Different Effects of High and Low Shear Stress on Platelet-Derived Growth Factor Isoform Release by Endothelial Cells

Consequences for Smooth Muscle Cell Migration

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Abstract—In the present study, we analyzed the effect of conditioned media (CM) from bovine aortic endothelial cells exposed to laminar shear stress (SS) of 5 dyne/cm^2 (SS5) or 15 dyne/cm^2 (SS15) for 16 hours on smooth muscle cell (SMC) migration. In response to CM from bovine aortic endothelial cells exposed to SS5 (CMSS5) and SS15 (CMSS15), migration was 45±5.5 and 30±1.5 cells per field, respectively ($P<0.05$). Similar results were obtained with SS of 2 versus 20 dyne/cm^2 and also when SS of 5 and 15 dyne/cm^2 lasted 24 hours. Platelet-derived growth factor (PDGF)-AA levels in CMSS5 and CMSS15 were 9±7 and 18±5 ng/10^6 cells for 16 hours, respectively ($P<0.05$); PDGF-BB levels in CMSS5 and CMSS15 were 38±10 and 53±10 ng/10^6 cells for 16 hours, respectively ($P<0.05$). PDGF receptor α (PDGFRα) and PDGF receptor β (PDGFRβ) in SMCs were phosphorylated by CMSS15>CMSS5. In response to CMSS15, a neutralizing antibody against PDGF-AA enhanced SMC migration to a level comparable to that of CMSS5; in contrast, antibodies against PDGF-BB abolished SMC migration. Transfection of SMCs with a dominant-negative PDGFRα or PDGFRβ increased or inhibited, respectively, SMC migration in response to CMSS15. Overexpression of wild-type PDGFRα inhibited SMC migration in response to CMSS5, CMSS15, or recombinant PDGF-BB ($P<0.001$). These results suggest that the ability of high SS to inhibit arterial wall thickening in vivo may be related to enhanced activation of PDGFRα in SMCs by PDGF isoforms secreted by the endothelium. **(*Arterioscler Thromb Vasc Biol*. 2002;22:405-411.)**

Key Words: shear stress ■ endothelial cells ■ smooth muscle cells ■ platelet-derived growth factors ■ platelet-derived growth factor receptors

Smooth muscle cell (SMC) migration and proliferation play key roles in neointimal accumulation,[1] and in native arteries and in vascular grafts with an intact endothelium, SMC function is modulated by shear stress (SS). In fact, intimal thickness is enhanced by low blood flow, whereas it is inhibited by high blood flow.[2,3] Endothelial cells (ECs) are directly in contact with the bloodstream, and in response to SS, they secrete a variety of growth factors, including platelet-derived growth factor (PDGF), a potent modulator of SMC migration and proliferation. Therefore, it is possible that the different effects of high and low SS on neointimal accumulation in the presence of an intact endothelium may be partially due to PDGF secreted by ECs.[4–6]

PDGF isoforms consist of homodimers and heterodimers of A, B, C, and D chains, and at least 5 PDGF isoforms have been identified so far: AA, AB, BB, CC, and DD.[7,8] They bind PDGF receptor (PDGFR) α or β subunits (PDGFRα and PDGFRβ, respectively) with different affinities, inducing receptor dimerization and autophosphorylation. Receptor dimer $\alpha\alpha$ binds AA, AB, BB, and CC dimers; $\beta\beta$[9] binds BB and DD dimers; and receptor dimer $\alpha\beta$ binds BB and AB dimers. Once activated, PDGFRs retain distinct chemotactic properties.[10] In fact, cells expressing $\beta\beta$-receptor dimers migrate toward PDGF-BB,[11] whereas activation of $\alpha\alpha$-receptor dimers does not elicit SMC chemotaxis.[12] The molecular basis for these different responses is not completely clarified but may be the consequence of the activation of different signaling pathways.[11] In fact, several reports indicate that $\alpha\alpha$- and $\beta\beta$-receptor dimer signaling relies on a differential usage of intermediate signal transducers, such as Ras and phosphatidylinositol 3′-kinase (PI3K).[11,13,14]

In the present study, it is shown that the chemotactic response of SMCs to conditioned media (CM) from ECs exposed to high SS is lower than that induced by CM from

Received July 9, 2001; revision accepted November 8, 2001.

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Arterioscler Thromb Vasc Biol. **is available at http://www.atvbaha.org** **DOI: 10.1161/hq0302.104528**

cells exposed to low SS and that this effect is partially due to enhanced PDGFRα activation. These results provide a new insight into how SS may modulate SMC migration in the presence of an intact endothelium.

Methods

Cell Isolation

Bovine aortic ECs and SMCs were isolated as previously described.[15] Cells between passages 3 and 10 were used in all experiments. ECs and SMCs were characterized by immunofluorescence staining for factor VIII and anti-SMC α-actin (Dako A/S), respectively. Cell populations >95% pure were used in all experiments.

SS Apparatus

Confluent EC monolayers in DMEM without FCS were exposed to laminar fluid SS of 2, 5, 15, or 20 dyne/cm^2 (SS2, SS5, SS15, and SS20, respectively) for 8, 16, or 24 hours in a cone-and-plate apparatus[16] maintained at 37°C in humidified air with 5% CO_2. Control ECs were kept under static conditions. CM from static control cells (CMC) or from cells exposed to SS of different intensity (CMSS2, CMSS5, CMSS15, and CMSS20) was used as chemoattractant in SMC migration assays.

