BASIC RESEARCH STUDIES

Pressure distention compared with pharmacologic relaxation in vein grafting upregulates matrix metalloproteinase-2 and -9

Ada W. Y. Chung, PhD,[a] Pooja Rauniyar, BSc,[a] Honglin Luo, MD,[a] York N. Hsiang, MD,[b] Cornelis van Breemen, PhD,[a] and **Elena B. Okon, PhD,[a]** *Vancouver, British Columbia, Canada*

Objective: **Autogenous vein bypasses are a common and effective method to treat occlusive disease. During surgical preparation, veins are routinely pressure distended to overcome vasospasm and twists. Distention, however, is believed to promote vascular remodeling and contribute to decreased graft patency. Pharmacologic vasorelaxation with a combination of effective vasodilators has been suggested as an alternative to pressure distention. The extracellular matrix (ECM)-degrading matrix metalloproteinases (MMPs) have been implicated in vascular remodeling and neointima formation. The purpose of the present study was to compare the effects of pressure distention with pharmacologic vasorelaxation on graft remodeling and regulation of MMP-2 and MMP-9 in porcine vein grafts.**

Methods: **Carotid artery bypass utilizing internal jugular veins was performed in eight female white pigs. Jugular veins were randomized to receive pressure distention (300 mm Hg for 2 minutes) or a combination of vasodilators (the α-adrenergic antagonist phenoxybenzamine, 10 μmol/L; the Rho-kinase inhibitor HA-1077 [fasudil], 50 μmol/L; and the calcium-channel blocker nicardipine, 1 μmol/L) for 30 minutes and then were grafted into the carotid arteries. Two weeks after surgery, vein graft samples were analyzed for vessel intimal and medial area, lumen diameter, and ECM composition. Molecular analysis using reverse transcription-polymerase chain reaction, Western immunoblotting, gelatin zymography, and reverse zymography were performed to study the expression and activation of MMP-2 and MMP-9, and tissue inhibitors of MMP (TIMP)-1 and TIMP-2.**

Results: **Pressure distention irreversibly overstretched the porcine jugular vein and increased MMP-2 and MMP-9 proteolytic activity by 40% and 77%, respectively. Two weeks of vein grafting in the carotid arterial bed induced vessel wall thickening, ECM modification, and neointima formation, which were more pronounced in the distended grafts ($P < .05$) and accompanied by an increase in MMP expression and activity. Distended grafts demonstrated higher percentages of active MMP-9 (17.8% ± 1.0%) and higher activities of latent (35.5% ± 3.3%) and active MMP-2 (69.6% ± 8.8%) than the pharmacologically treated grafts. Protein expression of TIMP-1 and TIMP-2 was downregulated after arterial grafting, but the pharmacologically treated grafts expressed significantly more TIMP-1 protein (by 36.8% ± 4.1%) than the distended ones. The activities of TIMPs were markedly decreased after grafting, contributing to the upregulated MMP activity.**

Conclusions: **Pressure distention of vein grafts before implantation, compared with pharmacologic vasodilatation, stimulates neointima formation and augments MMP activities. Pharmacologic vasorelaxation may be clinically superior to distention in attenuating graft remodeling and possibly improving graft patency. (J Vasc Surg 2005;42:747-56.)**

Clinical Relevance: **Autogenous vein bypasses are a common and effective method to treat occlusive disease. This study demonstrated that pressure distention, a common preparatory procedure in bypass surgery, upregulates extracellular matrix-degrading matrix metalloproteinases, which predisposes vein grafts to extensive remodeling and contributes to neointima formation and graft occlusion. The topical application of a combination of vasodilators to the vein graft before implantation may be clinically superior to pressure distention in attenuating graft remodeling and may possibly improve graft patency and reduce secondary surgical interventions.**

Arterial reconstruction with autologous tissue by using vein grafts is an effective and durable treatment for peripheral artery occlusion.[1-3] During preparation for grafting, the vein usually undergoes pressure distention to overcome vasospasm resulting from surgical trauma. This maneuver is believed to promote vascular remodeling and contribute to

From The James Hogg iCAPTURE Center, St. Paul's Hospital,[a] and the Department of Surgery, University of British Columbia.[b]

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Reprint requests: Ada W. Y. Chung, PhD, Cardiovascular Science, Room 2099, 950 West 28th Avenue, Vancouver, British Columbia V5Z 4H4, Canada (e-mail: achung@mrl.ubc.ca).

