Matrix metalloproteinase expression in vein grafts: role of inflammatory mediators and extracellular signal-regulated kinases-1 and -2

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Matrix metalloproteinases (MMPs) play key roles in vascular remodeling. We characterized the role of inflammatory mediators and extracellular signal-regulated kinases (ERKs) in the control of arterialized vein graft expression of MMP-9, MMP-2, and membrane-type 1-MMP (MT1-MMP) and of the tissue inhibitor of metalloproteinases-2 (TIMP-2). For this purpose we used a canine model of jugular vein to carotid artery interposition graft and analyzed the vein grafts at various postoperative times (30 min to 28 days) using the contralateral vein as a control. To study the role of ERK-1/2, veins were incubated with the mitogen-activated protein kinase kinase (MEK-1/2) inhibitor U0126 for 30 min before being grafted. Vein graft extracts were analyzed for MMPs, TIMP-2, tumor necrosis factor-α (TNF-α), polymorphonuclear neutrophil (PMN) infiltration, myeloperoxidase (MPO), and thrombin activity, and for ERK-1/2 activation. Vein graft arterIALIZATION resulted in rapid and sustained (8 h to 28 days) upregulation of vein graft-associated MMP-9, MMP-2, MT1-MMP, thrombin activity, and TNF-α levels with concomitant TIMP-2 down regulation. MMP-2 activation preceded MT1-MMP upregulation. PMN infiltration and vein graft-associated MPO activity increased within hours after arterIALIZATION, indicating a prompt, local inflammatory response. In cultured smooth muscle cells, both thrombin and TNF-α upregulated MT1-MMP expression; however, only thrombin activated MMP-2. Inhibition of ERK-1/2 activation blocked arterIALIZATION-induced upregulation of MMP-2, MMP-9, and MT1-MMP. Thus, thrombin, inflammatory mediators, and activation of the ERK-1/2 pathway control MMP and TIMP-2 expression in arterialized vein grafts.

Inflammation; mitogen-activated protein kinase; vascular remodeling

VEIN GRAFT exposure to arterial circulation often results in intimal hyperplasia and medial hypertrophy, with eventual luminal stenosis and reocclusion. The process of vein graft stenosis involves migration and proliferation of smooth muscle cells (SMCs), along with excess deposition of extracellular proteins such as collagen (44).

Matrix metalloproteinases (MMPs), a family of zinc- and calcium-dependent proteinases, collectively degrade all protein components of the extracellular matrix (ECM; 21). SMCs and macrophages in atherosclerotic and balloon-injured arteries express increased levels of MMP-9, MMP-2, and membrane-type 1 metalloproteinase (MT1-MMP) (30). MMP-2 appears to have particular requirements for activation, entailing interaction with plasma membrane-tethered MMPs (the MT-MMPs) and formation of a trimolecular complex consisting of proMMP-2, MT-MMP, and tissue inhibitor of metalloproteinases-2 (TIMP-2) (38). MT1-MMP-bound MMP-2 is also activated by thrombin (23), plasmin, and neutrophil proteinases (22, 24).

Inflammation contributes to vascular remodeling through multiple mechanisms, including the control of MMP activities, and activated leukocytes play a central role in the development of intimal hyperplasia in both injured arteries and atherosclerotic plaque progression (43). Polymorphonuclear neutrophils (PMNs) are rich in MMP-9 (PMN gelatinase) (36), and PMN-derived proteinases activate MMP-2 and MMP-9 in vivo (7) and in cell culture (35). A variety of inflammatory mediators are also involved in vascular remodeling. Tumor necrosis factor-α (TNF-α), a cytokine implicated in the development of intimal hyperplasia and arterial remodeling (5), controls MMP-9 and MT1-MMP expression in a variety of cell types (28, 30, 40). Previous studies have described MMP-2 and MMP-9 expression in a porcine model of vein graft (37) and the use of MMP inhibitors to block intimal hyperplasia in an arterogenous-graft model (31).

