Fluid flow decreases preproendothelin mRNA levels and suppresses endothelin-1 peptide release in cultured human endothelial cells

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Endothelin-1, a 21-amino acid peptide secreted by endothelial cells, has constrictor and mitogenic activity for vascular smooth muscle cells, and its mitogenic activity is synergistic with that of platelet-derived growth factor. Endothelial cell–derived endothelin-1 might therefore contribute to intimal hyperplasia in reendothelialized segments of vascular grafts or of endarterectomy and angioplasty sites. Because intimal hyperplasia occurs most often at sites with disordered flow patterns and lower fluid shear stress, we tested the effects of static culture versus high laminar shear stress (25 dyne/cm^2) on endothelin-1 precursor (preproendothelin) gene mRNA transcript levels and endothelin-1 peptide release in cultured human endothelial cells. Primary cultures of human umbilical vein endothelial cells were subjected to controlled levels of shear stress in parallel plate flow chambers for 24 hours. To detect preproendothelin mRNA we applied a linked reverse transcriptase-polymerase chain reaction (RT/PCR) to RNA extracted from cultures. Southern blots of RT/PCR reaction products were hybridized with radioactive phosphorous (^{32}P) labeled probes for the amplified preproendothelin complementary deoxyribonucleic acid (cDNA). Detection by RT/PCR of mRNA for glyceraldehyde 3-phosphate dehydrogenase was used to measure a constitutively expressed control signal. Endothelin-1 release into culture medium was measured by radioimmunoassay. Application of 25 dyne/cm^2 of shear stress for 24 hours sharply reduced endothelial cell levels of precursor preproendothelin mRNA. High shear stress also decreased endothelin-1 peptide release rates into culture medium from 0.55 ± 0.02 and 0.49 ± 0.03 ng/10^6 endothelial cells/hour (n = 3 replicate cultures in each experiment) (static conditions) to values of 0.13 ± 0.03 and 0.28 ± 0.06 ng/10^6 cells/hour (shear stress) ($p < 0.01$). The flow-induced suppression of preproendothelin gene expression suggests that endothelin-1 release from endothelial cells might help mediate the inverse relationship between local fluid shear rates and the severity of neointimal hyperplasia. (J Vasc Surg 1991;14:1-9.)

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Endothelin-1 is a 21-amino acid peptide first found in the medium conditioned by cultured endothelial cells (ECs) and later shown by immunohistologic methods to be present in the EC layer of human arteries in vivo.[1-3] Endothelin-1 is made from a single copy gene by initial transcription and translation to produce the 202-amino acid precursor preproendothelin (PPET). Subsequent cleavages produce a 39-amino acid peptide (big endothelin) and the final 21-amino acid product endothelin-1.[2-5] Although other types of endothelin denoted endothelin-2 and endothelin-3 have been found, only endothelin-1 appears to be made by vascular endothelium.[4] Endothelin-1 is a potent vasoconstrictor effective at concentrations of as low as 10^{-9} mol/L[2].

Endothelin-1 is also a mitogen for vascular smooth muscle cells (SMCs) and Swiss 3T3 cells.[6-8] In addition, the mitogenic action of endothelin-1 on at least one cell type (Swiss 3T3) is synergistic with the effects of platelet-derived growth factor (PDGF), and basic fibroblast growth factor,[9] which can also be expressed in vascular wall cells in vivo and in vitro.[10-13] These reports suggested the hypothesis that endothelin-1 from ECs may act together with other growth factors from ECs or SMCs to induce proliferation in subjacent vascular SMC layers in vivo.

