followed by detection with [125I]-protein A (Dupont NEN Research).

Carotid Endarterectomy and Femoral Balloon Angioplasty

Carotid artery endarterectomy procedures were performed as previously described by Hanson et al.23 Briefly, after a midline neck incision, one common carotid artery was dissected free of surrounding tissue from the aortic arch proximally to the carotid bifurcation distally. Standard heparin (100 U/kg) was given intravenously, and the common carotid artery was occluded with atrumatic microvascular clamps placed at the end of the exposed vessel. The artery was then divided at a point 1 cm proximal to the distal clamp, and the proximal segment was everted over curved forceps to obtain exposure of the vessel intimal surface. The everted surface was held in place using continuous 7-0 polypropylene sutures, and the endarterectomy was begun 1 cm from the divided end of the artery and continued for 1 cm. The endothelium, internal elastic lamina, and approximately half of the thickness of the medial tissue layers were removed using microvascular forceps and a surgical operating microscope under 32× magnification. After the endarterectomy, the vessel was restored to its normal configuration, and an end-to-end anastomosis was accomplished using continuous 7-0 polypolypropylene sutures.

To perform balloon catheter denudation, an incision was made over the medial aspect of the distal thigh. A side branch on the distal superficial femoral artery, near the takeoff of the popliteal artery, was isolated without exposing the proximal superficial femoral artery and controlled using vessel loops. A 3F Fogarty balloon catheter was passed through the branch to the femoral bifurcation proximally, inflated to a diameter of approximately 4 mm by filling with sterile saline, and withdrawn throughout the length of the superficial femoral artery (to the insertion site) using a gentle twisting motion. A moderate but not strong resistance to the passage of the balloon was achieved in all cases. To ensure complete deendothelialization, this procedure was repeated 3 times with no instance of vessel rupture. After ballooning, the catheter was withdrawn, the side branch access vessel was ligated, and the incision site was closed.

Treatment and Control Groups

A total of 38 normal male juvenile baboons (Papio cynocephalus) weighing 8 to 36 kg were used in these studies; 15 were used as controls. Control animals were untreated but underwent the same procedures for vessel injury as the animals receiving anti-PDGFR antibodies. In the treated groups, 5 baboons were given anti-alpha PDGFR mAb 2H7C5 and 18 were treated with anti-beta PDGFR mAb 2A1E2. The animals were quarantined and observed to be disease-free for at least 90 days before initiating the studies. All procedures were approved by the Institutional Animal Care and Use Committee in compliance with National Institutes of Health guidelines (Guide for the Care and Use of Laboratory Animals, 1985). In control studies, 11 animals underwent bilateral carotid artery endarterectomy. Single vessels from 5 of these baboons were harvested after 7 days for bromodeoxyuridine (BrdU) staining; the remaining 17 vessels were harvested after 30 days for morphometric analysis. Two of the vessels harvested at 30 days were found to be occluded and in 1 additional vessel the injury procedure was deemed technically incomplete because histological evaluation showed only partial removal circumferentially of the internal elastic lamina and subjacent media; these vessels were not included in the subsequent morphometric analysis. Thus, in the control studies a total of 14 vessels from 10 different animals were patent at 30 days and available for morphometric analysis.

Five additional vessels from the treated groups, 9 of the 15 control animals also underwent bilateral femoral artery balloon angioplasty, performed concurrent with the endarterectomy procedures. Within this group of 18 injured arteries, 5 single vessels from individual animals were taken at 7 days for BrdU analysis. The remaining 13 vessels and an additional 4 vessels from animals who had balloon angioplasty of only 1 femoral artery were taken at 30 days for morphometric analysis.

Eighteen animals were treated with anti-beta PDGFR mAb 2A1E2. Of this number, 10 animals underwent bilateral carotid endarterectomy. In 5 of these 10 animals receiving bilateral endarterectomy, single vessels were harvested for BrdU analysis at day 7. Of the remaining 15 vessels, 4 endarterectomy procedures were considered technically inadequate. Thus, 11 carotid arteries from treated animals were patent and available for analysis at the 30-day point. Concurrently, 9 treated baboons also underwent bilateral balloon angioplasty of the femoral arteries. Five of these vessels were taken for BrdU analysis at 7 days. The remaining 13 vessels, and 7 additional vessels from animals who had undergone balloon angioplasty of only 1 femoral artery, were taken at 30 days for morphometric analysis.