Little is known, however, about the signal transduction cascade(s) involved in the control of MMP expression in arterialized vein graft. Growth factors and stress stimuli activate intracellular signaling pathways in which mitogen-activated protein kinases (MAPKs) play a pivotal role (2, 14). Three major MAPK pathways converging to extracellular signal-regulated kinases-1 and -2 (ERK-1/2), p38MAPK, and c-Jun NH2-terminal kinases (JNKs) have been identified. These signaling pathways control various cellular functions and phenotypes, including proliferation, migration, differentiation, and apoptosis via phosphorylation of both cytoplasmic and nuclear substrates.

Our previous studies have shown that vein graft preparation and arterIALIZATION cause differential activation of MAPK pathways (1, 33). Moreover, MAPK activation in saphenous vein and in arterialized vein grafts can be modulated by topical treatment with inhibitors of the extracellular signal-regulated kinase pathway (1, 27). In vitro studies have shown the role of ERK-1/2 in the control of MMP-1 and MMP-9 expression (17). Recently, we have shown that sustained activation of ERK-1/2 is required for the induction of MMP-3 by fibroblast growth factor-2 (FGF-2; 28). However, no studies have investigated the signal transduction pathways that control MMP...
expression in arterialized vein graft. Therefore, we studied the expression of MMP-2, MMP-9, and MT1-MMP, and the potential role of inflammatory mediators and of the ERK-1/2 signaling pathway in the control of MMP expression and activation in a canine model of arterialized vein graft.

MATERIALS AND METHODS

Materials. UO126 was purchased from (Promega, Madison, WI); gelatin-Septarhose from Amersham Pharmacia Biotech (Piscataway, NJ); and myeloperoxidase substrate (o-dianisidine), 4-aminophenylmercuric acetate, and bovine serum albumin (BSA) from Sigma (St. Louis, MO). Antibodies to MT1-MMP, TIMP-2, and myeloperoxidase were purchased from Chemicon (Temecula, CA); anti-MMP-2 and anti-MMP-9 antibodies from Oncogene Research Products (Boston, MA); antibodies to the active (phosphorylated) forms of ERK-1/2 from Cell Signaling Technology (Beverly, MA); antibodies to ERK-2 and to β-actin from Santa Cruz Biotechnology (Santa Cruz, CA); and anti-TNF-α antibody from NeoMarkers (Fremont, CA).

Vein interposition graft. Vein interposition grafts were performed in mongrel dogs as described (33). Briefly, after heparinization (100 U/kg iv), the external jugular vein was grafted to the ipsilateral carotid artery in an end-to-side fashion. Where indicated, veins were incubated in phosphate-buffered saline containing either 80 μM UO126 or, as a control, 0.8% (vol/vol) dimethylsulfoxide (DMSO; vehicle) 30 min before being grafted. Vein grafts and control contralateral external jugular veins were harvested under general anesthesia 30 min, 3 h, 8 h, 1, 7, 14, and 28 days postoperatively (3 dogs/time point). All grafts were patent upon reexploration. This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publications No. 85-23, Revised 1996). The study protocol has been approved by the New York University School of Medicine Institutional Review Board.

Processing of vein samples. Vein graft and control vein segments were frozen in liquid nitrogen (for RNA extraction) or placed into ice-cold lysis buffer and processed for protein extraction immediately after being harvested as previously described (33).

Gelatin zymography and Western blotting analysis. Vein extract protein (75 μg) or cell-conditioned medium was analyzed by gelatin zymography or Western blotting analysis as described (22, 33). As a control for equal loading and transfer, the blots were stripped and reprobed with antibodies to ERK-2 or to β-actin. Quantitative analysis of the immunocomplexes (Western blotting analysis) or of gelatin zymography bands was performed by scanning densitometry with Kodak 1D Image Analysis software (Kodak, Rochester, NY).

