Dexamethasone Arterializes Venous Endothelial Cells by Inducing Mitogen-Activated Protein Kinase Phosphatase-1

A Novel Antiinflammatory Treatment for Vein Grafts?

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Background—Vein grafting in coronary artery surgery is complicated by a high restenosis rate resulting from the development of vascular inflammation, intimal hyperplasia, and accelerated atherosclerosis. In contrast, arterial grafts are relatively resistant to these processes. Vascular inflammation is regulated by signaling intermediaries, including p38 mitogen-activated protein (MAP) kinase, that trigger endothelial cell (EC) expression of chemokines (eg, interleukin-8, monocyte chemotactic protein-1) and other proinflammatory molecules. Here, we have tested the hypothesis that p38 MAP kinase activation in response to arterial shear stress (flow) may occur more readily in venous ECs, leading to greater proinflammatory activation.

Methods and Results—Comparative reverse-transcriptase polymerase chain reaction and Western blotting revealed that arterial shear stress induced p38-dependent expression of monocyte chemotactic protein-1 and interleukin-8 in porcine jugular vein ECs. In contrast, porcine aortic ECs were protected from shear stress–induced expression of p38-dependent chemokines as a result of rapid induction of MAP kinase phosphatase-1. However, we observed with both cultured porcine jugular vein ECs and perfused veins that venous ECs can be protected by brief treatment with dexamethasone, which induced MAP kinase phosphatase-1 to suppress proinflammatory activation.

Conclusions—Arterial but not venous ECs are protected from proinflammatory activation in response to short-term exposure to high shear stress by the induction of MAP kinase phosphatase-1. Dexamethasone pretreatment arterializes venous ECs by inducing MAP kinase phosphatase-1 and may protect veins from inflammation. (Circulation. 2011;123:524-532.)

Key Words: endothelial shear stress ■ inflammation ■ MAP kinase ■ inflammatory activation

Vascular inflammation involves the capture of leukocytes from the bloodstream to the vessel wall via interactions with activated vascular endothelial cells (ECs) expressing adhesion proteins (eg, E-selectin, vascular cell adhesion molecule-1, intercellular adhesion molecule-1), chemokines (eg, monocyte chemotactic protein-1 [MCP-1], interleukin [IL]-8), and other proinflammatory molecules.1 This process is coordinated by the simultaneous activation of c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein (MAP) kinases2 and nuclear factor-κB (NF-κB)3 signaling pathways, which cooperate to induce and stabilize proinflammatory transcripts in vascular ECs.4,5

Clinical Perspective on p 532

The susceptibility of blood vessels to inflammatory processes varies according to their anatomic location. This has been attributed to local differences in hemodynamics6,7; differences in the structure and physiology of blood vessels also play an important role.8,9 This is exemplified in bypass grafting in which arteries and veins respond differently to arterial flow. Although vein grafts are commonly used as conduits in cardiac and vascular surgery,10 their use is commonly complicated by intimal hyperplasia and accelerated atherosclerosis, which cause late failure rates of up to...
40% of grafts within 10 years of surgery. The process of vein grafting leads to activation of MAP kinases, endothelial expression of adhesion molecules and chemokines, and recruitment of inflammatory cells within 6 to 24 hours after surgery. It has been suggested that the inflammatory process contributes to pathogenesis because vein graft disease can be reduced by depletion of macrophages or by genetic deletion of proinflammatory genes such as intercellular adhesion molecule-1 or the p55 tumor necrosis factor receptor. In contrast, arterial grafts are relatively resistant to inflammation, intimal hyperplasia, and failure.

To gain insight into intrinsic molecular mechanisms underlying the differential responses of arteries and veins to grafting, we compared the effects of arterial shear stress on proinflammatory activation of venous and arterial ECs. We found that arterial but not venous ECs were protected from proinflammatory activation in response to the rapid induction of high shear stress through negative regulation of p38 MAP kinase by MAP kinase phosphatase-1 (MKP-1). However, brief pretreatment with dexamethasone “arterialized” venous ECs by inducing MKP-1 and protected them from inflammatory activation.