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decreased graft patency.[4,5] Distention results in venous vasomotor dysfunction,[6,7] exaggerated platelet and leukocyte adhesion,[8] reduced adenosine triphosphate levels,[9] and impaired production of nitric oxide and prostacyclin,[10,11] the biologic mediators that suppress thrombosis and atherosclerotic transformation.

Surgical preparation of human saphenous vein, including manual distention, induces dedifferentiation of smooth muscle cells (SMCs), which is characterized by a shifting from the contractile to the synthetic phenotype.[12] Distention-stimulated SMC proliferation and migration are the major causes of graft wall thickening and neointima formation.[4,5] These processes require coordinated alterations in mitogenic signaling and the degradation and reorganization of extracellular matrix (ECM) by matrix metalloproteinases (MMPs). Proliferation and migration of SMCs have been shown to be closely related to stimulation of MMPs.[13,14] MMP-2 and MMP-9 (gelatinase A and B, respectively) are especially important regulators of vein graft architecture because of their specificity for elastin and collagen.[15]

Mechanical stretch and vascular injury are known to activate these MMPs and are associated with SMC migration and vascular remodeling.[13,16-20] Secretion, proteolytic activity, and immuno-positive staining of MMP-2 and MMP-9 have been shown to be upregulated in porcine vein grafts.[20] MMP activity is tightly controlled at the level of gene transcription, activation of latent enzyme, and inhibition by endogenous tissue inhibitors of MMPs (TIMPs).[13,15,21] Therefore, activation of MMPs and a shift in the balance of the MMP/TIMP system stimulated by pressure distention could contribute to SMC proliferation and migration, thus accelerating graft occlusion.

Unfortunately, because human saphenous vein is highly susceptible to vasoconstriction,[7] pressure distension during surgical preparation for grafting cannot be avoided without an alternative procedure to prevent vasospasm. We have previously suggested such an alternative strategy[7] based on the topical application of a combination of long-lasting vasodilatatory agents to the vein before grafting. This maneuver provides prolonged relief from vasospasm, which is effective for the critical time period during and after surgery until established arterial blood pressure prevents vasospasm in the grafted vein.

In the present study, we compared the effects of distention with pharmacologic treatment on the activity of MMPs and ECM integrity in the porcine jugular vein before and after grafting into the carotid artery. For pharmacologic treatment, vein grafts were exposed to a combination of vasodilatatory drugs: the α-adrenergic antagonist phenoxybenzamine, the Rho-kinase inhibitor HA-1077 (fasudil), and the calcium-channel blocker nicardipine. Together, these drugs block the main mechanisms of vasoconstriction in human vessels.[7] We hypothesized that pressure distention upregulates MMP activity and enhances neointima development during grafting and that pharmacologic relaxation is beneficial for preserving ECM integrity and attenuating graft remodeling.

METHODS

Animal surgical procedures. All animal procedures were approved by the University of British Columbia Animal Ethics Board. Animals were cared in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1996). Reversed vein grafts utilizing jugular veins were grafted into the carotid arteries of eight female white pigs that weighed 60 to 65 kg. Pigs were given a general anesthetic (a single dose of ketamine, 800 to 1400 mg), and under sterile conditions, a midline neck incision was made to expose both common carotid arteries and internal jugular veins.