Migration Assay

SMC migration was evaluated in a modified Boyden chamber assay as previously described.[17] Briefly, bovine aortic SMCs were detached with trypsin, counted, centrifuged, and resuspended at 2×10^5 cells/800 μL in DMEM containing 0.1% BSA. Cells were plated on the upper side of a gelatin-treated polycarbonate filter (8.0-μm pores, Nucleopore Costar Scientific Corp). In the lower chamber of the Boyden apparatus, either human recombinant PDGF-AA (1 or 5 ng/mL, Collaborative Research), PDGF-BB (5 or 10 ng/mL, Collaborative Research), or CM from ECs exposed to SS were used as chemoattractants.

In migration-inhibition experiments, neutralizing antibodies against PDGF-AA and PDGF-BB were placed in the lower chamber of the Boyden apparatus at a concentration of 40 μg/mL. After 4 hours of incubation, cells on the filter were fixed with ethanol and stained with toluidine blue. Cells from 5 randomly chosen high-power (magnification×400) fields on the lower side of the filter were counted.

Determination of PDGF Isoforms in CM

PDGF-AA and PDGF-BB in CM was assayed by an inhibition antibody binding assay. Fixed amounts of polyclonal rabbit anti–PDGF-AA and anti–PDGF-BB were incubated with aliquots of CM in Eppendorf tubes precoated with PBS supplemented with 2% gelatin (PBS-gelatin 2%). After 20 hours of incubation at 4°C, *Staphylococcus aureus* protein A (Sigma Chemical Co) was added, and immunoaggregates were removed by centrifugation. The residual antibody-binding activity in the supernatant was measured by direct ELISA, as previously described.[18]

Immunoprecipitation and Western Blot Analysis

SMCs were incubated for 10 minutes with CM from ECs subjected to SS. Cells were rinsed twice with ice-cold PBS and lysed with 1% Triton X-100, 1 mmol/L phenylmethylsulfonyl fluoride, 100 mmol/L NaCl, 10 μg/mL leupeptin, and 10 μg/mL pepstatin. Lysates were incubated overnight at 4°C with 1 μg anti-PDGFRα or anti-PDGFRβ antisera (Santa Cruz) with an orbital shaker. Subsequently, 50 μL of protein A–Sepharose (Sigma) was added, and orbital shaking was continued for additional 16 hours at 4°C. The immunocomplexes were washed 10 times with lysis buffer and then subjected to 6% SDS-polyacrylamide electrophoresis. For Western blotting, proteins were transferred from the gel to a nitrocellulose membrane and then blocked in PBS containing 5% nonfat dry milk, washed, and incubated with anti-phosphotyrosine antibody (4G10, Upstate Biotechnology). The membranes were then stripped and incubated with anti-PDGFRα or anti-PDGFRβ. Specific signals were visualized by using the ECL Western Blot Detection Kit according to manufacturer's instructions (Amersham Pharmacia Biotech).

Plasmids and Transfection Methodology

SMCs (1.8×10^6) were transfected by using the Lipofectamine Plus reagent (GIBCO-BRL), as previously reported,[19] either with 15 μg of DNA encoding dominant-negative PDGFRα (DN-PDGFRα), dominant-negative PDGFRβ (DN-PDGFRβ), wild-type PDGFRα, or PDGFRβ mutants in which single or double tyrosine residues were mutated to phenylalanine at amino acid positions 1009, 1009/1021, or 740/751. Controls received equal amounts of pCDNA3 empty vector. All transfections were performed in the presence of 5 μg (3:1 ratio) cotransfected enhanced green fluorescent protein (GFP)-N1 vector (pEGFP-N1) (Clontech).

Because cotransfection with 2 independent vectors results in the internalization of both plasmids by the same cell,[20] migrated cells were counted by using fluorescence microscopy to evaluate only GFP-positive cells to overcome the limitations of low transfection efficiency, ie, transfection of 5% to 10% of the total population.

Statistical Analysis

Continuous variables were analyzed by the Student *t* test and ANOVA. Post hoc tests according to the Student-Newman-Keuls methods were used when the *P* value (by ANOVA) indicated a statistically significant difference among groups. Data are expressed as mean±SD. A value of $P<0.05$ was deemed statistically significant.

Results

Modulation of SMC Migration

In these experiments, the chemotactic effect of CM from ECs kept under static conditions (CMC) or exposed to different levels of SS on SMC migration was examined (Figure 1A).

In the Boyden chamber assay, all CM from ECs exposed to SS enhanced EC migration compared with CMC ($P<0.05$). However, the ability of CMSS15 and CMSS20 to induce SMC migration was ≈40% lower than that of CMSS2 and CMSS5 ($P<0.05$). The chemotactic effect of CMSS5 versus CMSS2 was enhanced ($P<0.05$), whereas there was no significant difference between CMSS15 versus CMSS20. It is noteworthy that the chemotactic effect of CMSS2 and CMSS5 was close to that of 10 ng/mL PDGF-BB, which, in prior studies, has been shown to be the concentration of PDGF-BB that elicits maximal or near-maximal SMC migration in the Boyden chamber assay.[21,22] In other experiments, the time course of the effect of SS on SMC migration was examined. After 8 hours of exposure to SS, it was found that CMSS15 versus CMC enhanced SMC migration, but this effect did not achieve statistical significance, whereas a significant difference ($P<0.05$) was observed after 16 and 24 hours of exposure to SS (Figure 1B). In addition, after 24 hours of exposure to SS, the chemotactic effect of CMSS5 was still stronger than that of CMSS15 (Figure 1B).