**Methods**

**Reagents and Antibodies**

Anti–phosphorylated-p38 Tyr180/Thr182 (Cell Signaling Technology, Danvers, MA), anti-p38 (Cell Signaling Technology), anti–phosphorylated JNK, anti-JNK (Cell Signaling Technology), anti–RelA (Santa Cruz Biotechnology Inc, Santa Cruz, CA), anti–MKP-1 (Santa Cruz), anti–Nrf2 (Santa Cruz), and anti–MCP-1 (Abcam, Cambridge, MA) antibodies; DBA lectin (Vector Laboratories, Burlingame, CA); TO-PRO-3 (Invitrogen, Carlsbad, CA); pharmacological inhibitors of p38 (SB202190 and SB203580; Calbiochem, San Diego, CA) and JNK (SP600125; Calbiochem); human recombinant tumor necrosis factor-α (R&D Systems, Minneapolis, MN); and a mammalian expression vector containing enhanced green fluorescent protein (Clontech Laboratories, Inc, Palo Alto, CA) were obtained commercially. An adenovirus containing a dominant-negative form of NrT2 (Ad-NrT2-DN) was generously provided by Professor Jeffrey A. Johnson (University of Wisconsin–Madison) and has been described previously.

A mammalian expression vector containing a FLAG epitope-tagged version of MKP-1 (pCMV-FLAG-MKP-1) was generously supplied by Dr Andrew Clark (Imperial College London) and has been described previously.

**Detection of Proteins in Cultured ECs**

Cell lysates were prepared with the Nuclear Extraction Kit (Active Motif, Carlsbad, CA). Western blotting was carried out with primary antibodies followed by suitable horseradish peroxidase–conjugated secondary antibodies and chemiluminescent detection. Alternatively, levels of MKP-1, NrT2, MCP-1, or phosphorylated p38 in cultured cells were measured by immunostaining of methanol-fixed cells with specific antibodies and Alexa Fluor 568–conjugated secondary antibodies followed by laser-scanning confocal microscopy.

**Detection of Proteins in Ex Vivo Perfusion of Veins**

Internal jugular veins were dissected using aseptic technique from necks of adult pigs, which were obtained from a local abattoir. Heparin was administered systemically, and thoracic surgery was performed as described previously.

**Transplant Transfection**

Cell cultures that were 80% to 90% confluent were transfected with plasmids by microinjection (Digital Bio Technology) following the manufacturer’s instructions and then incubated in growth medium without antibiotics for 48 hours before analysis.

**Comparative Reverse-Transcriptase Polymerase Chain Reaction**

Transcript levels were quantified by comparative reverse-transcriptase polymerase chain reaction (RT-PCR) with gene-specific primers for MKP-1 (sense, 5′-GGCAAATCAACCTGTTTGCA-3′; antisense, 5′-TAGATCGTACCAAGGGAGGTTTTGTTT-3′), IL-8 (sense, 5′-CTCTGTTCTGCAGCTTCCT-3′; antisense, 5′-GACCTTTTCTTCCATTG-3′), MCP-1 (sense, 5′-TCACCTGCTGCTATACACTFAC-3′; antisense, 5′-ATCACTCTGCTTCTTTGAGGACTTGC-3′), E-selectin (sense, 5′-CATAGAGGACCATCATATAC-3′; antisense, 5′-TTCAAAATCTCCTCTAC-3′), intercellular adhesion molecule-1 (sense, 5′-ATCAATGGAACCAGAAG-3′; antisense, 5′-CACCCTTGCTACTGGTAG-3′), vascular cell adhesion molecule-1 (sense, 5′-CAGTACAGGACGATGATATGC-3′; antisense, 5′-ATCACTGCTCTTTGAGGACTTGC-3′), cyclophilin-A (sense, 5′-ATTTGTAGTGAAGATTTATCAC-3′; antisense, 5′-ATGCCTCTCCCTTCTC-3′), and antioxidant factor-2 (sense, 5′-GCTCCCAAGGTTGCT-3′; antisense, 5′-AAGCAGTATTGATGACCAAA-3′). Extraction and reverse transcription of total RNA and real-time PCR were carried out as described previously.