After dissection, veins were removed, reversed, and randomized to pressure distension or immersion into a vasodilatatory solution. Veins allocated to pressure distention were distended with isotonic heparinized saline solution at 300 mm Hg for 2 minutes. A piece of the distended vein was taken as a sample. For vein grafts allocated to immersion, a piece of the vein was taken as the nondistended sample, and the rest of the vein was placed in a bath containing a combination of vasodilatatory drugs (phenoxybenzamine, 10 μmol/L; HA-1077 (fasudil), 50 μmol/L; nicardipine, 1 μmol/L)[7]in a heparinized (1:250 concentration) isotonic saline solution at room temperature for 30 minutes. An end-to-end anastomotic technique was used to graft the veins to the divided carotid artery as an interposition vein graft.

After 2 weeks, the seven pigs that survived the surgery were given another general anesthetic, and the interposition vein grafts were removed. All animals were then euthanized. Schemes of experimental design and sampling handling are presented in Appendix Figs E1 and E2 (online only).

Histology. Vessel segments were formalin fixed, embedded in paraffin, and 3-μm cross-sections were prepared and stained with modified Movat pentachrome. Image acquisition and processing was performed by using a MicroPhot microscope (Nikon Inc, Kanagawa, Japan.). Images were captured by a SPOT digital camera (Diagnostic Instruments, Sterling Heights, Mich), and analyzed with ImagePro-Plus5 software (Media Cybernetics, Silver Spring, Md) (Appendix, online only).

Reverse transcription-polymerase chain reaction. Total RNA and protein was extracted from flash-frozen samples of vessels by TRIzol reagent according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, Calif). Total RNA (1 μg) was reverse-transcribed into complementary DNA (cDNA) using Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies). Reverse transcription was performed in a Gene Amp thermocycle (Gene Amp PCR System 9700, Applied Biosystems, Foster City, Calif) for 60 minutes at 37°C, then for 15 minutes at 70°C. First-strand cDNA (5 μL) was used for polymerase chain reaction (PCR) as previously described (Appendix, online only).

Western immunoblotting. Measurement of MMP expression by Western immunoblotting was previously de-

scribed.[22] In brief, 10 μg of each protein sample was separated on 8% (for MMP-2 and MMP-9) or 12% (for TIMP-1 and TIMP-2) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinyldifluoride membranes (Bio-Rad Laboratories, Hercules, Calif). Membranes were first incubated with primary antibodies: mouse monoclonal anti-MMP-2, anti-MMP-9 (dilution 1:250) (Oncogene Science, San Diego, Calif) and anti-TIMP-1 or anti-TIMP-2 (dilution 1:400) (Calbiochem, San Diego, Calif) antibodies, and then with immunoglobulin G peroxidase-conjugated secondary antibodies (dilution 1:2500). Immunoreactive proteins were visualized by an enhanced chemiluminescence kit (Amersham Life Sciences, Buckinghamshire, England). To normalize the protein level, membranes were stripped and reprobed with β-actin antibody.

Gelatin zymography. Gelatinolytic activity of MMPs was measured as previously described.[22] Briefly, protein extract (2 μg) was electrophoresed in 8% SDS-PAGE copolymerized with gelatin (2 mg/mL). Gels were washed with 2.5% Triton X-100, then the enzymatic activity was developed by incubating the gels in enzyme assay buffer at 37°C for 48 hours. Gels were then stained and destained, and the gelatinolytic activities were identified as transparent bands against the blue background. Images were captured by digital camera (Olympus Optical Co, Tokyo, Japan).

Reverse zymography. Activities of TIMP-1 and TIMP-2 in tissue extract (2 μg) were determined by electrophoresis in 12% SDS-PAGE copolymerized with 1 mg/mL gelatin and 50 ng/mL human recombinant MMP-2 or MMP-9 (Calbiochem). Gels were rinsed in 2.5% Triton X-100 and then incubated in the enzyme assay buffer. After staining and destaining the gels, the TIMP activity was visualized as dark bands against the clear background.

Materials. All other reagents were the highest molecular grade purchased from Sigma (St Louis, Mo), unless specifically stated in the text.