Five additional animals who received anti-alpha PDGFR mAb 2H7C5 were also studied. In these baboons, balloon angioplasty was performed on 1 femoral artery only. All animals in this group were sacrificed at 30 days, at which time all vessels were patent and taken for subsequent morphometric analysis.

Administration of mAbs

mAb 2H7C5 or 2A1E2 was given by intravenous bolus at 20 mg/kg beginning 2 hours before surgery and once every 24 hours thereafter at 1.0 mg/kg for a total of 6 doses. Plasma samples were obtained just before and 10 minutes after each dose of antibody. Plasma concentrations of mAbs were determined by standard ELISA using purified alpha or beta PDGFR extracellular domain immobilized in microtiter plates.24,25

Immunohistochemistry and Morphometric Analysis

Carotid and femoral arteries were harvested fresh at 7 days or 30 days after injury. The contralateral femoral artery or a region adjacent to the endarterectomy site of the carotid artery was used to provide uninjured control sections. All tissues were fixed for 2 hours in 4% paraformaldehyde, transferred to 15% sucrose buffer overnight, embedded in orbihine carbasylm-transferase (Miles Inc) and stored at −80°C before sectioning. Sections 10 μm thick were placed on slides, thawed at 25°C, dried 1 to 3 hours at 60°C, and rehydrated in PBS for 10 minutes at 25°C before antibody staining. Rehydrated vessel sections were blocked in 5% donkey sera for 5 to 10 minutes at 25°C and then incubated with 10 μg/mL of rabbit anti-alpha or anti-beta PDGFR IgG24 in 1% donkey sera, 0.01% Brij 35, and 0.01% NaN, in PBS at pH 7.4 overnight at 4°C. After rinsing the slides with PBS, biotin-conjugated donkey anti-rabbit IgG
Histologic cross-sections of carotid and femoral arteries were stained with rabbit polyclonal IgG directed against the alpha or beta PDGFR extracellular domain. Proliferating cells were identified in adjacent sections by staining with antibodies to PCNA or BrdU. Uninjured segments of carotid artery did not stain positive for beta PDGFR and contained very few BrdU-positive nuclei (Figure 1A and 1D). In contrast, at 7 days after injury, sections of the carotid artery that had undergone endarterectomy stained strongly for beta PDGFR and cell proliferation in baboon carotid artery. Staining for beta PDGFR (A through C) or proliferating cells (D through F) from uninjured carotid artery (A and D) was compared with vessels at 7 days after injury (B and E) or 30 days after injury (C and F). Proliferating cell nuclei were stained for BrdU (D and E) or for PCNA (F). Double staining for PCNA and beta PDGFR (F) was performed on endarterectomy sections taken 30 days after injury; arrows indicate proliferating cells.

**Results**

**Effect of Vascular Injury on Expression of Beta PDGFR and Cell Proliferation**

Histologic cross-sections of carotid and femoral arteries were harvested 30 days after injury. Vessel cross-sections were treated with Verhoeff-van Gieson elastic stain to facilitate the identification of neointima. Morphometric measurements were performed on femoral and carotid arteries using a Nikon Optiphot-2 microscope (Nikon Inc) coupled with a Hitachi HV-C 20 U color video camera (Hitachi Inc). Two sections from each site of carotid endarterectomy (1 cm in length) and 2 sections from the adjacent uninjured portion of each carotid artery were analyzed to determine medial and neointimal areas (millimeter squared) using Image-Pro Plus software (Media Cybernetics, Inc). Injured femoral arteries (approximately 5 cm in length) were divided into multiple segments longitudinally. An average of 8 sections were analyzed from each vessel to determine the vessel-averaged medial and intimal areas (millimeter squared).

**Statistics**

The lesion sizes for vessels taken at each location were analyzed with a statistical analysis of covariance procedure that afforded an efficient comparison of lesion size in the control and treated groups, as well as between-animal and between-vessel components of variability. In addition, because animal weights in the different study groups varied approximately 3-fold, analysis of covariance was used to assess whether weight was significantly related to the neointima/media ratio while simultaneously accounting for potential treatment effects and animal-to-animal variability. Computations were performed in Systat on an IBM-compatible PC. All values are reported as the mean±SD.
at this time. Coincident with beta PDGFR expression was a significant increase in the number of BrdU-positive nuclei, which were most frequently observed in regions of receptor staining but were also present in the adventitia (Figure 1E). At 30 days after injury, a concentric neointima was readily observed, the luminal region of which stained strongly for beta PDGFR (Figure 1C). Nuclei that stained positively for PCNA were found primarily in the region of beta PDGFR staining although the number of proliferating cells was decreased compared with the findings at 7 days after injury (Figure 1E and 1F).