**Ex Vivo Perfusion of Veins**

Internal jugular veins were dissected using aseptic technique from necks of adult pigs, which were obtained from a local abattoir. Human saphenous veins were harvested from patients undergoing coronary artery bypass grafting using a “no touch” technique, and portions that were not required for grafting were studied. The study was approved by the Hammersmith Hospital Research Ethics Committee; informed consent was provided by all subjects. Veins were cannulated with 2-olive-tip needles, submerged in M199 cell culture medium (containing 10% FCS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin), and connected to a perfusion circuit consisting of a peristaltic pump (Stockert SIII double-head pump, Sorin Group, Milan, Italy), polyvinyl chloride tubing, and a Tycos pressure monitor (Figure IA in the online-only Data Supplement). The flow system was placed in an incubator and maintained at 37°C. Veins were perfused under mean arterial pressure (65 mm Hg) with M199 medium (containing 20% FCS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin), which was oxygenated (oxygen content, 20 mL/L) and perfused at 50 mL/min. Ultrasound imaging with color and spectral Doppler and contrast-enhanced imaging with nonlinear imaging
were performed to measure the internal diameter of the vein and to confirm that the flow pattern was laminar (Figure IB in the online-only Data Supplement). Using the diameter of the vessel and viscosity of the perfusate and assuming laminar flow in a cylindrical tube, we calculated the relationship between flow rate and shear stress with Hagen-Poiseuille equations. Thus, we used a flow rate of 100 mL/min to generate a wall shear stress of 12 ± 0.2 dynes/cm² in perfused veins.

En Face Immunostaining
The expression levels of specific proteins were assessed in vein ECs by en face staining. Veins were cut longitudinally, pinned to dental wax to keep flat, and fixed with 4% paraformaldehyde. Fixed veins were tested by immunostaining with specific primary antibodies and Alexa Fluor 568–conjugated secondary antibodies. ECs were identified by costaining with DBA lectin conjugated to the fluorophore FITC. Nuclei were identified with a DNA-binding probe with far-red emission (TO-PRO-3). Stained veins were mounted before visualization of endothelial surfaces with confocal laser-scanning microscopy (LSM 510 META, Zeiss). Isotype-matched monoclonal antibodies raised against irrelevant antigens or preimmune rabbit sera were used as experimental controls for specific staining (data not shown). The expression of particular proteins was assessed by quantification of fluorescence intensity for multiple cells (at least 100 per site) with LSM 510 software (Zeiss).

Statistics
For comparisons between ≥2 groups, a factorial approach was used with 1 (1-way ANOVA) or 2 (2-way ANOVA) independent variables followed by posthoc pairwise comparisons with the Bonferroni correction for multiple comparisons. Normal distribution of each variable was assessed with the Kolmogorov-Smirnov test. The cutoff value for significance was 0.05. All P values calculated are presented. Statistical analyses were performed with the SPSS 17.0 program (SPSS Inc, Chicago, IL).

Results
Arterial Shear Stress Activates Venous But Not Arterial ECs
The effects of arterial shear stress on proinflammatory activation in venous and arterial ECs were compared. We observed by quantitative RT-PCR that the application of LSS for 4 hours activated PJVECs, as shown by expression of the chemokines MCP-1 and IL-8 (Figure 1A). In contrast, arterial shear stress had relatively modest effects on chemokine expression in PAECs (Figure 1A) and had little or no effect on intercellular adhesion molecule-1, vascular cell adhesion molecule-1, or E-selectin expression in either PJVECs or PAECs (Figure II in the online-only Data Supplement).

To determine the molecular basis for the differential responses of venous and arterial ECs to flow, we studied the effects of arterial shear on p38 MAP kinase and NF-κB signaling pathways, which are essential for EC activation. We observed by Western blotting that rapid induction of arterial shear led to activation of p38 MAP kinase by phosphorylation in PJVECs but not in PAECs (Figure 1B). In contrast, arterial shear stress had relatively modest effects on chemokine expression in PAECs (Figure 1A) and had little or no effect on intercellular adhesion molecule-1, vascular cell adhesion molecule-1, or E-selectin expression in either PJVECs or PAECs (Figure II in the online-only Data Supplement).