Statistics. Data were reported as a mean ± standard error from five to eight independent experiments. A paired *t* test was performed to compare the differences between the two groups. A value of $P < .05$ was considered significant.

RESULTS

Vessel morphology and ECM composition. Pressure distension of 300 mm Hg irreversibly overstretched the veins, resulting in a 104% ± 2% increase in lumen diameter-to-thickness ratio (Fig 1, *A* and *B*; Appendix Fig E3, online only) and a reduction of intimal-medial thickness (Appendix Fig E4, online only). In native veins, elastin was presented as continuous bands; in distended vessels, it appeared as discontinuous fragments (Fig 1, *C* and *D*).

After 2 weeks of implantation, arterial hemodynamics had a pronounced impact on proliferative and synthetic processes in the grafts, resulting in wall thickening and neointima formation (Fig 1, *E* and *F*; Fig 2, *A*; Appendix Figs E3 and E4, online only). The pressure-distended graft in one of the seven pigs was completely occluded (Fig 1, *H*), and one was critically narrowed, whereas all the pharmacologically treated grafts had clear lumens.

There was no detectable intima between endothelial and medial layers before grafting, but after grafting, a profuse neointima had developed. In pressure-distended grafts, the ratio of intima to media area was 112% ± 9% higher than in vein grafts treated with vasodilatators (Fig 2, *A*). The neointima consisted of radially oriented proteoglycan fibers and SMCs (Appendix, online only).

Collagen and elastin composed about 60% and 17% of the jugular vein body, respectively (Fig 2, *B*). In the pressure-distended grafts, significant reductions in the proportions of elastin (by 49.1% ± 1.5%) and collagen (by 30.7% ± 2.0%) were observed. These changes were less pronounced ($P > .05$) in pharmacologically treated grafts. A significant increase in proteoglycans (by 350% ± 26% in the drug-treated grafts and by 148 ± 15% in the distended grafts), which compose about half of the total neointima area, was evident in both grafts (Fig 1, *G*; Fig 2, *B*).

Expression and activity of MMPs. The expression and activities of MMPs were measured because MMP degradation of ECM can facilitate SMC proliferation and migration as well as enhance wall thickening and neointima development. Distention did not induce immediate changes in gene transcription or apparent protein expression of MMP-2 or MMP-9, but 2 weeks of grafting dramatically upregulated transcription of MMP-2 in both drug-treated and pressure-distended grafts (by 209% ± 13% and 149% ± 14%, respectively) but not of MMP-9 (Fig 3, *A*).

Western blotting detected two immunoreactive bands for MMP-2 and MMP-9: 68 kDa-(latent MMP-2), 62 kDa-(active MMP-2), 92 kDa-(latent-MMP-9), and 83 kDa-(active MMP-9) (Fig 3, *B*). Grafting roughly doubled the percentage of active MMP-2 in both types of grafts. The proportion of active MMP-9 increased by 27.7% ± 1.3% in the distended grafts, being significantly higher than that in the drug-treated grafts. The expression of latent forms of both MMP-2 and MMP-9 was not significantly altered by grafting and distention (Fig 3, *B*).

The protein levels of MMP-1 and MMP-3 were also measured in this study, but their expression was very low and was not altered by distention and arterial grafting (data not shown).

In contrast to MMP expression, the activity of the proteinases was acutely affected by distention of the vein. Gelatinolytic activity of both MMPs was low in the nondistended veins (Fig 4, *zymogram*); however, distention instantly upregulated the activities of latent MMP-9 by 39.9% ± 2%, latent MMP-2 by 77.1% ± 6.6%, and active MMP-2 by 187% ± 4% (Fig 4, *bar graphs*).