Both SMC proliferation and intimal hyperplasia tend to occur in vivo in regions of disordered flow such as graft anastomoses, carotid endarterectomy sites, or angioplasty sites with residual stenoses.[14-18] Animal studies have shown an inverse relationship between local fluid shear stress and the severity of intimal hyperplasia, suggesting that fluid shear might affect growth factor gene expression in vascular wall cells.[19-24] One study reported that exposure of cultured and passaged porcine aortic ECs to high fluid shear stress decreased the precursor preproendothelin mRNA transcript level, although the absolute level of fluid shear was not specified.[2] A later report, however, described the effect of fluid shear stress on fifth to tenth passage cultured porcine aortic ECs as a very different effect of transient *stimulation* of endothelin mRNA levels and of endothelin-1 peptide release at low shear stresses of 5 dyne/cm^2. Initial stimulation between 1 and 4 hours after shear stress initiation was followed by a gradual decline in endothelin mRNA levels over the next 12 to 24 hours, whereas higher shear stress exposure (of over 8 dyne/cm^2) was reported to have no effect on EC levels of endothelin mRNA compared to static culture.[25] We have studied the response of mRNA levels and secretion rates for endothelin-1 in human umbilical vein endothelial cells (HUVECs) either maintained in static culture or exposed to arterial levels of steady laminar shear stress (25 dyne/cm^2) for 24-hour periods. In contrast to the report cited above,[25] we found that high shear stress strongly and persistently suppresses both (1) the mRNA transcript levels for endothelin measured at 24 hours and (2) the rate of endothelin-1 peptide release at all times of measurement starting 2 to 4 hours after initial shear stress exposure.

METHODS AND MATERIAL

Use of primary cultures with small cell numbers to study changes in preproendothelin mRNA transcript levels. Patterns of EC gene transcription and translation of proteins for growth factors, growth factor receptors, antithrombotic substances, and cytoskeletal components are distorted in cultured ECs serially passaged and exposed to exogenous growth factors.[26-28] For this reason we studied ECs in primary culture without added growth factors. Such small primary cultures ($< 3 \times 10^6$ cells per experiment), however, contain insufficient total RNA for quantitation of multiple gene mRNA levels by conventional Northern blot methods. We therefore used the method of linked reverse transcription and polymerase chain reactions (RT/PCR) to amplify mRNA signals from these cultures as described in recent reports.[29-32]

Endothelial cell harvest and plating. Umbilical cords were stored at 4° C and used for HUVEC harvest within 6 hours of delivery by a modification of the method of Gimbrone.[33] The vein was flushed with sterile phosphate-buffered saline (PBS) at 37° C, filled with a sterile solution of 40 to 50 units/ml of sterile type CLS I collagenase in PBS, closed at both ends, and incubated at 37° C for 30 minutes by immersion in PBS. The vein lumen was flushed with 100 ml PBS, and the pooled effluent was centrifuged for 10 minutes at 100 *g*. The cell pellet was resuspended in M199 with 0.1 mg/ml penicillin, 0.1 mg/ml streptomycin, 0.20 mg/ml neomycin, 0.3 mg/ml L-glutamine, and 20% heat-inactivated fetal calf serum. Cells were seeded on 35 × 75 mm glass slides at initial plating densities estimated to range from 2×10^4 to 5×10^4 cells per cm^2 for various primary harvests. Each experiment was performed with replicate monolayers plated from the same primary cell harvest. Before cell seeding, slides were cleaned in 0.5 mol/L NaOH for 2 hours, rinsed with deionized water, and autoclaved. At 24 hours after plating, slides were washed three times with 10 ml warm PBS to remove unattached cells. Monolayers of confluent HUVECs formed on the slides were used for experiments 3 to 4 days after seeding.

Experimental apparatus. Replicate slides plated from the same primary harvest were exposed to shear stress in individual (one slide per chamber) parallel plate flow chamber systems with a constant volume of recirculating medium driven by a constant hydrostatic pressure head. A peristaltic pump recirculated medium to the upper reservoir but did not drive flow through the flow chamber.[34-36] Three independent parallel plate flow chambers and recirculation loop systems, each filled with 15 ml of complete medium, were set up to maintain wall shear stresses of 25 dyne/cm^2 on the HUVEC monolayer, which formed one plate of the flow chamber. Each chamber had a gap thickness of 200 μm and a monolayer surface

area of 15 cm^2. Replicate monolayers were incubated under static conditions as controls. Flow systems were kept at 37° C with an air curtain incubator. Medium pH was maintained at 7.4 by continuous gassing with a mixture of 5% CO_2 in air. Shear stress was calculated from the formula (shear stress) = 6(Q) (u)/(B^2) (w); where Q = flowrate in cm^3/sec, u = viscosity (in poise), B = gap thickness (in centimeters), and w = chamber width (in centimeters). These conditions were chosen to produce a purely laminar flow without microzones of turbulence and with fluid mechanics at the cell surface dominated by viscous forces (local cell surface Reynolds number < 0.005), since they resulted in a macroscopic Reynolds number of < 20, an entrance length distance less than 1 mm, and cellular height variations of the order of only 1 μm allowing the cell surface to be treated as a flat layer for purposes of calculation.[34,36]