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Figure 1. Effects of arterial shear stress on proinflammatory signaling in venous and arterial ECs. PAECs or PJVECs were cultured under static conditions or exposed to LSS for 4 hours (A) or 90 minutes (B). A, Transcript levels of MCP-1 and IL-8 were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown. B, Expression and phosphorylation of p38 were assessed by Western blotting of total cell lysates with specific antibodies. Nuclear localization of NF-κB was measured by Western blotting of nuclear lysates with anti-RelA (NF-κB) antibodies or with anti–Lamin B antibodies to control for equal loading. Images shown are representative of 3 independent experiments. C, PJVECs were pretreated with a p38 inhibitor (either SB202190 [20 μmol/L] or SB203580 [25 μmol/L]) or with vehicle alone for 45 minutes and were then exposed to LSS for 4 hours or cultured under static conditions. Transcript levels of MCP-1 and IL-8 were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown.
venous ECs are sensitive to p38-dependent induction of MCP-1 and IL-8 by arterial shear stress, whereas arterial ECs are resistant.

**Arterial Shear Stress Induces MKP-1 in Arterial But Not Venous ECs**

We hypothesized that PAECs may be more resistant to the proinflammatory effects of short-term arterial shear stress resulting from elevated expression of MKP-1, a negative regulator of p38 that is expressed at high-shear regions of the murine arterial tree. Comparative RT-PCR revealed that MKP-1 transcripts were induced in PAECs by the application of arterial shear stress for 1 hour (Figure 2A). The effects of short-term shear stress on MKP-1 transcripts in PAECs were paralleled by increased MKP-1 protein levels as demonstrated by immunostaining (Figure 2B) and Western blotting (Figure 2E; compare 1 and 2). In contrast to arterial ECs, the application of prolonged (12 to 24 hours) but not short-term (1 to 4 hours) shear stress induced MKP-1 in PJVECs (Figure III in the online-only Data Supplement and Figure 2A), indicating that the kinetics of MKP-1 induction by LSS were delayed in venous compared with arterial ECs.

Given that shear stress activates vascular endothelial growth factor receptor 

**Figure 2. MKP-1 protects arterial ECs from inflammatory activation in response to shear stress.** A, PAECs or PJVECs were exposed to LSS for 1 hour or were cultured under static conditions. MKP-1 transcript levels were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown. B, PAECs were exposed to LSS for 90 minutes or were cultured under static conditions. MKP-1 expression levels were assessed by immunofluorescence staining with anti–MKP-1 antibodies, quantified in multiple ECs, and averaged for each experimental group. Representative images, values from triplicate independent experiments, and mean values (horizontal line) are shown. Nuclear counterstain (DNA). C, PAECs or PJVECs were exposed to LSS for 30 minutes or were cultured under static conditions. Expression and phosphorylation of JNK were assessed by Western blotting of total cell lysates with specific antibodies. Images shown are representative of 3 independent experiments. D, PAECs were pretreated with a JNK inhibitor (SP600125 [10 μmol/L]) or with vehicle alone for 45 minutes and were then exposed to LSS for 1 hour or cultured under static conditions. Transcript levels of MKP-1 were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown. E and F, PAECs were transfected with MKP-1–specific siRNA (MKP-1) or with a scrambled control (Scr) as indicated. Cells were then exposed to LSS for the indicated times or were cultured under static conditions. E, MKP-1 expression and p38 expression and phosphorylation were assessed by Western blotting of total cell lysates with specific antibodies. Images shown are representative of 3 independent experiments. F, Transcript levels of MKP-1, MCP-1, and IL-8 were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown.
activated rapidly by shear stress in PAECs but not in PJVECs (Figure 2C). Comparative RT-PCR revealed that PJVECs expressed higher levels of A20, a negative regulator of JNK, compared with PAECs (Figure IV in the online-only Data Supplement), thus potentially explaining the resistance of PJVECs to JNK activation by shear stress. We studied the function of JNK in PAECs using a pharmacological inhibitor that suppressed the induction of MKP-1 by LSS (Figure 2D), indicating that short-term induced MKP-1 in arterial ECs via a JNK-dependent mechanism.

MKP-1 Protects Arterial ECs From Proinflammatory Activation in Response to Shear Stress
The function of MKP-1 in PAECs was assessed with a prevalidated MKP-1–specific siRNA26 that suppressed MKP-1 protein and messenger RNA expression in sheared PAECs, whereas a nontargeting scrambled control had no effect (Figure 2E [compare 2 and 3] and 2F). We observed that silencing of MKP-1 enhanced p38 activation (Figure 2E) and elevated the expression of MCP-1 and IL-8 transcripts (Figure 2F) in PAECs exposed to short-term LSS, indicating that MKP-1 plays an essential role in the suppression of p38 activation and proinflammatory molecules in sheared arterial EC.