Femoral artery injury by balloon angioplasty also caused the induction of beta PDGFR expression in association with cell proliferation (Figure 2). Immunohistochemistry of uninjured femoral artery cross-sections did not show beta PDGFR staining, and the level of cell proliferation, as measured by BrdU staining, was very low, as expected (Figure 2A and 2D). However, sections obtained at 7 days after injury stained strongly for beta PDGFR in the luminal region of the media, and proliferating cells were detected in the same region by BrdU staining (Figure 2B and 2E). At 30 days after injury, most of the femoral neointima stained strongly for beta PDGFR and contained most of the BrdU-positive nuclei, which were now infrequent (Figure 2C and 2F). Interestingly, no alpha PDGFR was detected in control vessels, and injury of either the carotid or femoral arteries failed to induce its expression as determined by staining with anti-alpha receptor IgG (data not shown). In control experiments, IgG obtained from rabbits before immunization with beta PDGFR failed to stain vessel sections, whereas anti-beta PDGFR mAbs gave the same pattern of staining as the immune polyclonal IgG (data not shown). These results indicate that alpha and beta PDGFR expression is low in normal baboon vessels, but that injury produced by endarterectomy or balloon dilatation causes a selective upregulation of beta PDGFR in regions of the vessel undergoing cellular proliferation.

**Anti-PDGFR mAbs Block Baboon PDGFR Autophosphorylation**

mAbs 2H7C5 and 2A1E2 are potent selective antagonists of the alpha and beta PDGFR, respectively.24 Both mAbs block PDGF binding, receptor autophosphorylation, and mitogenic signaling.24 The relative potency of mAb 2H7C5 against human and baboon alpha PDGFR was compared using the human sarcoma cell line MG63 and baboon primary lung fibroblasts. As shown in Figure 3, treatment of each cell line with 50 ng/mL PDGF AA resulted in the anti-phosphotyrosine antibody detection of the 180-kDa alpha PDGFR band, which was not detected in unstimulated cells. Preincubation of each cell line with increasing concentrations of mAb 2H7C5 caused a reduction in alpha PDGFR phosphorylation with an IC50 of 1 to 3 nmol/L and >90% inhibition at 10 nmol/L (Figure 3).

Similarly, the ability of mAb 2A1E2 to inhibit human and baboon beta PDGFR phosphorylation was compared using...
These results demonstrate that mAbs 2H7C5 and 2A1E2 are equally potent for inhibiting human and baboon PDGFR activation at concentrations that should be readily achievable in vivo.

**Effect of mAb 2H7C5 or 2A1E2 Treatment on Neointima Formation in Baboons**

To examine the effect of alpha or beta PDGFR inhibition on neointima formation, mAb 2H7C5 or 2A1E2 was administered by IV bolus at 2 mg/kg 2 hours before injury and at 1 mg/kg every 24 hours thereafter for a total of 6 doses. Treated baboons had average daily trough and peak mAb 2H7C5 plasma concentrations of 107 and 207 nmol/L whereas these values for 2A1E2 were 122 and 207 nmol/L (Figure 5). These plasma levels, sustained during the first 7 days after injury, were 10 to 20 times those required to completely inhibit alpha or beta PDGFR activation in vitro (Figures 3 and 4).

**Results After Carotid Endarterectomy**

The results obtained from vessels after carotid endarterectomy are given in Table 1. The untreated control baboons were stratified into 2 groups that were homogeneous on the basis of body weight: a group with low body weight (average, 10.8 kg) and one with high body weight (average, 26.8 kg). To preclude animal weight as an independent variable in these measurements, animals were subsequently selected for treatment with anti-PDGFR mAb on the basis of size so that the treated group (average, 28.1 kg) was closely weight-matched with the group of larger control animals (Table 1). However, it is noteworthy that for control animals of all sizes the ratio of areas of injured media to normal media were similar, averaging 0.51 to 0.55, indicating that the endarterectomy procedure consistently removed approximately half of the medial thickness of the vessel wall (Table 1).