A total of six samples of 1 ml volume per sample were taken from each flow loop at intervals ranging from 4 to 6 hours over a period of 24 hours starting at the time defined as 0 hours (just after medium change but before flow initiation in the shear stress exposed cultures). Samples were centrifuged 10 minutes at 4° C at 3000 *g;* the supernatants were frozen at −80° C and later used for duplicate radioimmunoassay (RIAs) for endothelin-1 as described below. Whenever the need to sample multiple loops made the exact sampling times differ slightly, the exact times were used as x-axis points in plotting curves of endothelin-1 accumulation in the medium (Fig. 1). Removed sample volumes were replaced with 1 ml fresh medium; RIA measurements of endothelin-1 concentration were corrected for dilution of endothelin-1 level caused by this volume replacement. Human umbilical vein endothelial cell monolayers were photographed at 24 hours to record final cell density.

Measurement of endothelin-1 secretion rate into culture medium. Endothelin-1 release was measured by a RIA kit (Amersham, Corp., Arlington Heights, Ill.) by use of a rabbit antibody against synthetic human endothelin-1, which can detect either endothelin-1 or the variant endothelin-2, but does not cross react with the endothelin-3 subtype. The antiserum cross reacted with endothelin antigen in conditioned medium, could quantitate rises in conditioned medium endothelin content above the background levels from the serum used in fresh unconditioned complete medium, and showed a lower detection limit of 20 femtomoles/ml. A 100 mm^3 volume of undiluted conditioned medium sample or of standard was mixed with 100 mm^3 of 0.02 mol/L borate assay buffer (pH 7.4). A 100 mm^3 aliquot of rabbit antiendothelin serum was then added and the mixture allowed to stand 18 hours at 4° C. Endothelin-1 labeled with iodine 125 (^{125}I) tracer was then added to the tubes and equilibrated 18 hours at 4° C. By use of beads coated with donkey antirabbit antibody, the ^{125}I tracer was pelleted and counted for 1 minute. LOGIT binding plots were fitted by least squares regression analysis, showing correlation coefficients of $R^2 > 0.99$ in all cases.

RNA extraction from endothelial cultures after 24-hour periods of steady laminar shear stress. All solutions were made RNAse free by diethylpyrocarbonate (DEPC) treatment[37] or by filtration done twice through 0.2 μm nitrocellulose filters (Millipore, Corp., Bedford, Mass.).[37] After a 24-hour study, the EC monolayer on each slide was rinsed once with ice cold PBS, lysed in 1.2 ml of 5 mol/L guanidine thiocyanate with 25 mmol/L sodium citrate and 0.5% w/v sodium sarcosinate, sonicated, layered in 2 ml polyallomer tubes (Beckman Instruments, Palo Alto, Calif.) onto an 0.8 ml cushion of 5.7 mol/L CsCl in 2 mmol/L ethylenediamine tetraacetic acid and 25 mmol/L sodium acetate, and centrifuged 3 hours at 2×10^5 *g* (5.5×10^4 rpm) in a Beckman TL-100 (Beckman Instruments, Inc., Fullerton, Calif.) ultramicrocentrifuge with a TL-55 rotor.[38] The RNA pellet was dissolved in water, extracted twice with 25:24:1 phenol:chloroform:isoamyl alcohol and twice with 24:1 chloroform:isoamyl alcohol, vacuum dried, dissolved in H_2O, and precipitated using 1/10 volume 3 mol/L NaOAc and 2.2 volumes of 100% ethanol. The RNA pellet was then washed with 70% ethanol, vacuum dried, and resuspended in 30 mm^3 H_2O. As a final purification step 10 mol/L LiCl was added to bring the solution to a 2.5 mol/L concentration of LiCl, followed by 1.5 hour precipitation in an ice water bath and recentrifugation for 30 minutes at 4° C at 10^4 *g*. The RNA pellet was desalted with 70% ethanol, redried, and dissolved in H_2O. Ribonucleic acid concentrations were measured by ultraviolet spectroscopy before use; all RNA samples showed a final A_{260}/A_{280} absorbance ratio of 1.7 or greater.