The levels of PDGF-BB, PDGF-AA, and PDGF-AB were examined in CM from ECs kept under static conditions or exposed to either SS5 or SS15 for 16 hours (Table). Significant levels of PDGF-AA and PDGF-BB were found in all CM, and both PDGF isoforms were significantly increased in response to SS15 compared with SS5 or with the static condition ($P<0.05$). In contrast, the increase induced by SS5 versus the static condition was not statistically significant. PDGF-AB was either undetectable or extremely low in all CM.

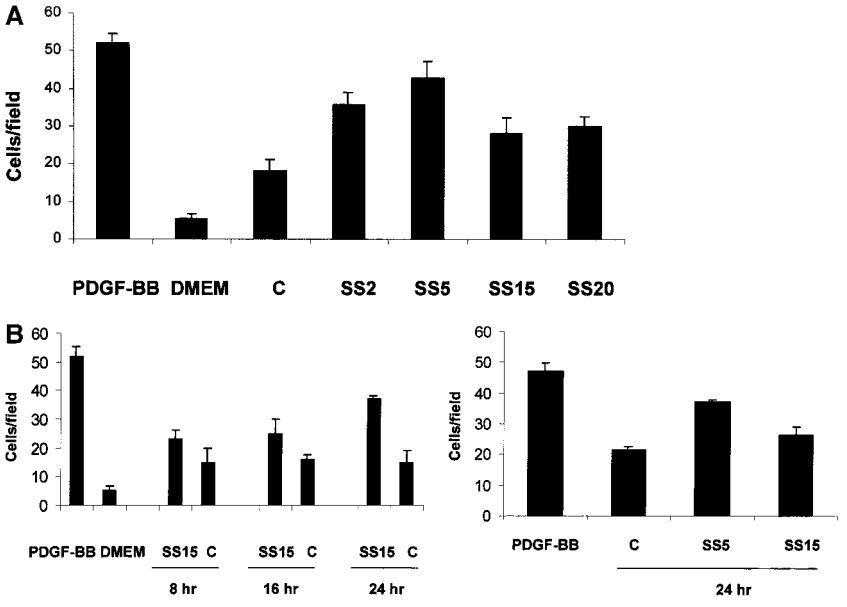

Figure 1. Effect of EC CM on SMC migration. CM from ECs exposed to SS2, SS5, SS15, or SS20 were used as chemoattractants. CM from bovine aortic ECs grown under static conditions was used as control (CMC). Human recombinant PDGF-BB (10 ng/mL) was used as positive control. DMEM not conditioned was used as negative control. A, Effect of SS of different intensities. SMC migration was markedly lower in response to CMSS15 and CMSS20 compared with CMSS2 and CMSS5 ($P<0.05$). The chemotactic effect of CMSS5 vs CMSS2 was enhanced ($P<0.05$), whereas there was no significant difference between CMSS15 vs CMSS20. In these experiments, exposure to SS lasted 16 hours. C indicates control endothelial cells. Results represent mean±SD of 4 independent experiments for each group. B, Time course experiments. Left graph shows that after 8 hours, the chemotactic effect of CMSS15 vs CMC was enhanced but did not achieve statistical significance; a significant difference was observed after 16 hours, and it persisted at 24 hours ($P<0.05$). Right graph shows that after 24 hours, the chemotactic effect of CMSS5 vs CMSS15 was still enhanced ($P<0.05$) and that the magnitude of this effect was comparable to that observed after 16 hours and shown in panel A. Results represent mean±SD of 3 experiments for each group.

Induction of PDGFR Activation

PDGF family members bind cell surface α- and β-tyrosine kinase receptors and induce receptor dimerization and *trans*-phosphorylation.[9] To investigate whether the EC CM induced receptor activation in SMCs, we examined PDGFRα and PDGFRβ phosphorylation by immunoprecipitation and Western blot analyses (Figure 2). CMSS5 enhanced α- and β-receptor phosphorylation ≈6-fold and 2-fold, respectively, compared with CMC, whereas CMSS15 enhanced PDGFRα and PDGFRβ phosphorylation ≈10-fold and 3-fold, respectively, compared with CMC (please see online Figure I, which can be accessed at http://atvb.ahajournals.org). These results indicate that PDGF isoforms present in the CM collected from ECs exposed to different SS levels activated PDGF receptors and that the activation of PDGFRα and PDGFRβ was higher in response to CMSS15 than to CMSS5.

Effects of PDGF-AA and PDGF-BB on SMC Migration

It has been shown previously that PDGF-AA inhibits the chemotactic effect of PDGF-BB on SMCs.[13] Therefore, the possibility was examined that the presence of PDGF-AA in the CM may inhibit PDGF-BB–directed SMC migration. To address this issue, anti–PDGF-BB and anti–PDGF-AA antibodies were used, and their effectiveness was evaluated in preliminary experiments with human recombinant PDGF-BB and PDGF-AA. In agreement with the results of prior studies, PDGF-BB exhibited a strong chemotactic effect on SMCs (Figure 3A). In contrast, SMC migration in response to PDGF-AA was markedly lower and comparable to that observed in the absence of a chemoattractant (not shown). The chemotactic effect of PDGF-BB was inhibited by PDGF-AA in a dose-dependent manner, and under these conditions, PDGF-AA antibodies reversed the inhibitory effect of 1 and 5 ng/mL PDGF-AA on SMC migration. In contrast, anti–PDGF-BB antibodies decreased the effect further when PDGF-BB and PDGF-AA were used in a 5:1 ratio (Figure 3A). When CMSS5, CMSS15, or CMC was used as a chemoattractant, anti–PDGF-BB antibodies inhibited SMC migration 57±0.6%, 50±1%, or 13±2%, respectively. This result suggests that the ability of EC CM to induce SMC migration is largely related to the presence of PDGF-BB in the CM. In contrast, anti–PDGF-AA antibodies enhanced the chemotactic effect of CMSS15, which achieved a level comparable to that of CMSS5 in the absence of any antibody, whereas the chemotactic effect of CMSS5 and CMC were not affected by the anti–PDGF-AA antibody (Figure 3B). Taken together, these results suggest that the weaker chemotactic effect of CMSS15 compared with CMSS5 is due to the presence of PDGF-AA in CMSS15.