Real-time reverse transcription-polymerase chain reaction. Total RNA, extracted from vein samples with Tri Reagent (Molecular Research Center, Cincinnati, OH) following the manufacturer’s instructions, was reverse transcribed using oligo (dT18) plus random hexamers and Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA) for 1 h at 37°C. MT1-MMP mRNA was measured with the real-time reverse transcription-polymerase chain reaction SmartCycler system (Cepheid, Sunnyvale, CA) using SYBR Green fluorescence and SmartCycler 1.2b software. PCR was performed using an initial 10-min hold at 94°C followed by 35 cycles (1 min denaturation at 94°C, 1 min annealing at 57°C, 1 min elongation at 72°C) and a final 10-min elongation step. PCR products were characterized by melting temperature curve analysis following the manufacturer’s instructions and by agarose gel electrophoresis using β-actin cDNA as a loading control. MT1-MMP mRNA levels were normalized to β-actin transcript in the same sample. The following primers were designed according to the published human sequences: MT1-MMP (NM-004995) sense: 5’ to 3’: TCGGCCCCAAAGCAGCACTTCTC, MT1-MMP antisense, 5’ to 3’: CCTCATGTTGTCGTGACATC.

RESULTS

MMP-2, MMP-9, and MT1-MMP upregulation and TIMP-2 downregulation in vein grafts. We have previously shown that in our canine model vein graft arterialization results in the generation of intimal hyperplasia as early as 14 days after grafting, with a pathological lesion characterized by a thick fibromuscular layer covering an expanded media (33). To study MMP-2 and MMP-9 expression in arterialized vein grafts, we used gelatin zymography to analyze protein extracts obtained from vein grafts and control veins at different times following arterialization. Both vein grafts and control veins showed 92-, 72-, and 62-kDa gelatinolytic bands consistent with proMMP-9, proMMP-2, and active MMP-2, respectively (Fig. 1). Vein graft levels of MMP-9 increased over control veins as early as 3 h after grafting. This effect peaked at 8 h and gradually decreased toward baseline by 28 days. Vein grafts also showed a strong increase in both total (72 kDa) and active β-actin sense: 5’ to 3’: GGGCCCATATGTTGGCATGGCGCG, β-actin antisense, 5’ to 3’: CCACTGGAATCCGTCGTGACATC.
(62 kDa) MMP-2 from day 1 to 28. In contrast, active MMP-2 was negligible in control, contralateral jugular veins. To investigate potential mechanisms of proMMP-2 activation in vein grafts, we analyzed MT1-MMP and TIMP-2 expression because these proteins are involved in MMP-2 activation (21). Western blotting analysis of vein extracts with anti-MT1-MMP antibody showed immunoreactive bands of 60, 58, and 43 kDa consistent with active MT1-MMP and cleavage products of this enzyme (Fig. 2A). Vein graft arterialization resulted in upregulation of MT1-MMP levels starting from day 4 (2.6 ± 0.5-fold increase over control). This effect was maximum at day 14 (4.1 ± 0.7-fold increase over control) and persisted until day 28 (2.7 ± 0.3-fold increase over control) (Fig. 2B). Quantitative real-time RT-PCR analysis showed higher (>8-fold increase over control) MT1-MMP mRNA levels in vein grafts than in control veins from day 1 to 14 (Fig. 2C). In contrast, TIMP-2 levels were dramatically downregulated in vein grafts relative to contralateral controls starting from day 1. Low TIMP-2 levels were maintained until day 14 and returned toward control level at day 28 (Fig. 3).

**ERK-1/2 CONTROL OF MMPS IN VEIN GRAFT ARTERIALIZATION**

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**Fig. 2. Time course of membrane-type-1 (MT1)-MMP expression in arterialized VG and C vein.**

A: representative Western blotting analysis of vein extracts with anti-MT1-MMP antibody. Rightmost lane, human HT1080 cell extract shown as a control (21). B: densitometric analysis of MT1-MMP bands (60, 58, and 43 kDa) in three independent Western blots of VG (○) and C (●) vein at different times after arterialization. One-way ANOVA showed overall increase ($P < 0.001$) of MT1-MMP in VG but not in C vein. Means ± SE are shown. *$P < 0.01$, VG vs. C. C: quantitative real-time RT-PCR analysis of MT1-MMP mRNA in VG (○) and C (●) vein at different times after arterialization. Bars represent SYBR green fluorescence units normalized to β-actin mRNA. Means ± SE of 3 samples per time point are shown. One-way ANOVA showed overall increase ($P < 0.001$) of MT1-MMP mRNA. *$P < 0.01$, VG vs. C vein.