Design of oligonucleotide primers and probes for reverse transcription, amplification, and detection of amplified preproendothelin complementary deoxyribonucleic acid (cDNA). We applied a computer program for PCR primer design[39] to the cDNA sequence for the human preproendothelin precursor of endothelin-1 to select sense and

antisense primers and an oligonucleotide probe sited between the primers (Table I).[40] To prevent amplification of any contaminating precursor preproendothelin genomic DNA from increasing the detected 441 bp length precursor preproendothelin mRNA signal strength, these primers were chosen to flank introns in the genomic endothelin DNA sequence.[41] The primers lie outside the precursor preproendothelin cDNA region encoding the final 21-amino acid product; however, endothelin-1 release does not involve prior cytoplasmic storage and is not preceded by postranscriptional rearrangement of precursor preproendothelin mRNA,[2,42] so that detection of a separate region of precursor preproendothelin mRNA can be used to measure endothelin gene transcript levels related to endothelin-1 release. Primers and a probe were also chosen from the cDNA sequence of glyceraldehyde-3 phosphate-dehydrogenase (GAPDH) as a constitutively expressed internal standard signal (Table I).[43] Primers and probes were made by the beta cyanoethyl phosphoramidite method.

Reverse transcription/polymerase chain reactions. Simultaneous reverse transcription of preproendothelin and GAPDH mRNAs to their respective cDNAs was carried out as previously described in a 25 mm^3 reaction volume with 500 units Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT, Bethesda Research Labs, Rockville, Md.), 0.5 μg each of precursor preproendothelin and GAPDH antisense primer, 0.5 mmol/L final concentrations each of dATP, dCTP, dGTP, and dTTP, and with final concentrations of 50 mmol/L Tris-HCl (pH 8.3), 75 mmol/L KCl, and 3 mmol/L $MgCl_2$.[29,32] Before adding RT enzyme each reaction mixture was adjusted with H_2O to a 22.5 mm^3 volume, heated for 6 minutes at 70° C to denature RNA secondary structure, and chilled on ice for 6 minutes to prevent renaturation before adding RT enzyme (500 units/2.5 mm^3) and incubating at 37° C for 30 minutes. The RT reaction was stopped by heating at 90° C for 10 minutes. Preproendothelin and GAPDH cDNA products were coamplified with a PCR in a total volume of 100 mm^3 in the same tube. To do this, a 75 mm^3 volume was added containing 0.5 μg of sense primer together with an additional 0.25 μg antisense primer for both preproendothelin and GAPDH, 6.25 mm^3 of a solution 10 mmol/L for each of four deoxynucleotide triphosphates (dNTPs), 10 mm^3 of ×10 replacement buffer for Taq DNA polymerase,[33,36] and H_2O for volume adjustment. Two units of recombinant Thermus Aquaticus DNA polymerase enzyme (Amplitaq, Perkin Elmer-Cetus, Inc., Norwalk, Conn.) dissolved in 5 mm^3 of ×1 Taq replacement buffer were then added, and the mixture was briefly vortexed, recentrifuged 5 seconds at 6000 rpm, and overlaid with 100 mm^3 mineral oil to prevent evaporation. A total of 24 cycles of PCR amplification in one set of samples and 30 cycles in a duplicate set were performed with settings of 90 C × 1.5 minutes, 50 C × 1 minute, and 72 C × 2 minutes.