PDGF Isoforms Released by Bovine Aortic ECs After Exposure to SS

	PDGF Levels, ng/10^6 Cells		
Treatment	PDGF-AA	PDGF-BB	PDGF-AB
CMC	8±7	27±10	0
CMSS5	9±7	38±10	0.065±0.033
CMSS15	18±5	53±10	0.065±0.028

Values are mean±SD and are expressed as nanograms per 10^6 cells over 16 hours.

PDGFRα and PDGFRβ in SMC Migration

Because previous studies[23,24] have demonstrated that PI3K and phospholipase C-γ (PLC-γ) are required for SMC migration, we examined whether PDGFRβ mutants, in which the tyrosines residues involved in the binding of PI3K and PLC-γ were changed to phenylalanine residues, could alter SMC migration. Cells transfected with the control plasmid revealed the same migratory capacity as did mock-transfected cells (Figure 1). In contrast, SMC migration was markedly

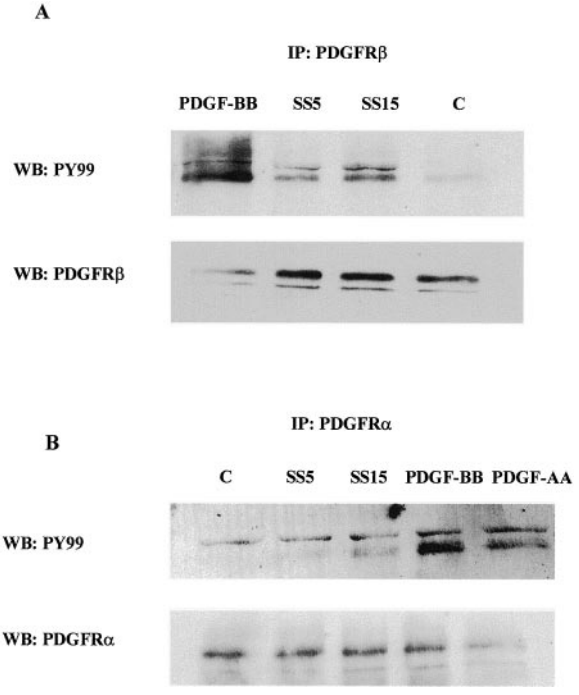

Figure 2. Effect of EC CM on PDGFRβ (A) and PDGFRα (B) phosphorylation. SMCs were incubated for 10 minutes with CMSS5, CMSS15, or CMC. PDGFRα and PDGFRβ were immunoprecipitated (IP) from SMC whole-cell extracts by using polyclonal antibodies recognizing either PDGFRβ or PDGFRα, separated on 6% SDS-polyacrylamide gel, transferred onto nitrocellulose membrane, and probed sequentially with antibodies against phosphotyrosine (PY99) and PDGFRβ(A) or PY99 and PDGFRα (B). Immunocomplexes were visualized by Western blot (WB) analysis and quantified by densitometric analysis (please see online Figure I at http://atvb.ahajournals.org). Data show that PDGFRβ and PDGFRα phosphorylation was enhanced when SMCs were incubated either with CMSS15 or with CMSS5 vs CMC and that the activation of both receptors was more marked in response to CMSS15 vs CMSS5. Exposure to 10 ng/mL PDGF-BB for 10 minutes was used as a positive control. Similar results were obtained in 4 independent experiments.

inhibited when cells were transfected with PDGFRβ mutants Y1009/Y1021F and Y1021F, which are unable to activate PLC-γ, or when cells were transfected with the Y740/751F mutant, which is unable to activate PI3K (please see online Figure II, which can be accessed at http://atvb.ahajournals.org). To further explore this aspect, we used DN-PDGFRα and DN-PDGFRβ containing the transmembrane and extracellular domains but lacking the cytoplasmic domains. SMCs transfected with DN-PDGFRβ showed a strong inhibition of the chemotactic response to PDGF-BB, CMSS5, and CMSS15 ($P<0.05$), whereas there was no significant inhibition in response to CMC (Figure 4A). Cells transfected with DN-PDGFRα exhibited an enhanced chemotactic response to CMSS15 ($P<0.05$), whereas the effect of CMSS5, CMC, and PDGF-BB was not modulated (Figure 4B). Furthermore, the overexpression of wild-type PDGFRα inhibited SMC migration under all conditions tested (Figure 4C), and the magnitude of this effect was comparable to that of DN-PDGFRβ (Figure 4A). These data are in agreement with prior studies in other cellular systems[21,25,26] showing that positive chemotactic signals are mediated only by PDGFRβ, whereas PDGFRα activates negative regulatory pathways. Our data suggest that the production of different PDGF isoforms by ECs in response to SS could be one of the mechanisms by which the endothelium modulates the biological responses of SMCs.