After 30 days, animals that had been treated with anti-beta PDGFR antibody 2A1E2 had a significant reduction in the area of neointima formed (Table 1). When 11 vessels from treated animals were compared with 9 arteries obtained from weight-matched control animals, there was a 37% reduction in neointimal area in the treated versus control group (1.85±0.86 mm² versus 2.92±1.28 mm²; P=0.05). Between these treated and control groups there was no difference in the area of media in normal vessel segments taken adjacent to the injury site (1.97±0.51 mm² versus 2.37±0.54 mm², respectively; P=0.15), indicating that vessel size was comparable in the 2 groups. Similarly, the ratios of areas of the injured media to adjacent normal media were not different between the treated and control groups (0.47±0.24 mm² versus 0.51±0.17 mm², respectively; P=0.56), indicating that the extent of vessel injury was equivalent between the groups (Table 1).

Although the endarterectomy procedure removed about half of the vessel media in each study group, in individual vessel segments the fraction of media removed varied from approximately 20% to 80%. Because the variable removal of media by this method invalidates normalization of neointimal areas by the area of underlying (ie, endarterectomized) media, the benefit of anti-beta PDGFR mAb 2A1E2 for reducing neointima formation was further documented by comparing the ratio of neointimal area to the medial area in normal vessels (Table 1).

Results After Carotid Endarterectomy

<table>
<thead>
<tr>
<th>Group</th>
<th>Area of Neointima (mm²)</th>
<th>Area of Media (mm²)</th>
<th>Neointima/Media Ratio</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Low</td>
<td>1.28±0.54</td>
<td>2.37±0.54</td>
<td>0.53±0.24</td>
<td>0.65</td>
</tr>
<tr>
<td>Control High</td>
<td>2.37±0.54</td>
<td>3.65±0.73</td>
<td>0.64±0.17</td>
<td>0.83</td>
</tr>
<tr>
<td>Treatment Low</td>
<td>0.76±0.24</td>
<td>2.37±0.54</td>
<td>0.32±0.08</td>
<td>0.019</td>
</tr>
<tr>
<td>Treatment High</td>
<td>1.42±0.56</td>
<td>3.65±0.73</td>
<td>0.39±0.17</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Subgroup analysis was also performed to assess the possible influence of other interventional procedures on neointima formation in carotid arteries harvested at 30 days. In these...
TABLE 1. Effect of Anti-β-PDGFR mAb in Carotid Artery Endarterectomy

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight, kg</th>
<th>Number of Animals</th>
<th>Number of Vessels</th>
<th>Area, mm²</th>
<th>Neointima</th>
<th>Media (injured)</th>
<th>Media (normal)</th>
<th>Media (injured)/Media (normal)</th>
<th>P value (vs weight-matched controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control animals</td>
<td>10.8±1.6</td>
<td>3</td>
<td>5</td>
<td>2.06±0.55</td>
<td>0.78±0.30</td>
<td>1.45±0.30</td>
<td>0.55±0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-β-PDGFR antibody-treated</td>
<td>26.8±6.0</td>
<td>7</td>
<td>9</td>
<td>2.92±1.28</td>
<td>1.26±0.59</td>
<td>2.37±0.54</td>
<td>0.51±0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28.1±4.0</td>
<td>7</td>
<td>11</td>
<td>1.85±0.86</td>
<td>0.94±0.48</td>
<td>1.97±0.51</td>
<td>0.47±0.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±1 SD.

Results After Femoral Artery Angioplasty

In Table 2 the results with 10 vessels from baboons weighing an average of 11.5 kg were compared with 5 vessels from weight-matched animals treated with anti-alpha PDGFR mAb 2H7C5 and with 7 vessels from weight-matched animals treated with anti-beta PDGFR mAb 2A1E2. Medial areas were equivalent in the 3 study groups (P>0.4). In the animals treated with 2H7C5, neither the area of neointima nor the neointima/media ratio was decreased versus the control results (P>0.5 in both cases, Table 2). Conversely, in animals treated with 2A1E2, the area of neointima was reduced by 48% (P=0.005 versus controls), and the neointima/media ratio was reduced by 43% (P=0.006 versus controls). Thus, a striking benefit for reducing femoral artery intimal thickening was shown after blockade of the beta PDGFR, but not the alpha PDGFR. In the 5 femoral arteries harvested after 7 days for quantitative evaluation of cell proliferation by BrdU staining, there was no difference in the 2A1E2-treated animals versus results in 5 control arteries in the percentage of BrdU-positive medial SMC (4.6±2.1% versus 5.5±3.8%, respectively; P>0.5).