Agarose gel electrophoresis and Southern hybridization. After PCR reactions, 90% of the oil was aspirated from tubes and residual oil extracted with 400 mm^3 chloroform. Then 10 mm^3 of the PCR reaction PCR mix was combined with 2 mm^3 of ×7 dye/buffer mix and electrophoresed in a 20 × 25 cm, 2.0% Seakem GTG Agarose gel (FMC Corporation, Rockland, Maine) in ×1 Tris-borate-EDTA (TBE) buffer at 100 volts using a BRL 123 ladder as a size standard. Gels were stained with ethidium bromide, photographed in ultraviolet light, denatured in 1.5 mol/L Tris/0.5 mol/L NaOH (pH 13) for 40 minutes, neutralized in 1.5 mol/L Tris/1.5 mol/L NaCl (pH 8) for 40 minutes, and blotted overnight onto 0.45 μm pore size nylon membranes (Nytran, Schleicher & Schuell, Keene, N.H.) in ×10 sodium chloride-sodium phosphate-EDTA (SSPE) buffer. Membranes were ultraviolet-light crosslinked for 2 minutes, baked 2 hours at 80° C in a vacuum oven, and prehybridized for 3 hours at 42° C in ×5 SSPE with ×5 Denhardt's solution and 0.1% sodium dodecylsulfate (SDS). Membranes were hybridized overnight at 42° C in the same solution with oligonucleotide probes for preproendothelin (Table I) end labeled with ^{32}P gamma adenosine triphosphate by use of T4 polynucleotide kinase.[44] Blots were washed 5 minutes at 22° C in ×2 SSPE with 0.1% SDS and then for 20 minutes at 42° C in the same solution, and exposed to Kodak (Kodak, Inc., Rochester, N.Y.) XAR or XRP X-ray film at −70° C for varying periods in cassettes with intensifying screens. Blots were stripped by boiling 5 minutes in 10 mmol/L Tris (pH 8) with 0.1% SDS and rehybridized with labeled probe for the GAPDH product.

RESULTS

Results of RT/PCR reaction and Southern blots for preproendothelin mRNA detection. Application of 25 dyne/cm^2 shear stress for 24 hours resulted in nearly complete loss of detectable signal for preproendothelin mRNA (Fig. 1). This result was similar whether amplification was done for 24 cycles of PCR (Fig. 2) or 30 cycles (data not

Table I. Oligonucleotide RT/PCR primers and probes for preproendothelin (PPET) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

Gene	*Oligonucleotide*	*Sequence*	*Position/reference*
GAPDH	Sense primer	5′-CCATGGAGAAGGCTGGGG-3′	386-403 (43)
	Antisense primer	5′-CAAAGTTGTCATGGATGACC-3′	580-561 (43)
	Probe	5′-CTAAGCAGTTGGTGGTGCA-3′	527-547 (43)
PPET	Sense primer	5′-AGAGTGTGTCTACTTCTGCC-3′	283-292 (40)
	Antisense primer	5′-TTGTGGGTCACATAACGC-3′	606-623 (40)
	Probe	5′-GGAACACCTAAGACAAACCAGG-3′	289-318 (40)

shown). Because endothelin-1 release measured by RIA continued at a lower level, however, (see below) the low mRNA signal might signify either (1) continued preproendothelin gene transcription at low levels or (2) a process whereby maximal suppression of preproendothelin mRNA transcription was only attained near the end of the 24-hour time period. In contrast to the preproendothelin mRNA signal, the GAPDH mRNA signal showed no comparably sharp changes under shear stress (Fig. 1), suggesting that the change in preproendothelin mRNA levels was not an artifact of random changes from tube to tube in reaction efficiency or RNA loading.

Secretion rate of endothelin-1 into culture medium as a function of shear stress. Application of shear stress caused a decrease of 60% to 70% in the rate of endothelin-1 release into the culture medium, which was detectable within 4 hours of shear stress initiation and was sustained over the 24-hour period of study (Fig. 1 and Table II). Variables other than shear stress that might have produced these findings, such as final cell density and efficiency of RNA extraction, were not significantly different between shear stressed and static cultures (Table II).

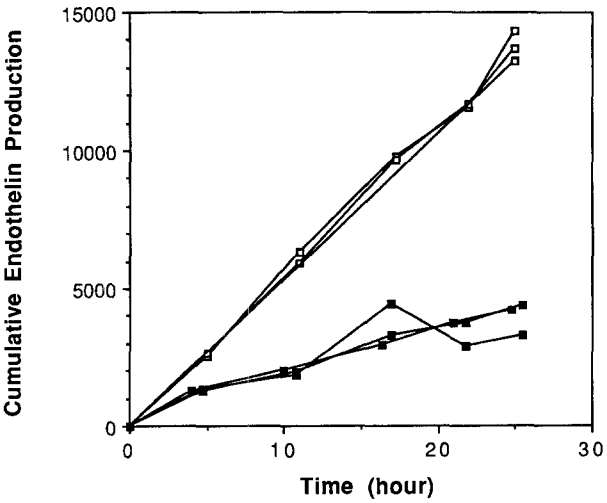

Fig. 1. Cumulative production of endothelin-1 in picograms/10^6 cells produced by HUVEC held in static conditions *(open squares)* or exposed to steady laminar shear stress (25 dyne/cm^2) *(black squares)*. Each point is the mean of duplicate RIA determinations. Endothelin-1 release rates in Table II are slopes of least-squares straight line plots of these data.