Discussion

In the present study, we report that SMC migration is lower in response to the CM of ECs exposed to high SS compared with low SS. The increase in SS from 5 to 15 dyne/cm^2 enhanced PDGF-BB and PDGF-AA secretion. In addition, the degree of phosphorylation of PDGFRα and PDGFRβ in SMCs was enhanced when the cells were incubated with CM from ECs exposed to low SS, and it was increased even further when the cells were incubated with CM from ECs exposed to high SS. Inhibition of PDGFRα-mediated effects either by overexpressing DN-PDGFRα or by a PDGF-AA antibody enhanced SMC migration in response to CMSS15; in contrast, these interventions failed to modulate SMC migration when CMSS5 was used as a chemoattractant. Taken together, these results indicate that PDGFRα activation on SMCs is enhanced when the cells are treated with the CM of ECs exposed to high SS and that, via this mechanism, PDGFRβ-directed SMC migration is inhibited. PDGF-BB binds $\beta\beta$-, $\alpha\beta$-, and $\alpha\alpha$-receptor dimers, whereas PDGF-AA selectively binds $\alpha\alpha$ dimers; it has been shown that PDGF-AA inhibits PDGF-BB–directed SMC migration,[13,22,25,26] and this effect has been attributed to different signaling mechanisms between PDGFRβ and PDGFRα.[14] In fibroblasts, PDGF-AA inhibits the increase in cytosolic Ca^{2+} concentration triggered by PDGF-BB, which is a key signal in SMC migration.[27] Furthermore, α- and β-receptors activate extracellular signal–regulated kinases, whereas only PDGFRα activates c-Jun NH_2-terminal kinase 1, which inhibits the PDGFRβ-induced phenotypic transformation of NIH 3T3 cells.[28] Previously, it has been shown that PDGFRβ, on exposure to high concentration PDGF-BB, can also inhibit SMC chemotaxis.[19] However, under the conditions of the present study, the PDGFRβ-mediated inhibition of SMC migration did not appear to play a role, inasmuch as the cells overexpressing DN-PDGFRβ or cells treated with anti–PDGF-BB neutralizing antibody failed to exhibit an enhanced chemotactic response; rather, they showed a decrease in migration in response to CMSS5 and CMSS15.

In humans and in animal models, intimal-medial thickness in the presence of an intact endothelial layer is modulated by SS[29,30] as well as by some risk factors for atherosclerosis.[31] Studies with noninvasive ultrasound techniques, performed either on human autopsy specimens or in vivo,[32,33] have shown that in elastic and in muscular arteries, low SS is associated with enhanced arterial wall thickness. Similar conclusions have been reached in studies using animal models.[29] In endothelialized balloon vascular grafts, a reduction in flow and SS initiates subendothelial SMC proliferation, which ultimately leads to severe neointimal accumulation.[34–36] In one in vivo study, the roles of PDGFRα and PDGFRβ in neointimal accumulation in vascular grafts were addressed[34–36] by treating the animals with blocking antibodies either to PDGFRβ or to PDGFRα.[36,37] Baboons treated with the antibody to PDGFRα exhibited a decrease in SMC nuclear density, consistent with the known effect of PDGFRα on SMC proliferation; however, despite this effect on cell number, there was not a decrease in neointima. SMC prolif-