Also studied were animals of substantially higher body weight, both as controls (6 animals) and after treatment with 2A1E2 (9 animals). These findings are given in Table 2. When compared with the results obtained in control animals, treatment with the anti-beta PDGFR mAb reduced the area of neointima by 46% (P=0.015) and the neointima/media ratio by 52% (P=0.0003), results that were consistent with the observed benefit of 2A1E2 in animals of smaller size, as noted above. The overall comparison of individual data (neointima/media ratio) from all treated and control animals is provided in Figure 7. When all of the data from animals having different body weights were grouped, analysis of covariance documented that the benefit of treatment was independent of animal size (P=0.969), thereby reinforcing the generality of these results.

The possible influence on neointima formation of performing several interventional procedures in individual animals was also assessed. In these studies, femoral arteries were analyzed from animals that underwent (1) bilateral carotid endarterectomy and bilateral femoral angioplasty with 1 carotid artery and 1 femoral artery harvested at 7 days, and the remaining vessel pair harvested at 30 days (5 control animals, 5 treated with 2A1E2); (2) bilateral carotid endarterectomy and bilateral femoral angioplasty with all vessels harvested at 30 days (4 control animals, 5 animals treated with 2A1E2); or (3) angioplasty of 1 femoral artery with all vessels harvested at 30 days (4 control animals, 7 animals treated with 2A1E2). When vessels from animals studied under the 3 interventional protocols were compared, control values for the neointima/media ratio at 30 days were equivalent for animals of comparable body weight (P>0.5 in each case). Within each procedural group, the benefit of therapy was also equivalent (range, 44% to 48% reduction in the neointima/media ratio), indicating that surgical procedural variables (ie, concurrent endarterectomy, bilateral angioplasty, or the harvesting of some vessels at 7 days) did not
affect femoral artery neointima formation when measured 30 days after balloon angioplasty.

**Discussion**

This study demonstrates that in nonhuman primates the PDGF response to vascular injury is mediated by beta PDGFR and that its antagonism can effectively inhibit neointima formation. The biological activity of PDGF is mediated by 2 PDGFRs, alpha and beta, which interact differentially with PDGF isoforms. PDGF BB and, to a much lesser extent, PDGF AB activate beta PDGFR, whereas all PDGF isoforms efficiently activate alpha PDGFR. Therefore, the responsiveness of vascular SMC to PDGF will be determined by the PDGFR subtype that is expressed and by the ligand isoforms that are present. Immunostaining failed to detect alpha or beta PDGFR in our uninjured baboon vessels, but after balloon injury or endarterectomy there was a sharp increase in beta, but not alpha, PDGFR (Figures 1 and 2, and data not shown). This finding is consistent with previous observations showing beta PDGFR expression in injured rat carotid arteries, in injured pig coronary arteries, in the neointima of vascular grafts in baboons, in human atherosclerotic plaques, and in human coronary arteries after angioplasty. Although alpha PDGFR was not detected in baboon arteries in the present study, low levels of this receptor have been detected in the media of the rat carotid artery and in the neointima of healing vascular grafts in baboons. Alpha PDGFR expression by medial SMC could be functionally important because PDGF A chain, which only interacts with the alpha receptor, has been routinely observed in atherosclerotic plaques and in medial cells after injury.