DISCUSSION

We have found large reductions in the level of preproendothelin mRNA and the secretion rate of the endothelin-1 peptide after exposure of HUVECs to arterial levels of shear stress. In more recent experiments, application of venous levels of shear stress (4 dyne/cm^2) has resulted in a correspondingly smaller (15% to 20%) but similarly sustained decrease of endothelin-1 release over a 24-hour time period (data not shown). Evidence that the change in preproendothelin autoradiographic signal was a genuine response to shear was provided both by the comparative constancy of the signal level for GAPDH and by the contrasting finding of large shear-induced *increases* in mRNA levels and protein release for a different gene (tissue plasminogen activator) using the same RNA samples.[32] Although we only studied mRNA levels for preproendothelin at 24 hours after shear stress initiation, our RIA data showed a decrease in endothelin-1 secretion rate starting within 4 hours. Since the mRNA for preproendothelin has a half-life of only 15 minutes and endothelin-1 release is controlled by transcription without significant intracellular storage,[45] these findings suggest that fluid shear stress can control endothelin-1 release from human ECs by early suppression of preproendothelin gene transcription.

Our results agree with the initial report of shear stress effects on endothelin mRNA levels in ECs but differ from a later study's finding that shear stress produced only transient *increases* in endothelin-1 mRNA levels in porcine ECs grown through multiple passages in culture.[2,25] This may reflect both species differences and distortions of phenotype

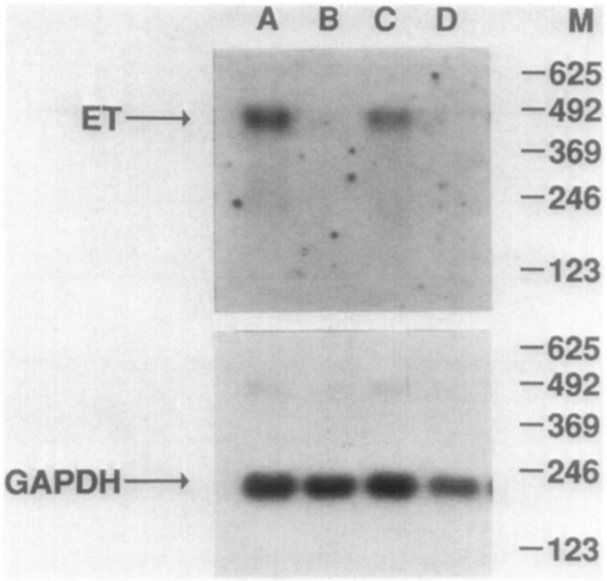

Fig. 2. Southern blot of RT/PCR products for preproendothelin and GAPDH mRNA in HUVEC in static culture (lanes **A, C**) or exposed to shear stress (lanes **B, D**). Each experiment was done with an independent primary cell harvest (experiment 1, lanes **A** and **B**; experiment 2, lanes **C** and **D**).

caused by culture and serial passage of porcine cells. In addition, previous studies did not specify the exact levels of higher shear stresses produced by the flow apparatus. This prior study also used addition of dextran to culture medium to study one range of shear stress values but did not use dextran at lower values[25]; this change in medium content may have affected comparison of the responses to variable shear stress.