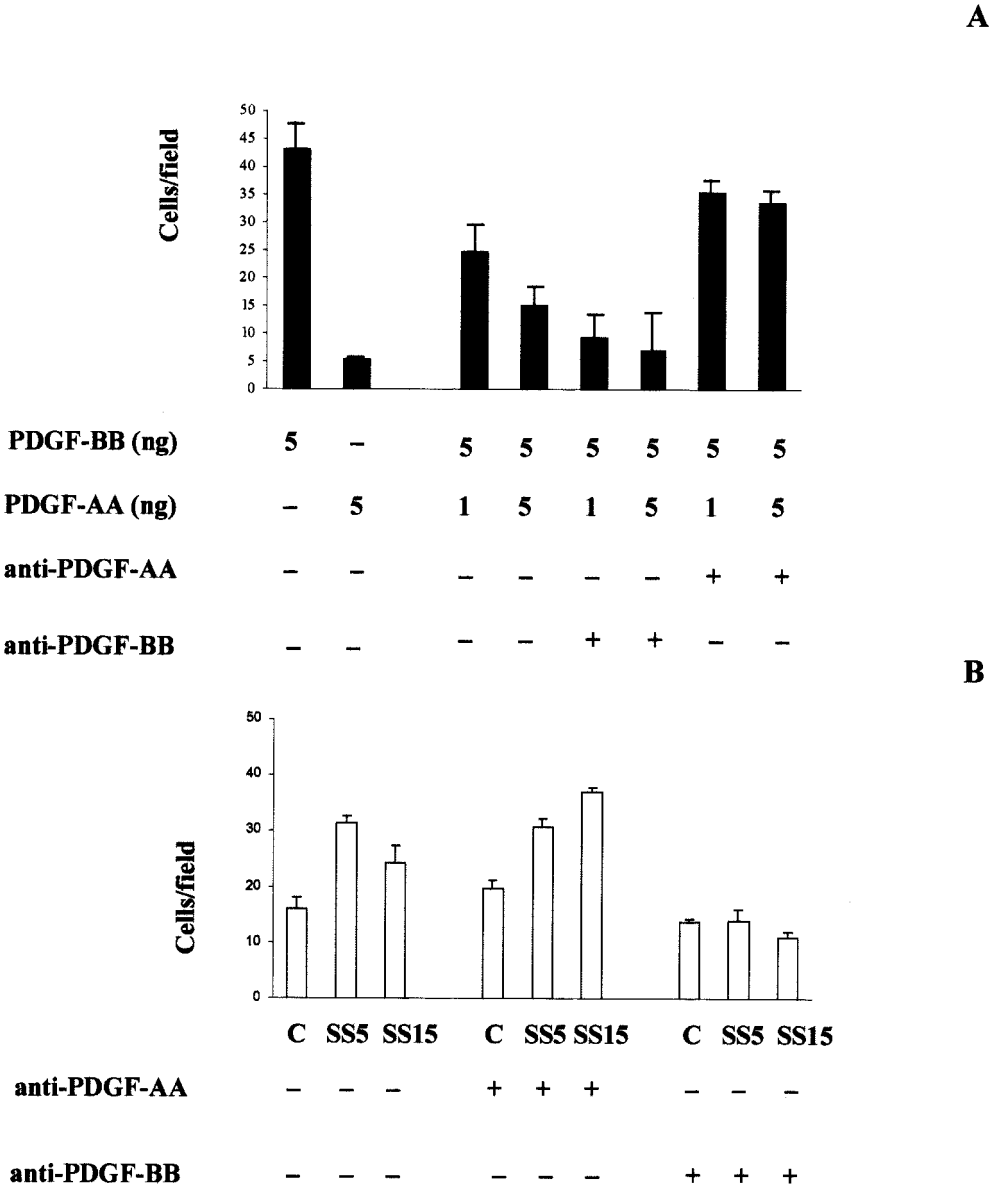

Figure 3. Effect of anti–PDGF-BB and anti–PDGF-AA antibodies on SMC migration. A, SMCs were exposed to PDGF-BB (5 ng/mL) and PDGF-AA (1 or 5 ng/mL) either alone or in a 5:1 or a 1:1 ratio. PDGF-AA (1 ng/mL and 5 ng/mL) exhibited a dose-dependent effect to inhibit PDGF-BB–directed SMC migration. Furthermore, the chemotactic effect of PDGF-BB/PDGF-AA was inhibited by anti–PDGF-BB antibodies (40 μg/mL) when PDGF-BB/PDGF-AA was used in a 5:1 ratio, whereas it was enhanced by anti–PDGF-AA antibodies (40 μg/mL) when PDGF-BB/PDGF-AA was used in a 5:1 or a 1:1 ratio ($P<0.05$). The number of migrated cells was determined after 4 hours of exposure to PDGF-BB, PDGF-AA, or PDGF-BB/PDGF-AA. Results represent mean±SD of 3 independent experiments for each group. B, Cell migration in response to different CM was evaluated in the presence of anti–PDGF-AA (40 μg/mL) or anti–PDGF-BB (40 μg/mL) antibodies. Anti–PDGF-AA enhanced SMC migration in response to CMSS15 ($P<0.05$), whereas it had no significant effect on migration in response to CMSS5 and CMC. In contrast, anti–PDGF-BB antibody inhibited SMC chemotactic response to CMSS5 and CMSS15 ($P<0.05$), whereas it had no effect on CMC. Results represent mean±SD of 3 independent experiments in each condition.

eration and migration from the media to the intima and extracellular matrix production are key events in arterial wall remodeling. However, the mechanisms by which blood flow affects SMC function within the arterial wall, in the presence of an intact endothelium, are still poorly characterized. Recently, it has been shown that ECs exposed to flow produce and secrete plasminogen activator inhibitor-1 and, thus, inhibit SMC migration in vitro.[38] The potential role of PDGF has been examined in several prior studies because this growth factor is a powerful SMC chemotactic agent and also enhances SMC proliferation and matrix metalloproteinase-2 and -9 expression in SMCs.[39] Hsieh et al[40] reported that SS transiently increases PDGF-A and -B mRNA. Specifically, PDGF-A mRNA increased when SS was enhanced from 6 to 51 dyne/cm^2, whereas PDGF-B mRNA was upregulated at significantly lower SS rates, ranging between 0 and 6 dyne/cm^2. This effect of SS on PDGF gene expression has been confirmed by other studies,[40–44] and more recently, an SS response element has been identified in the promoter region of several genes, including PDGF-B and PDGF-A.[43] These results on PDGF gene expression were corroborated by PDGF-BB measurements in CM of ECs; in these experi-