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**Fig. 3. Time course of tissue inhibitor of metalloproteinase-2 (TIMP-2) expression in VG and C vein.**

A: representative Western blotting analysis of vein extracts with anti-TIMP-2 antibody. B: densitometric analysis of TIMP-2 bands in three independent Western blots. Bars represent ratio of densitometry readings of VG vs. C bands. One-way ANOVA showed overall change in TIMP-2 ($P < 0.001$). Means ± SE are shown. *$P < 0.01$, VG vs. C vein.
Increase in thrombin activity precedes MMP-2 activation in vein graft. MMP-2 activation in vein grafts was observed as early as 1 day postoperatively (Fig. 1), a time when MT1-MMP levels were not significantly higher than in control veins (Fig. 2). This finding indicated that the early activation of MMP-2 was not mediated by increased MT1-MMP expression. To investigate alternative mechanisms of MMP-2 activation, we analyzed thrombin activity in vein grafts and control vessels because thrombin can activate MMP-2 (23). Thrombin activity dramatically increased in vein grafts as early as 8 h after grafting. This effect peaked at day 1 (3.5 ± 0.5-fold over control) and gradually decreased to baseline until it was not significantly different from the control by day 7 (Fig. 4, A). The peak of thrombin activity coincided with the onset of MMP-2 activation (Fig. 1 and Fig. 4, A and B).

Inflammatory cell infiltration peaks with MMP-9 upregulation. Immunohistochemical analysis of vein grafts and contralateral veins showed an intense recruitment of myeloperoxidase (MPO)-positive inflammatory cells in vein grafts within 8 h from grafting (Fig. 5). Similarly, MPO activity, a PMN marker (18), rapidly increased in vein graft extracts as early as 3 h after surgery. This effect was maximum at 8 h and gradually decreased to control levels by day 7 (Fig. 6, A). The rapid increase in MPO activity paralleled the early upregulation of MMP-9 in vein grafts, and MPO peaked together with MMP-9 upregulation (Fig. 1 and Fig. 6, A and B). Because PMN granules contain large amounts of MMP-9, these data indicated that PMN infiltration could be the cause of the rapid MMP-9 increase observed in vein grafts (36).

TNF-α and thrombin differentially activate MMP-2 and MT1-MMP in cultured SMCs. TNF-α levels markedly increased in vein graft extracts as early as 3 h after surgery. This effect was maximum at 8 h and gradually decreased to control levels by day 7 (Fig. 6A). The rapid increase in MPO activity paralleled the early upregulation of MMP-9 in vein grafts, and MPO peaked together with MMP-9 upregulation (Fig. 1 and Fig. 6, A and B). Because PMN granules contain large amounts of MMP-9, these data indicated that PMN infiltration could be the cause of the rapid MMP-9 increase observed in vein grafts (36).
thrombin activity (Fig. 4) during the inflammatory response to injury.

Increased MMP expression and activation is controlled by ERK-1/2 activation. Vein graft arterialization results in MAPK activation (33), and ERK-1/2 activation controls MMP expression and/or activity in several experimental settings (20, 28, 34). To characterize the potential role of the ERK-1/2 pathway in the control of MMP expression in arterialized vein grafts, we inhibited ERK-1/2 activation with the synthetic MAPK kinase (MEK-1/2) inhibitor UO126. For this purpose excised veins were preincubated at room temperature with UO126 (80 μmol/l) for 30 min before implantation, an average incubation time between harvesting and grafting of saphenous veins in most vascular bypass procedures. This treatment resulted in strong inhibition of ERK-1/2 phosphorylation (27) (Fig. 9) and in the parallel downregulation of proMMP-2 and active MMP-2 at days 1 and 4, decreased MMP-9 at day 1 and MT1-MMP at day 4 after vein graft arterialization (Fig. 10 A–D). These results showed that the arterialization-induced increase in the expression of these MMPs is mediated by activation of the ERK-1/2 pathway.