We also studied the effects of shear stress on the expression and intracellular localization of the transcription factor Nrf2, which can suppress p38 activation by enhancing the catalytic activity of MKP-1.31 Immunofluorescence staining revealed that Nrf2 was induced by short-term LSS in both PAECs and PJVECs but was localized predominantly in the nucleus of arterial ECs and predominantly in the cytoplasm of venous ECs (Figure VA in the online-only Data Supplement). Because the active form of Nrf2 localizes to the nucleus,32 we examined the function of Nrf2 in sheared PAECs using an adenosivirus containing a dominant-negative form (Ad-Nrf2-DN). Comparative real-time PCR revealed that the induction of IL-8 and MCP-1 by shear stress was enhanced modestly by pretreatment with Ad-Nrf2-DN compared with an empty adenosivirus, but these differences did not reach statistical significance (Figure VB in the online-only Data Supplement). Thus, we conclude that PAECs are protected from inflammatory activation in response to short-term arterial shear via the induction of MKP-1, whereas shear stress–mediated activation of Nrf2 plays a relatively minor role in protection.

Dexamethasone Pretreatment Suppressed Proinflammatory Activation of Venous ECs by LSS Via MKP-1 Induction
We reasoned that venous ECs may be susceptible to flow-induced proinflammatory activation because of the absence of MKP-1 induction in response to shearing. Therefore, strategies to induce MKP-1 in venous ECs may suppress vascular inflammation. Consistent with this idea, we observed that overexpression of MKP-1 in PJVECs reduced p38 phosphorylation and MCP-1 induction by LSS, whereas expression of an irrelevant protein (green fluorescent protein) had no effect (Figure VI in the online-only Data Supplement), indicating that MKP-1 can suppress inflammatory activation of venous ECs by short-term LSS. We also examined the effects of pharmacological induction of MKP-1 using dexamethasone, a synthetic glucocorticoid that can induce MKP-1 in multiple cell types.33 We first validated the approach by examining the effects of varying doses of dexamethasone on PJVEC responses to tumor necrosis factor-α. Pretreatment with 10 μmol/L dexamethasone was considered optimal because it was the lowest dose that induced MKP-1 and reduced phosphorylation of p38 and induction of MCP-1 and IL-8 in response to tumor necrosis factor-α, but it did not influence NF-κB activation (Figure VII in the online-only Data Supplement). A 30-minute pretreatment with 10 μmol/L dexamethasone significantly altered PJVEC responses to LSS by enhancing the expression of MKP-1 at both the messenger RNA (Figure 3A) and protein (Figure 3B and 3C; compare 2 and 3) levels and by suppressing the phosphorylation of p38 (Figure 3C; compare 2 and 3) and the induction of MCP-1 and IL-8 (Figure 3D). We concluded that MKP-1 is essential for the suppression of PJVEC activation by dexamethasone because p38 activation in response to shear stress was restored by gene silencing with MKP-1–specific siRNA (Figure 3C; compare 3 and 5).

Discussion
Hemodynamics influence the inflammatory process by controlling leukocyte margination and adhesive interactions6,9 and by generating shear stress, which alters EC physiology.7 Here, we show that venous and arterial ECs respond differently to shear stress, despite in vitro culture for up to 4 cell divisions. Thus, we suggest that the susceptibility of veins and arteries to the inflammatory process is regulated by an interaction between local hemodynamics and stable vessel-specific EC phenotypes. Our findings are particularly relevant to grafting of veins into the arterial circulation, a procedure...
that exposes veins to new forces that can cause EC injury or activation. Stretching of veins during harvesting and preparation for grafting can lead to partial loss of ECs, whereas a “no touch” technique for vein harvest leads to the preservation of ECs. Veins grafted into the arterial circulation are also exposed to a sudden increase in blood flow, which elevates shear stress at the vascular wall. Venous ECs are activated rapidly by the application of arterial shear stress, and it is likely that this process contributes to early inflammatory processes in vein grafts. Our observation that arterial shear stress induces proinflammatory transcripts in venous ECs through a p38-dependent signaling pathway provides a potential mechanism for this process. On the other hand, we observed that arterial ECs were resistant to p38 activation and expression of proinflammatory genes in response to flow, which is consistent with previous observations that arterial grafts are relatively resistant to inflammation and the development of intimal hyperplasia.