Intimal hyperplasia is a common cause of obstructive stenosis after many kinds of vascular reconstruction.[14-18] Both intimal hyperplasia and early atherosclerotic lesions occur most often in areas of flow disturbance such as anastomotic sites and in areas with abnormally low fluid shear.[14-16,19-24]

These pathologic processes involve SMC proliferation and often occur at vessel injury sites where platelets adhere in the early postinjury period. One theory about intimal hyperplasia postulates that PDGF released from platelets causes SMC proliferation at intimal injury sites.[46] If growth factors from platelets were the sole cause of SMC proliferation, then the inverse relationship between fluid shear and hyperplasia might be explained by the hypothesis that high fluid shear reduced both the adhesion and the residence time of platelets at vessel injury sites and thus reduced or diluted locally released PDGF from

Table II. Comparison of cell density, total RNA extracted, and ET-1 production rates for ECs in static versus shear stress conditions

Experiment	*Cell density (10^4 cells/cm^2)*	*Total RNA* (μg)*	*Endothelin production (ng per 10^6 cells/hr)*
Control	7.7 ± 0.8	21.6	0.55 ± 0.02 (n = 3)
Shear	7.1 ± 0.9	24.8	0.13 ± 0.03 (n = 3)**
Control	6.7 ± 0.8	29.1	0.49 ± 0.03 (n = 3)
Shear	7.3 ± 0.8	29.2	0.28 ± 0.06 (n = 3)**

*Final yield (measured by ultraviolet A_{260}) after LiCl from a total of three pooled slides with a total confluent EC area of 45 cm^2.
**$p < 0.01$

platelets.[47] But many more studies suggest that PDGF from platelets cannot be the sole cause of neointimal hyperplasia. Late postoperative intimal hyperplasia involving vascular prostheses occurs most often at the perianastomotic areas that are most rapidly resurfaced with confluent ECs from pannus ingrowth and which therefore attain the earliest protection against platelet deposition.[14-16,48-51] In addition, late postoperative subendothelial vascular SMC proliferation occurs in both 60 μm internodal polytetrafluoroethylene (PTFE) arterial grafts in baboons and in Dacron grafts seeded with autologous ECs in dogs despite confluent EC linings free of adherent platelets.[52-56] In addition, both ECs and SMCs express genes and release protein products for PDGF in vitro.[10,11,57] Increased PDGF mRNA levels are found in carotid plaques and atherosclerotic lesions and in the neointima of healing PTFE prostheses in baboons at sites of abnormal SMC proliferation.[12,54,55,58,59] Finally, the highest rates of SMC proliferation in healing 60 μm internodal PTFE grafts in baboons are found in those SMCs immediately beneath the EC lining,[55] a finding consistent with the observation that confluent ECs in vitro secrete PDGF in a basal rather than an apical direction.[60]

These data suggest that PDGF from ECs and/or SMCs might help cause intimal hyperplasia. However, PDGF is unlikely to be the only mitogen released from ECs. Anti-PDGF antibody can neutralize only 30% to 50% of the mitogenic activity of medium conditioned by cultured ECs or by confluent ECs ECs lining PTFE grafts in baboons.[55,57] Non-PDGF-like factors must thus account for a large fraction of EC secreted–mitogen activity.[61]

Several EC products may account for EC secretion of non-PDGF-like mitogenic activity. Human ECs can express genes for basic fibroblast growth factor and transforming growth factor-β1.[11] Other non-PDGF-like mitogens produced by ECs in vitro have been designated as EC-derived growth factor and connective tissue activating factor V.[62-64]

Endothelin-1 may be an important candidate for the role of a non-PDGF-like EC-produced mitogen causing SMC proliferation in vivo because the decrease in endothelin gene expression at high shear stress is consistent with the known inverse relationship between fluid shear and intimal hyperplasia.[19-22] It is also notable that significant reductions in intimal hyperplasia have been found in animal trials of angiotensin-converting enzyme inhibiting agents,[65] since angiotensin II stimulates EC production of endothelin-1, whereas both endothelin-1 and angiotensin II induce increased SMC transcription of the PDGF A chain gene in vitro.[66] It would be useful to know if specific blockade of either endothelin-1 release or endothelin-1 action on SMCs was partly responsible for these effects of angiotensin-converting enzyme inhibitors. Our results also suggest that designing anastomoses to minimize areas of low shear stress and disturbed flow might reduce the severity of intimal hyperplasia.

These data suggest the need to find out if high shear stress levels will also suppress preproendothelin mRNA levels and endothelin-1 release in vivo, and to see if increased preproendothelin gene expression and endothelin-1 release by ECs can cause more severe levels of intimal hyperplasia in animal models.

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