To address the relative importance of alpha and beta PDGFR in the vascular response to injury, we used the isotype-matched neutralizing mAbs 2H7C5 and 2A1E2 directed against alpha and beta PDGFRs, respectively. These mAbs have very similar properties in that they recognize epitopes within the PDGF binding domain, and they block PDGF binding, receptor autophosphorylation, and mitogenic signaling. More importantly, they show almost complete inhibition of baboon PDGFR phosphorylation at concentrations >10 nmol/L (Figures 3 and 4). Treatment of baboons with mAb 2A1E2 for 6 days after injury resulted in a significant 37% to 48% reduction in neointima formation 30 days after femoral artery balloon injury or carotid artery endarterectomy, whereas such treatment with mAb 2H7C5 had no measurable effect (Figures 6 and 7; Tables 1 and 2). This result implies that alpha receptor signaling is not required for the PDGF response to vascular injury in baboons. Beta PDGFR may also play a predominant role in the rat carotid artery because its level of expression and autophosphorylation were increased after injury, whereas alpha PDGFR was unaffected. Also, periadventitial treatment of the rat carotid artery with antisense oligonucleotide that blocked beta PDGFR expression caused a 60% to 80% reduction in neointima formation. Taken together, these data strongly support the conclusion that beta PDGFR is important in the response to vascular injury, and that the alpha PDGFR is unlikely to play a major role.

**Table 2. Effect of Anti-β-PDGFR mAb in Femoral Artery Balloon Angioplasty**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight, kg</th>
<th>Number of Animals</th>
<th>Number of Vessels</th>
<th>Neointima</th>
<th>Media</th>
<th>Neointima/Media</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low body weight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.5 ± 1.2</td>
<td>7</td>
<td>10</td>
<td>0.23 ± 0.071</td>
<td>1.57 ± 0.35</td>
<td>0.15 ± 0.042</td>
</tr>
<tr>
<td>Anti-α-PDGFR</td>
<td>8.9 ± 0.46</td>
<td>5</td>
<td>5</td>
<td>0.27 ± 0.17</td>
<td>1.57 ± 0.37</td>
<td>0.16 ± 0.073</td>
</tr>
<tr>
<td>Anti-β-PDGFR</td>
<td>9.6 ± 0.63</td>
<td>7</td>
<td>7</td>
<td>0.12 ± 0.042</td>
<td>1.37 ± 0.21</td>
<td>0.085 ± 0.033</td>
</tr>
<tr>
<td><strong>High body weight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>29.2 ± 1.3</td>
<td>6</td>
<td>7</td>
<td>0.41 ± 0.12</td>
<td>1.98 ± 0.37</td>
<td>0.20 ± 0.041</td>
</tr>
<tr>
<td>Anti-β-PDGFR</td>
<td>29.8 ± 4.11</td>
<td>9</td>
<td>13</td>
<td>0.22 ± 0.14</td>
<td>2.16 ± 0.37</td>
<td>0.097 ± 0.052</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SD.

**Figure 7. Effect of anti-beta PDGFR mAb (2A1E2) and anti-alpha PDGFR mAb (2H7C5) on baboon femoral artery neointima formation at 30 days after balloon angioplasty.** The ratio of the area of neointima to the area of media at sites of femoral balloon angioplasty is shown. A, Results for baboons having body weights averaging 9 to 12 kg (Table 2). B, Results for animals having body weights averaging 29 to 30 kg. A consistent benefit of therapy is seen with anti-beta PDGFR mAb, but not anti-alpha PDGFR mAb.
at the site of injury, was used.23 Therefore, a higher local concentration of PDGF would be expected at this site, perhaps making PDGFR antagonism more difficult. This could explain the reduced effectiveness of mAb 2A1E2 that was seen in this setting compared with the balloon angioplasty model (Figures 5 and 7). After platelet deposition has subsided, a continuous source of PDGF BB is provided by macrophages and the regenerating endothelium.1,2,4,7,29,30,39,40 In the denuded rat carotid artery, endothelial cells at the wound edge begin to express PDGF B within 8 hours; by several days after injury, when the endothelium is undergoing regrowth and migration, it is present at and near the leading edge of the regenerating monolayer.7 This localized source of PDGF could act on the underlying SMC to promote intimal thickening. Interestingly, even 6 weeks after injury, when replication is no longer detectable, PDGF B mRNA is still being expressed in the denuded zone.7 In our baboon model of restenosis, endothelial regeneration and neointima formation continued for 30 days or longer,23 and in rats, arterial wall myointimal hyperplastic potential has been shown to persist long after the initial injury.34 Therefore, extending the treatment period of PDGFR antagonism beyond the 6 days used in the present study might inhibit lesion formation to an even greater extent.