The differences that we observed between the responses of venous and arterial ECs to shear stress may reflect an epigenetic memory of the markedly different hemodynamic environments that these cell types were exposed to before isolation. Arteries are exposed to higher shear stresses (10 to 20 dynes/cm²) compared with veins (<5 dynes/cm²). It is therefore plausible that cultured arterial ECs resisted activation in response to short-term arterial shear stress because they were preadapted to arterial hemodynamics, whereas venous ECs were susceptible because they were adapted to relatively low shear stress conditions. Nonhemodynamic factors are also likely to contribute to arterial-venous heterogeneity, including signaling pathways and epigenetic modifications that control the differentiation of arterial and venous ECs in embryonic vessels before the establishment of circulating blood.

To understand the molecular mechanism that underlies differences in susceptibilities of venous and arterial ECs to flow-mediated activation, we examined the effects of flow on the expression of the antiinflammatory molecule MKP-1. We observed that MKP-1 was induced rapidly by LSS in arterial ECs and was essential for the resistance of arterial cells to flow-mediated proinflammatory activation. Our studies revealed that flow induces MKP-1 in arterial ECs via a JNK-dependent signaling pathway, an observation that is consistent with a previous report that JNK can positively regulate MKP-1 expression. In contrast to arterial ECs, MKP-1 expression was induced in venous ECs exposed to prolonged (>12 hours) LSS but not in cultures exposed to short-term (1 to 4 hours) LSS. Thus, we suggest that the absence of JNK-dependent MKP-1 induction in venous ECs exposed to short-term LSS may contribute to the susceptibil-

Figure 3. Dexamethasone suppressed activation of cultured venous ECs by shear stress via MKP-1. A and B, PJVECs were pretreated with dexamethasone (10 μmol/L) for 60 minutes or remained untreated and were then exposed to LSS for 60 minutes (A) or 90 minutes (B). A, MKP-1 transcript levels were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown. B, MKP-1 expression levels were assessed by immunofluorescence staining with anti–MKP-1 antibodies, quantified in multiple ECs, and averaged for each experimental group. Representative images, values from triplicate independent experiments, and mean values (horizontal line) are shown. Nuclear counterstain (DNA). C, PJVECs were transfected with MKP-1-specific siRNA (MKP-1) or with a scrambled control (Scr) as indicated. Cells were then treated with dexamethasone (10 μmol/L) for 60 minutes or remained untreated and were then exposed to LSS for 90 minutes or cultured under static conditions. Expression of MKP-1 and expression and phosphorylation of p38 were assessed by Western blotting of total cell lysates with specific antibodies. Images shown are representative of 3 independent experiments. D, PJVECs were pretreated with dexamethasone (10 μmol/L) for 60 minutes or left untreated and were then exposed to LSS for 4 hours. Transcript levels of MCP-1 and IL-8 were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown.
ity of these cells to p38-dependent inflammatory activation. Interestingly, venous ECs expressed relatively high levels of A20 compared with arterial ECs, and it is plausible that this negative regulator of JNK may suppress MKP-1 induction by short-term LSS. Aside from MKP-1, it is likely that other antiinflammatory signaling molecules are differentially expressed in venous and arterial ECs (e.g., Kruppel-like factors), and genome-wide studies should now be carried out to identify the molecules that determine the differential responses of arterial and venous ECs to flow.

Because inflammation of vein grafts leads to intimal hyperplasia and failure, strategies to suppress early inflammatory responses to arterial hemodynamics may prolong graft survival. Here, we provide proof of principle that induction of MKP-1 with dexamethasone can prevent veins from exhibiting an inflammatory phenotype in response to arterial shear stress. Our findings suggest that the known beneficial effects of dexamethasone treatment in the prevention of intimal hyperplasia may involve local suppression of EC activation, although therapeutic alterations of smooth muscle cell proliferation and leukocyte activation may also occur. They also suggest that a brief pretreatment with dexamethasone may be sufficient to protect veins from early inflammatory responses to grafting. Perioperative treatment of veins may therefore provide an attractive novel therapeutic strategy to reduce graft inflammation while avoiding the undesirable side effects associated with systemic glucocorticoid treatment.