Arterial grafting caused further activation of both MMPs. In the distended grafts, activities of latent and active forms of MMP-2 were 35.5% and 69.6%, respectively, higher than the drug-treated grafts (Fig 4, *lower bar graph*). Although the latent MMP-9 activity was about doubled in two postgrafts (Fig 4, *upper bar graph*), we did not detect changes in the activity of active MMP-9

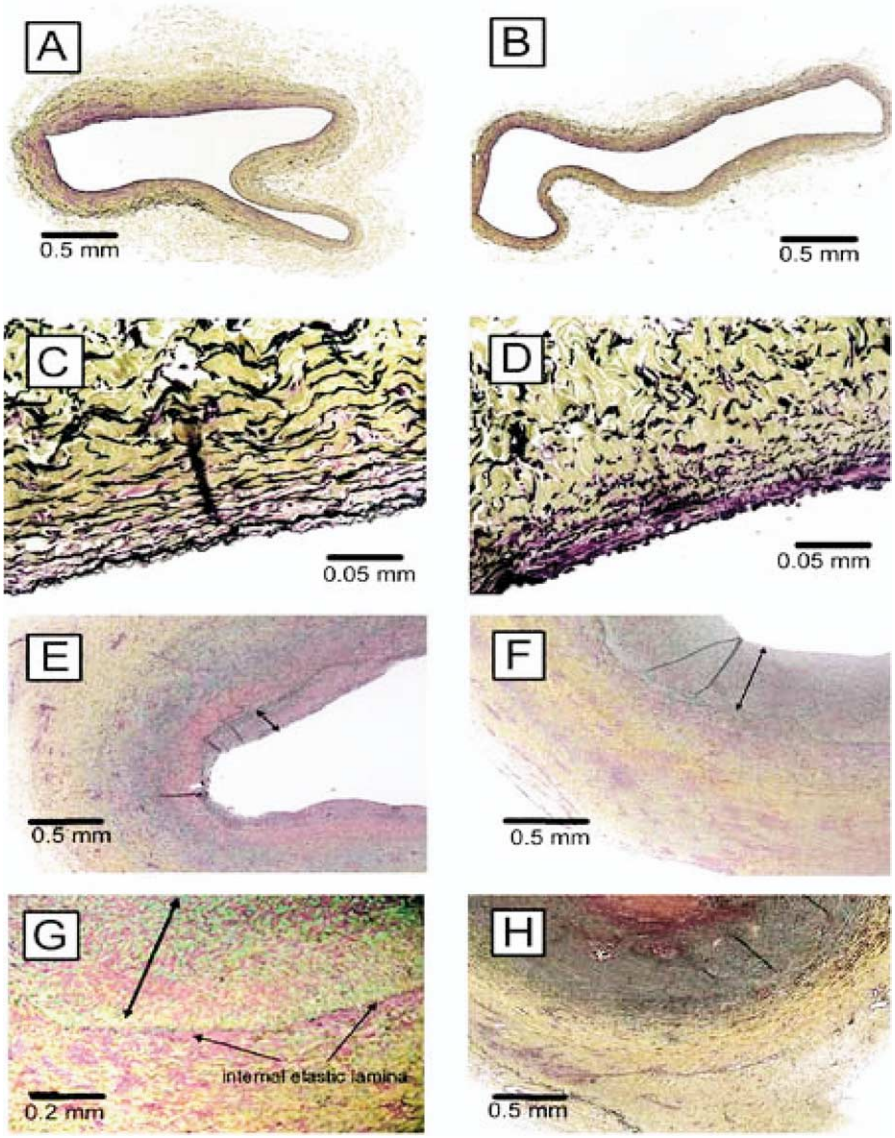

Fig 1. Representative Movat stained photomicrographs. **A** to **D,** Nondistended and distended veins with different magnification; (**E** and **F**) pharmacologically treated and distended grafts; **(G)** area with representative extracellular matrix composition; and **(H)** occluded distended graft. **G,** The extracellular matrix components are elastin, *black*; collagen, *yellow*; and proteoglycan, *green*. Smooth muscle cells are *purple*. *Arrows* indicate neointima. Representative samples from five to eight independent experiments.

throughout the experiments, probably because of the low intensity of the bands.