DISCUSSION

The reported data show that vein grafting into the arterial system results in rapid upregulation of MMP-2, MMP-9, and MT1-MMP expression and in downregulation of TIMP-2 levels, concomitant with rapid infiltration of MPO-positive inflammatory cells (PMNs) and increase in vein graft-associated thrombin activity and TNF-α levels. The increased expression of MMP-2, MMP-9, and MT1-MMP is controlled by MAPK activation and can be inhibited by treatment of the vein graft with a synthetic MEK-1/2 inhibitor before arterialization.

Previous studies have shown increased levels of vein graft-associated MMP-9 and MMP-2 and the effect of MMP inhibitors in animal models of vein graft (31, 37). Our study is the first to indicate a role for inflammatory mediators and MAPK activation in MMP expression and activation in arterialized vein graft. In our animal model, MMP-9 upregulation was detected very early (hours) following vein grafting. Vein graft-associated MMP-9 levels peaked together with MPO activity, a marker of PMN infiltration. Previous studies have shown inflammatory infiltrates predominantly consisting of mononuclear infiltrates in atherosclerotic plaques (25) and in developing hyperplastic lesions of vein grafts 1 to 4 wk after arterialization (45). In our vein graft model, we found that an intense inflammatory response mediated by PMN occurs within few hours of vein graft arterialization. Because PMNs contain large...
injury (15). Our finding of a very rapid increase in vein indicates that vein grafts are subjected to a chronic form of increases on rabbit vein graft model, vessel-bound thrombin activity in-
ticated in the development of intimal hyperplasia (12). In a
(16, 23), and tissue factor-generated thrombin has been impli-
activate alternative mechanisms of MMP-2 activation. Thrombin
those in control veins. This observation prompted us to inves-
tigate alternative mechanisms of MMP-2 activation. Thrombin
high levels of MT1-MMP, the physiological activator of
MMP-2, have been described in human atherosclerotic plaques
(30) and in allotransplanted coronary arteries (39). Our results
show that MT1-MMP mRNA and protein levels are rapidly
upregulated following vein grafting. However, at day 1 post-
arterialization, when MMP-2 activation occurred, MT1-MMP
protein levels in vein grafts were not significantly higher than
those in control veins. This observation prompted us to inves-
tigate alternative mechanisms of MMP-2 activation. Thrombin
activates MT1-MMP-bound MMP-2 in cultured vascular cells
(16, 23), and tissue factor-generated thrombin has been impli-
cated in the development of intimal hyperplasia (12). In a
rabbit vein graft model, vessel-bound thrombin activity in-
creases on day 7 and remains elevated for more than 14 days,
indicating that vein grafts are subjected to a chronic form of
injury (15). Our finding of a very rapid increase in vein
graft-associated thrombin activity coincident with MMP-2 ac-
tivation indicates that, besides chronic inflammation, vein
grafts are also subjected to a significant acute form of injury.
The early rise in thrombin activity could then be responsible
for the rapid activation of MMP-2 we observed in vein grafts.
In addition, as thrombin upregulates MT1-MMP expression in
cultured vascular cells (16), it can also induce the upregulation
of MT1-MMP expression that occurs in vein grafts at later time
points. This hypothesis is also supported by our results show-
ing that thrombin can both induce MT1-MMP expression and
activate MMP-2 in cultured vascular SMC. These findings
indicate thrombin as a potential pharmacological target for
preventing intimal hyperplasia and vein graft failure.