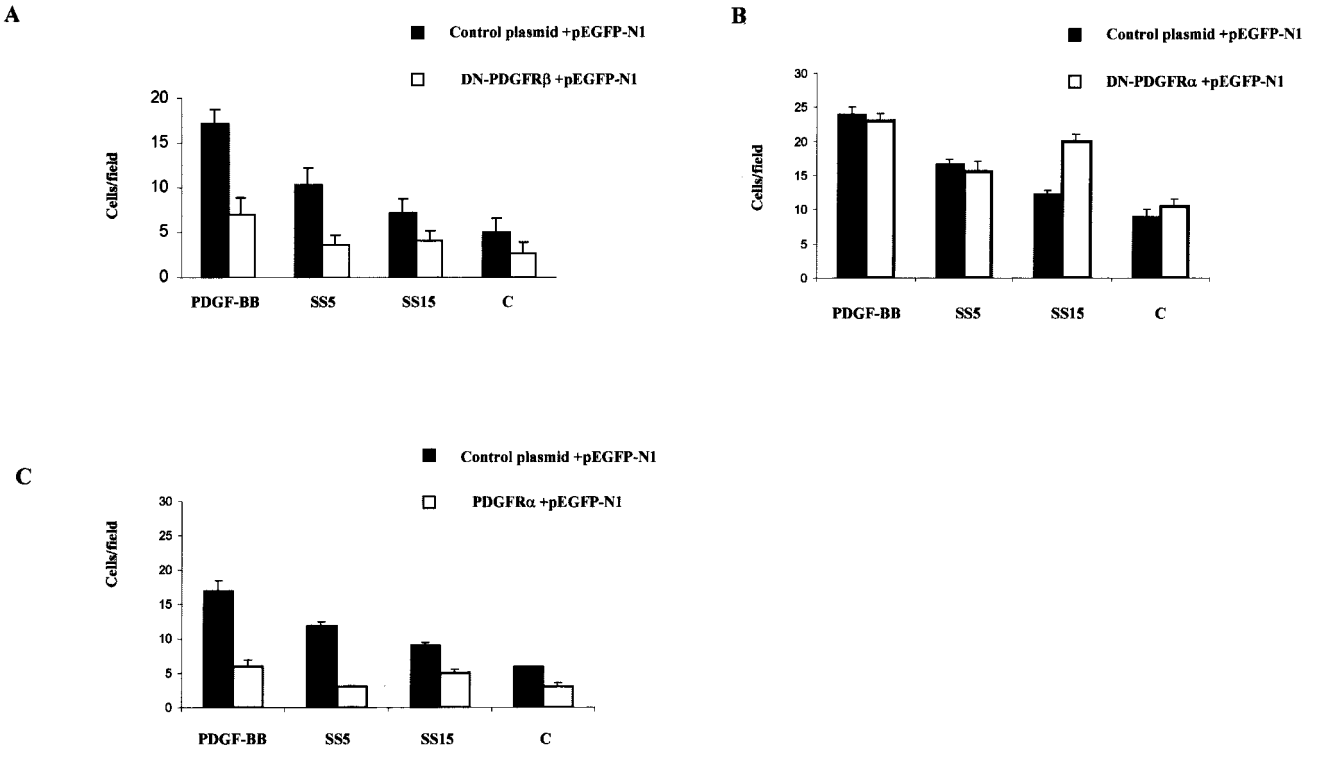

Figure 4. Effect of CM on migration of SMCs transfected with DN-PDGFRβ, DN-PDGFRα, wild-type PDGFRα, and equal amount of pEGFP-N1 (see Methods). A, Cells transfected with DN-PDGFRβ exhibited a significant decrease in migration compared with cells transfected with control plasmid in response to PDGF-BB, CMSS5, and CMSS15 ($P<0.05$). In contrast, the decrease in response to CMC did not achieve statistical significance. B, The chemotactic effect of CMSS15 was enhanced in SMCs transfected with DN-PDGFRα, whereas the chemotactic effect of CMSS5, CMC, and PDGF-BB was not modulated by PDGFRα overexpression. C, Cells transfected with wild-type PDGFRα exhibited a decrease in migration in response to CMSS5, CMSS15, CMC, and PDGF-BB. In contrast, cells transfected with control plasmid showed a chemotactic response similar to that reported in Figure 1. These experiments were performed with cells cotransfected with GFP and DN-PDGFRβ, DN-PDGFRα, wild-type PDGFRα, or control expression vectors pcDNA3; only GFP-positive cells were counted in the migration assay. PDGF-BB (10 ng/mL) was used as positive control. All results represent mean±SD of 4 independent experiments in each condition. $P<0.001$.

ments, SS of 3 to 9 dyne/cm^2 enhanced PDGF-BB secretion.[41] Furthermore, in vivo studies have shown that flow reduction enhances endothelial PDGF-A and -B gene expression in the rat carotid.[45] The present report confirms that SS modulates PDGF secretion by the endothelium; in addition, it shows that different levels of SS lead to the production of different PDGF isoforms, which, in turn, exhibit different chemotactic effects on SMCs. Under the experimental conditions of the present study, SMC migration in response to the CM of ECs exposed to 15 or 20 dyne/cm^2 versus 2 or 5 dyne/cm^2 was ≈40% lower. It is difficult to establish the biological significance of this finding and extrapolate it to in vivo studies that have shown a protective effect of higher SS on arterial wall thickness.[32] Nevertheless, these results, without excluding other contributing mechanisms, establish proof of the principle that SS levels comparable to those found in the human arterial system[32] determine which PDGF isoform is preferentially secreted by the endothelium; because PDGF isoforms exhibit different binding affinities for PDGFRα and PDGFRβ, signaling via these receptors may be responsible, at least in part, for the different behavior of SMCs underlying ECs exposed to high versus low SS.

Acknowledgments

This work was partially supported by the Italian Ministero della Salute. Dr Palumbo was partially supported by an EMBO Fellowship. Dr Rönnstrand holds a position as Senior Researcher funded by the Swedish Research Council. We thank Alessandra Cucina, PhD, and Valeria Borrelli, PhD, for PDGF quantification. We acknowledge Gabriella Ricci Cinzia Carloni and Susanna Rulli for excellent secretarial assistance.