Previous attempts to establish a direct role for PDGF in the vascular response to injury have been conducted primarily using the rat carotid injury model.17,18,35,41 In this model, balloon injury initially causes some cell death followed by 15% to 25% of medial SMC undergoing proliferation by 48 to 72 hours.42–46 This medial cell proliferation was inhibited by 85% with a neutralizing anti–basic fibroblast growth factor antibody administered before injury.47 This antiproliferative effect may have been caused by the neutralization of basic fibroblast growth factor that was released on cell death. In contrast, anti-PDGF antibodies have little effect on this initial phase of cell proliferation.17 However, the subsequent SMC migration across the internal elastic lamina, which leads to neointima formation, is strongly mediated by PDGF.17,48 For example, neutralizing antibodies against PDGF were found to reduce SMC migration across the internal elastic lamina at 4 days after injury by 80% and inhibited subsequent neointima formation by 40%.17,48 Conversely, infusion of PDGF BB caused a 20-fold increase in neointima formation with little change in medial SMC proliferation.49 In the baboon, PDGF may act similarly because beta PDGFR antagonism by mAb 2A1E2 had no detectable effect on the rate of medial cell proliferation in either carotid or femoral arteries at 7 days after injury but inhibited vascular lesion formation by 48% at 30 days after injury. These results support the view that enhanced SMC migration is an important mechanism by which PDGF mediates vascular lesion formation. SMC migration across the internal elastic lamina requires the formation of plasmin and the activation of matrix metalloproteinases, which have been shown to be regulated by PDGF.48,50–54 It is noteworthy that inhibitors of matrix metalloproteinases and tissue-type plasminogen activator effectively block PDGF-induced SMC migration after balloon injury,48,50,52 but not the subsequent neointima formation.52 Therefore, other effects of PDGF, including its ability to induce synthesis of extracellular matrix,55–59 protect SMC against apoptosis60,61 and possibly enhance intimal SMC proliferation41 may contribute to neointima formation.

The rat carotid artery model of restenosis has proven to be of limited value for identifying agents to prevent restenosis after coronary angioplasty in humans.19 Some agents that tested positive and then failed to show benefit in the clinic include heparin, calcium-channel blockers, antiplatelet drugs and angiotensin-converting enzyme (ACE) inhibitors.19,62–67 When tested in the baboon model, ACE inhibitors did not decrease intimal thickening after balloon angioplasty or endarterectomy.23 Heparin, the only other of these agents tested in baboons, did not inhibit lesion formation at the usual antithrombotic doses that were effective in the rat.68 Thus, the factors mediating the vascular response to injury in humans and nonhuman primates are different from those in rodents. Interspecies differences that could be of considerable consequence include the greater propensity for thrombus formation at arterial injury sites in primates compared with rodents, which would entail a larger accumulation of platelets in primates and most likely cause more PDGF to be released at the injury site. Also, rat platelets contain only the PDGF BB isoform, whereas human and baboon platelets also contain significant levels of the PDGF AB and PDGF AA isoforms.36 Although the potential biological significance of having multiple PDGF isoforms is unknown, this characteristic is unique to primates.36

A major drawback of all commonly used animal models of restenosis is that they do not duplicate the underlying disease state that exists in human atherosclerotic vessels. The response to acute mechanical injury of normal arteries in animals primarily involves SMC proliferation and migration. In humans, restenosis after angioplasty is a considerably more complex process involving multiple components, including plaque compression, vasoconstriction, thrombosis, recoil, matrix production, inflammatory cell reactions, and vascular remodeling, as well as SMC proliferation and migration.69 The use of coronary stent implantation as a method of revascularization potentially eliminates problems caused by vasoconstriction, recoil, and vascular remodeling, which could account for the reported reduction in restenosis rates compared with those observed after balloon angioplasty without stenting.70,71 Nevertheless, restenosis after vascular stent placement still occurs in 20% to 30% of cases, and the occlusive hyperplastic lesions that develop are highly cellular and arise from SMC migration and intimal proliferation.72–76 Therefore, beta PDGFR blockade may be most effective for prevention of in-stent restenosis, a possibility we are currently exploring. In summary, the studies reported here indicate that pharmacological antagonism of beta PDGF may provide a therapeutic strategy for limiting clinical restenosis after interventional procedures for coronary ischemic syndromes and after other procedures for peripheral artery revascularization or repair.

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References


The Role of Alpha and Beta Platelet-Derived Growth Factor Receptor in the Vascular Response to Injury in Nonhuman Primates
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