Our findings show that multiple mechanisms can be in-
volved in proMMP-2 activation in vein grafts. Thrombin and
PMN-derived serine proteinases rapidly activate MMP-2,
whereas upregulation of MT1-MMP expression can mediate
this process at later time points. Our study also shows vein
graft downregulation of TIMP-2 coincident with upregulation
of MT1-MMP, MMP-2, and MMP-9. Similar inverse levels of
TIMP-2 and MMPs have been reported after stent implantation
and balloon injury in rabbits (6). Likewise, gene trans-
fer-mediated upregulation of TIMP-2 results in attenuation of
neointimal growth in arterialized vein graft (11). The decrease
in TIMP-2 levels correlates with increased vein graft expres-
sion of TNF-α, a cytokine that downregulates TIMP-2 synthe-
sis in vascular SMC (42). TNF-α also upregulates MMP-9 and
MT1-MMP expression in SMCs (29) and has been implicated
in the pathogenesis of vein graft intimal hyperplasia (5). Our
experiments with cultured vascular SMC show that TNF-α
does indeed upregulate MT1-MMP efficiently; however,
TNF-α does not effect MMP-2 activation. This finding is
inconsistent with previous reports (9) that TNF-α induces
MMP-2 activation in full-thickness human skin or cultured
dermal fibroblasts. This effect requires 72–96 h of treatment
with TNF-α, whereas we characterized MMP-2 activation by
TNF-α or thrombin after a much shorter incubation (6 h). We
do not know whether in our experimental model TNF-α acti-
vates MMP-2 after 72–96 h of incubation. However, our
finding that thrombin does activate MMP-2 after a much
shorter time (6 h) indicates that thrombin is a much more
efficient, and likely physiological, activator of MMP-2 in vein
graft than TNF-α.

Fig. 8. Effect of thrombin and TNF-α on smooth muscle cell MMP-2 and MT1-MMP. Subconfluent human coronary artery smooth muscle cells, starved in basal medium containing 0.1% fetal calf serum for 18 h, were incubated for 6 h in basal medium without (control) or with addition of either purified human thrombin (10 U/ml) or human recombinant TNF-α (20 ng/ml). Conditioned medium was tested by gelatin zymography (A) and cell extracts by Western blotting analysis with MT1-MMP antibody (B). Bottom, densitometric analysis of three zymograms (left; open bars: 72-kDa proMMP-2; black bars: 62-kDa active MMP-2) and Western blotting immunoreactive bands (right). Means ± SE are shown. *P < 0.01, thrombin or TNF-α vs. control.

Fig. 9. UO126 inhibits arterialization-induced activation of ERK1/2 in VG. Western blotting analysis of ERK-1/2 activation in control femoral vein (C), arterialized VG pretreated with 0.8% (vol/vol) DMSO (VG) or with 80 μmol/l of UO126 (VG/UO) 3 h and 4 days after arterialization. Blot was probed with antibody to phosphorylated (active) ERK-1/2 and, after the antibody was stripped, with antibody to ERK-2 as a loading and transfer control (bottom).
We have previously shown that vein graft arterialization in our canine model results in ERK-1/2 activation, which does not occur after vein-to-vein or artery-to-artery bypass surgery (33). Inhibition of the ERK-1/2 pathway inhibits MMP-9 expression in cultured cells (32) and TNF-α-mediated MMP-9 upregulation in keratinocytes (10). Studies with cultured cells have also shown that MMP-9 upregulation can be mediated by parallel signaling pathways involving phosphatidylinositol 3-kinase-Akt and ERK-1/2 and that constitutive activation of these pathways mediates MMP-9 expression (32).

In our study, we tested the effects of topical delivery to vein grafts of the MEK-1/2 inhibitor UO126, a synthetic inhibitor shown to be effective in the topical treatment of inflammatory edema in a mouse model (13). Our results show that the ERK-1/2 pathway controls MT1-MMP, MMP-2, and MMP-9 expression in arterialized vein grafts. Surprisingly, we found that a short pretreatment of the vein graft with UO126 shows inhibitory effects at least 4 days after vein graft arterialization, despite the relatively short-lived (6–8 h) efficacy of UO126 (28). This finding suggests that late effects of vein graft arterialization (33) are controlled by the ERK-1/2 activation that occurs very early after vein grafting. For example, ERK-1/2 activation may control the expression of growth factors or cytokines that, in turn, induce effects (e.g., MMP expression) at later times. Blocking the initial ERK-1/2 activation, therefore, results in inhibition of the late events.