Regulation on the endogenous inhibitory system of MMPs-TIMPs. TIMPs represent one of the mechanisms regulating MMP activity. The most abundant TIMPs present in vascular tissue are TIMP-1 and TIMP-2.[13,21] Their transcription was low in the intact veins and was not altered by distention and arterial grafting (Fig 5, *A*); whereas protein levels were markedly downregulated after arterial grafting (Fig 5, *B*). However, TIMP-1 protein levels in the pharmacologically treated grafts were significantly higher (by 36.8% ± 4.1%) than those in distended grafts. No difference was seen in the level of TIMP-2. The activities of both TIMP-1 and TIMP-2 were markedly suppressed after grafting, but there was no difference between the two postgrafts (Fig 5, *C*).

DISCUSSION

The present study describes the effects of pressure distention, compared with a vasodilatory cocktail, and arterial hemodynamics on vascular remodeling and MMP activities in a porcine vein graft model. We showed that pressure distension of the vein enhanced the degree of grafting-induced wall thickening and neointima formation

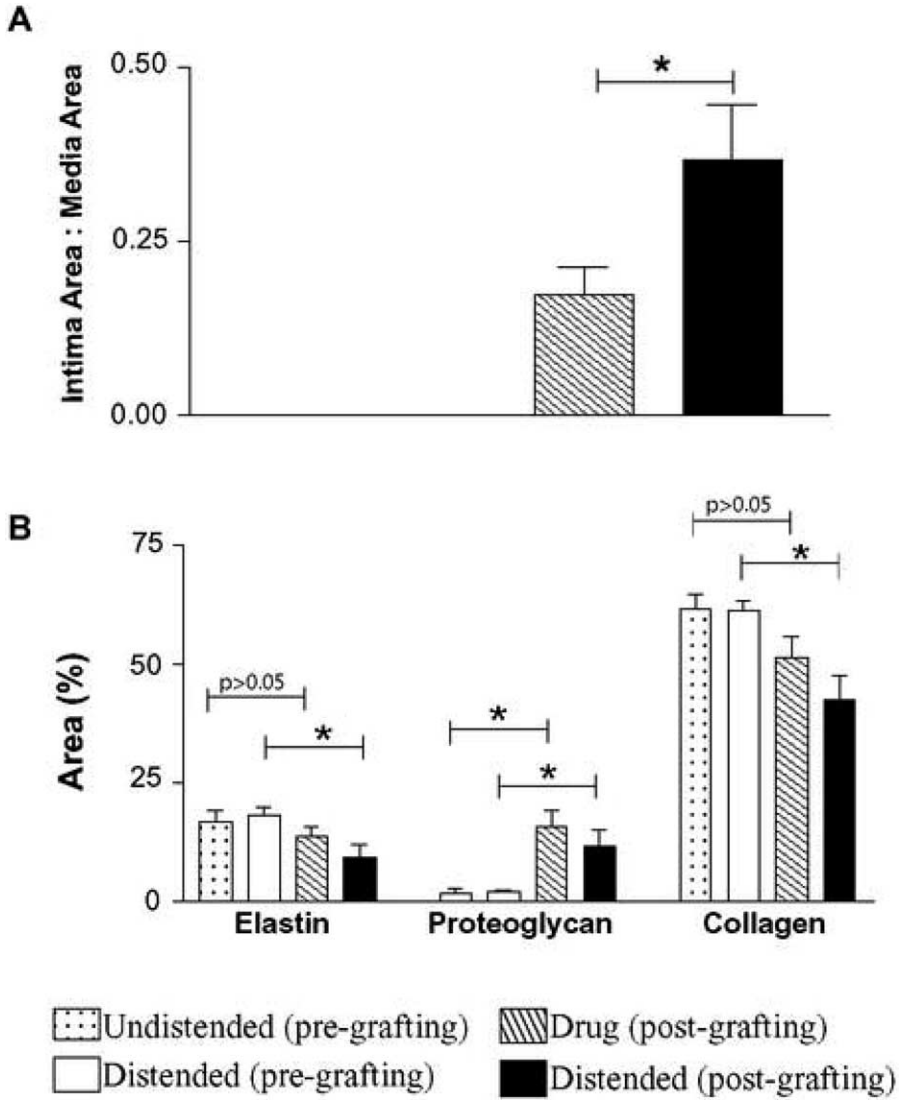

Fig 2. Morphometric analysis and extracellular matrix composition of vein and graft segments measured from Movat stained cross-sections. **A**, Shows ratio of intima area to media area. **B**, Bar graph presents the percent area of elastin, proteoglycan, and collagen. $*P < .05$, n = 5 to 8.

compared with pharmacologic treatment. This vascular remodeling is associated with elevated activities of MMP-2 and MMP-9 and modification of ECM and could account for the early venous graft occlusion.