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**Disclosures**

None.
References


Vein or artery grafts are used as conduits in cardiac and vascular surgery. Vein grafts are susceptible to inflammation, intimal hyperplasia, and accelerated atherosclerosis, which cause late failure rates of up to 40% of grafts within 10 years of surgery, whereas artery grafts are relatively resistant. Blood flow influences the physiology of vascular endothelial cells by exerting a frictional force, shear stress, on the vascular wall. To elucidate the molecular mechanisms that control differential responses of arteries and veins to grafting, we compared the effects of arterial flow on proinflammatory activation of venous and arterial endothelial cells. We demonstrate that arterial but not venous endothelial cells are protected from proinflammatory activation in response to arterial flow through the induction of mitogen-activated protein (MAP) kinase phosphatase-1, which negatively regulated inflammatory p38 MAP kinase signaling. Because inflammation of vein grafts leads to intimal hyperplasia and failure, strategies to suppress early inflammatory responses to arterial hemodynamics may prolong graft survival. A key observation from our study is that pharmacological induction of MAP kinase phosphatase-1, via a brief treatment with dexamethasone, can arterialize endothelial cells in porcine or human veins, thus protecting them from inflammatory activation in response to arterial flow. We suggest that brief perioperative treatment of veins using dexamethasone may therefore provide an attractive novel therapeutic strategy to reduce graft inflammation while avoiding the undesirable side effects associated with systemic glucocorticoid treatment.
Dexamethasone Arterializes Venous Endothelial Cells by Inducing Mitogen-Activated Protein Kinase Phosphatase-1: A Novel Antiinflammatory Treatment for Vein Grafts?
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LEGENDS FOR SUPPLEMENTARY FIGURES

Supplementary Figure 1.

Validation of flow apparatus for perfusion of veins ex vivo. (A) Schematic representation of the flow system used to perfuse veins. (B) Porcine internal jugular veins were mounted on the flow system and perfused under arterial pressure at a flow rate of 100 ml/minute using M199 medium containing 20% FCS. The flow field was assessed by ultrasound after introduction of ultrasound contrast agent (4.8 ml/L; SonoVue, Bracco SPA, Milan) and scanning using non-linear imaging mode and conventional fundamental modes with 2D acquisitions using an L9-17 MHz probe (Logiq E9, GE Healthcare). A representative image is shown with flow velocity false-coloured. Our analysis indicated that flow was laminar and that the velocity profile was parabolic.

Supplementary Figure 2

Shear stress did not influence expression of adhesion molecules. PAEC or PJVEC were cultured under static conditions or exposed to LSS for 4 hours. Transcript levels of ICAM-1, VCAM-1 and E-selectin were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown.

Supplementary Figure 3

Prolonged shear stress induced MKP-1 in venous EC. PJVEC were cultured under static conditions or exposed to LSS for 2-24 hours. Transcript levels of MKP-1 were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown.
Supplementary Figure 4

**A20 is expressed at higher levels in venous EC compared to arterial EC.** Transcript levels of A20 were measured in static cultures of PAEC or PJVEC by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown.

Supplementary Figure 5

**Nrf2 reduced inflammatory activation of PAEC exposed to acute LSS.** (A) PAEC or PJVEC were cultured under static conditions or exposed to LSS for 1 hour. Nrf2 protein expression was assessed by immunofluorescence staining using anti-Nrf2 antibodies (red). Levels of nuclear Nrf2 were quantified in multiple EC and averaged for each experimental group. Representative images, values from triplicate independent experiments and mean values (horizontal line) are shown. (B) PAEC were transduced with Ad-Nrf2-DN or with an empty adenovirus and incubated for 48h. Cells were then exposed to LSS for 4 hours or were cultured under static conditions. Transcript levels of IL-8 and MCP-1 were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown.

Supplementary Figure 6

**MKP-1 suppressed p38 activation and MCP-1 induction in response to acute shear stress.** PJVEC were transfected with pCMV-FLAG-MKP-1 or pEGFP (or remained untransfected) and were incubated for 48h. Cultures were then exposed to LSS for 1 hour (A) or 4 hours (B) or were cultured under static conditions. Levels of phosphorylated p38 (Phospho-p38) or MCP-1 were measured by immunofluorescence staining using specific antibodies (red), FLAG-MKP-1 was detected by co-staining using anti-FLAG-FITC antibodies (green).
and cell nuclei were identified using TO-PRO-3 (DNA; purple). Representative images from three independent experiments are shown.