MMP activity in arterialized veins can be controlled by growth factors and/or cytokines produced during injury-induced vascular remodeling, as well as by high intraluminal pressure (19) or shear force (26), or a combination of these factors. In addition, MMP-2 expression and localization vary with pressure changes (3). However, a recent study has shown that, although cyclic strain significantly increases MMP-2 expression, MAPK inhibition abolishes the strain-induced MMP-2 response (41). Therefore, it is conceivable that both direct hemodynamic changes and biological factors affect MMP activity during vein graft arterialization and that MAPKs are the converging intracellular point of control for these different extracellular stimuli.

In our experimental vein graft protocol, the animals were maintained on a normal, nonhypercholesterolemic diet, which might appear as a limitation of our study. Intimal hyperplasia, however, develops in vein grafts even with a standard diet, although to a lesser extent than in hypercholesterolemic animals (4). Moreover, most studies of MMP expression in animal models of vein bypass grafting did not use a hypercholesterolemic diet regimen (8, 37, 39).

In conclusion, the data presented in this study provide a comprehensive characterization of MMPs during vein graft arterialization, and both our in vivo and in vitro observations support the following mechanisms. Shortly after vein grafting...

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**Fig. 10.** UO126 inhibits arterialization-induced upregulation of MMP-2, MMP-9, and MT1-MMP in VG. Before grafting, jugular veins were incubated for 30 min at room temperature with either UO126 (80 μM) or DMSO (0.1%, vehicle). **A:** representative gelatin zymogram of arterialized VG and C vein extracts obtained at indicated times. **B:** densitometric analysis of proMMP2 (□) and active MMP-2 (■) bands in extracts of control vein or VG treated with UO126 (UO) or untreated. Results are shown as relative band intensity, measured by considering the densitometric reading of the proMMP-2 band in the untreated, day 1 C sample equal to 1. Histograms represent means ± SE of three zymograms (*P < 0.01). **C:** densitometric analysis of 92-kDa MMP-9 bands with (□) and without (■) UO126 treatment. Results are shown as relative band intensity, measured by considering the densitometric reading of the MMP-9 band in the untreated, day 1 C sample equal to 1. Histograms represent means ± SE of three zymograms (*P < 0.05). **D:** Western blotting analysis of MT1-MMP in day 4 extracts of VG and control C treated with or without UO126. Two sets of UO126-treated and untreated samples derive from different animals with different baseline levels of MT1-MMP expression. Results show strong inhibition of MT1-MMP at day 4 after VG arterialization.
arterialization, PMNs infiltrate into the graft wall, where they discharge the content of their granules, thus providing a rapid source of MMP-9 and serine proteinases. The inflammatory response, secondary to vein graft arterialization, is characterized by high local levels of thrombin and TNF-α. Thrombin rapidly activates MMP-2 and stimulates MT1-MMP expression in concert with TNF-α. High levels of MT1-MMP provide a cell membrane binding site for the MMP-2-TIMP-2 complex necessary for MMP-2 activation. TNF-α contributes to TIMP-2 downregulation. Thus the marked increase in MMP-2 activation and parallel decrease in TIMP-2 result in a net increase of the proteolytic activity required for the vessel remodeling that follows vein graft arterialization.

The arterialization-induced upregulation of MMP expression in vein grafts is mediated by activation of the ERK-1/2 pathway. Our data show that activation of this signaling pathway and the resulting increase in MMP levels can be efficiently inhibited by ex vivo pretreatment of vein grafts with the synthetic inhibitor UO126. This pharmacological approach affords a convenient and efficient method of drug delivery and reperfusion: role of selectins.


Pintucci G, Yu PJ, Sharony R, Baumann FG, Saponara F, Frasca A, Galloway AC, Moscatelli D, and Mignatti P. Induction of stromelysin-1...