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Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Different Effects of High and Low Shear Stress on Platelet-Derived Growth Factor Isoform Release by Endothelial Cells: Consequences for Smooth Muscle Cell Migration

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Arterioscler Thromb Vasc Biol. 2002;22:405-411
doi: 10.1161/hq0302.104528
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636

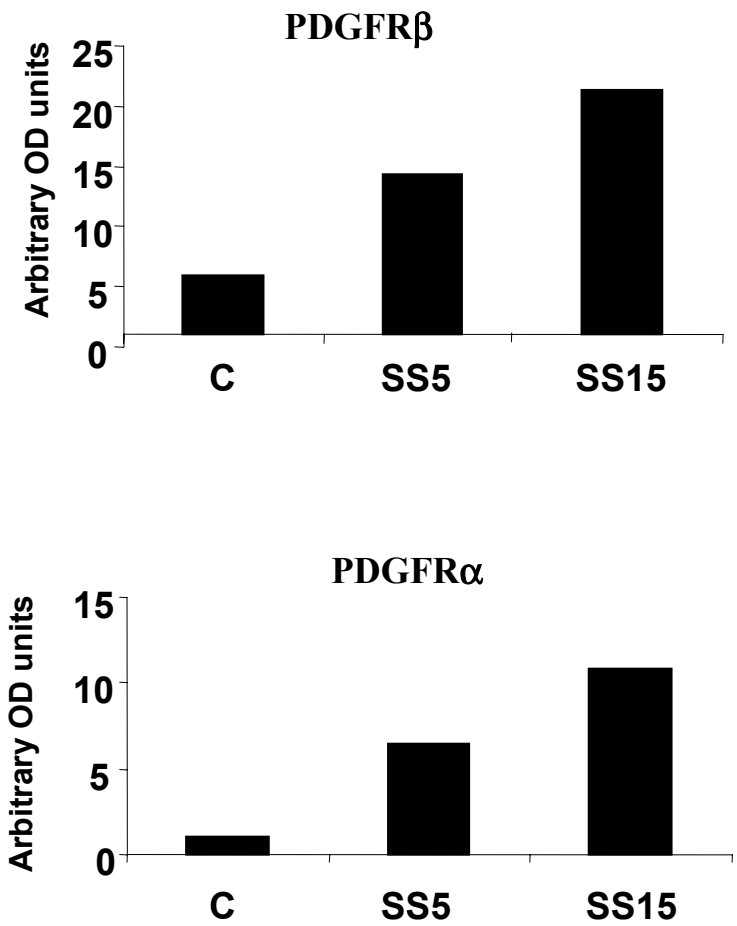
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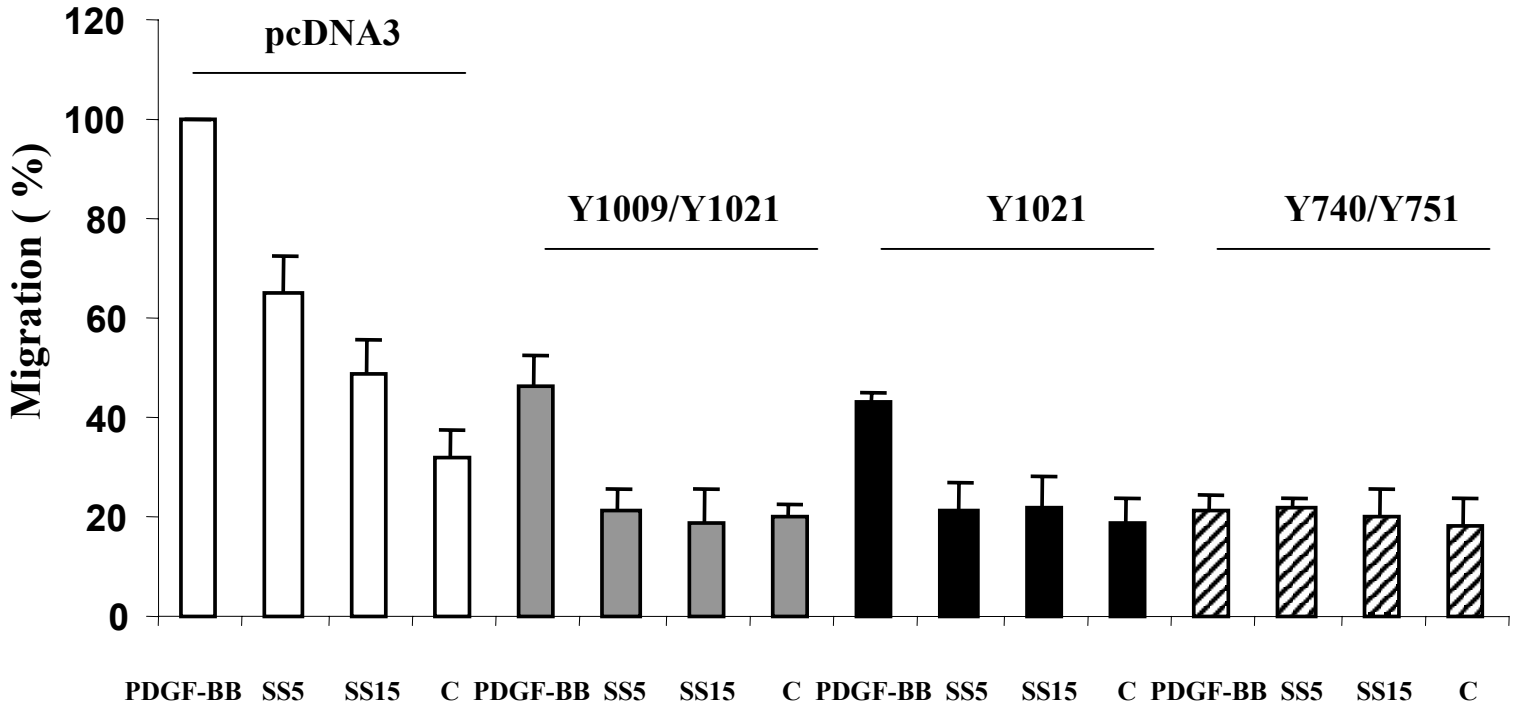
Figure I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20
0
Migration (%)
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Y1021
Y740/Y751
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Figure II