**Supplementary Figure 7**

**Dexamethasone inhibited pro-inflammatory activation in response to TNFα in a dose-dependent manner.** PJVEC were pre-treated with varying doses of dexamethasone (5, 10 or 25 µM) for 60 minutes or remained untreated, and were then exposed to TNFα (10 ng/ml) for 90 minutes (A) or 4 hours (B). (A) MKP-1 protein expression and p38 expression and phosphorylation were assessed by Western blotting of total cell lysates using specific antibodies. Nuclear localisation of NF-κB was measured by Western blotting of nuclear lysates using anti-RelA (NF-κB) antibodies or using anti-Lamin B antibodies to control for equal loading. Images shown are representative of two independent experiments. (B) Transcript levels of MCP-1 and IL-8 were measured by comparative RT-PCR. Values from triplicate independent experiments and mean values (horizontal line) are shown.

**Supplementary Figure 8.**

**Pre-treatment of human saphenous veins with dexamethasone suppressed EC activation by shear stress.** Human long saphenous veins were either pre-treated with dexamethasone (10 µM for 60 minutes) or remained untreated. They were then mounted on a perfusion apparatus and exposed to LSS for 90 minutes (panels 1-6) or 6 hours (panels 7-9) or were maintained under static conditions as a control. Expression levels of MKP-1, phosphorylated-p38 (Phos-p38) or MCP-1 were assessed by en face staining using specific antibodies, quantified in multiple EC and averaged for each experimental group. Representative images,
values from triplicate independent experiments and mean values (horizontal line) are shown. Endothelial marker (Lectin); nuclear counterstain (DNA).

**Supplementary Figure 9. Expression of p38 in porcine veins was not influenced by dexamethasone or shear stress.** Porcine internal jugular veins were either pre-treated with dexamethasone (10 µM for 30 minutes) or remained untreated. They were then mounted on a perfusion apparatus and exposed to LSS for 90 minutes or were maintained under static conditions as a control. Expression levels of p38 were assessed by en face staining using specific antibodies, quantified in multiple EC and averaged for each experimental group. Representative images, values from triplicate independent experiments and mean values (horizontal line) are shown. Endothelial marker (Lectin); nuclear counterstain (DNA).
Supplementary Figure 1

A

B

Vessel wall

Flow

Perfusate reservoir

Oxygenator

Pump

pressure monitor

Cannulated vein

Fluid-filled bath

FLOW

75 cm/s

0 cm/s
Supplementary Fig. 3
Supplementary Fig. 4

A20 mRNA levels

PAEC  PJVEC
Supplementary Figure 5

A

PAEC

PJVEC

static
LSS

B

IL-8

MCP-1

mRNA levels
mRNA levels

Adenovirus empty
empty Nrf2-DN

Adenovirus empty
empty Nrf2-DN

Nuclear Nrf2 levels (MFI)

NS
###

NS

NS

NS
Supplementary Figure 6

A

Untransfected  |  GFP  |  MKP-1
---|---|---
Phos-p38
LSS  |   -   |   +   |   +   |
merge | merge | merge |

B

Untransfected  |  GFP  |  MKP-1
---|---|---
MCP-1
LSS  |   -   |   +   |   +   |
merge | merge | merge |
Supplementary Fig. 7

A

MKP-1
Phos-p38
p38
α-tubulin
NF-κB
Lamin-B

Total cell lysates

Dex (µM) 0 0 5 2 10 25
TNFα – + + + +

Nuclear lysates

B

MCP-1

IL-8

mRNA levels

mRNA levels

Dex (µm) 0 0 5 2 10 25
TNFα – + + + +

Dex (µm) 0 0 5 2 10 25
TNFα – + + + +
Supplementary Figure 8

[Images of flow cytometry data showing differences in marker expression under static and LSS conditions with and without Dex treatment.]
Supplementary Figure 9

Static | LSS | LSS

Vehicle | Dex

Flow | – | + | +
Dex | – | – | +

MFI

Vehicle

Lectin | p38
DNA

Lectin | p38
DNA

Lectin | p38
DNA

MFI

NS

NS

NS