Grafting of veins in the arterial bed is known to induce wall thickening and intimal hyperplasia, leading to graft stenosis.[4,5] The main cause for this is high and pulsatile blood pressure in the arterial circulation to which the vein was not exposed in its original location concomitant with increased production of cytokines and growth factors. High and pulsatile pressure stimulates SMC proliferation and ECM production for the adaptation of grafts to the arterial circulation.[4,5] Some conditions during surgery, such as pressure distention together with ischemia and increased cytokine secretion, exacerbate these processes and accelerate graft failure. Each of these conditions could be a target for improving vein graft patency, especially distension, which is usually done with pressures exceeding arterial pressure.

Unlike ischemia and cytokine production, which are due to surgical trauma, distention can be avoided in the surgical preparation of the vein for grafting. The current technique of vein graft preparation requires distention of the vein to overcome vasospasm; however, an alternative procedure could be adopted to prevent vasospasm during and after surgery. We have suggested such an alternative procedure[7] that is based on the topical application of vasodilatators to the vein before grafting (Appendix, online only).

In the present study, we investigated the effects of distention, compared with pharmacologic treatment, on pig jugular veins before grafting and after a 2-week implan-

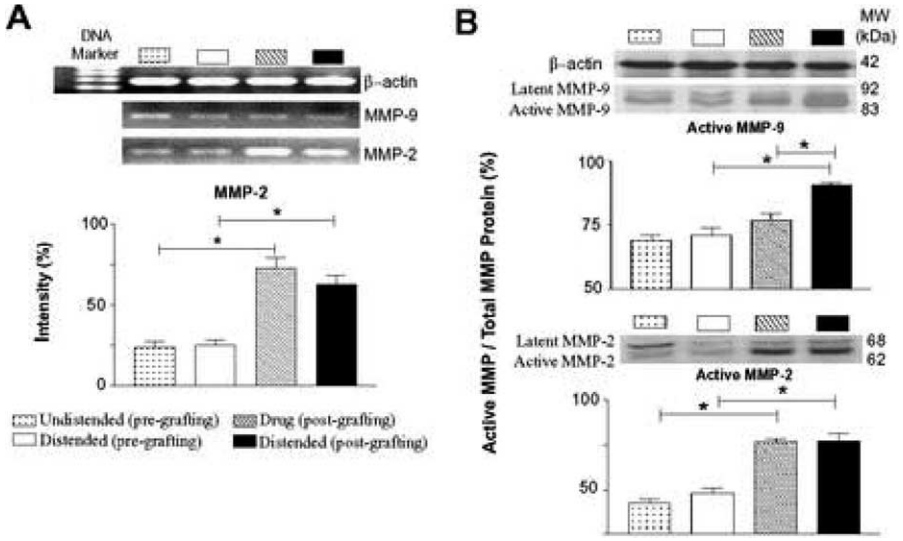

Fig 3. MMP-2 and MMP-9 gene transcription and protein expression in veins and grafts subjected to pressure-distention or drug treatment. **A**, Representative polymerase chain reaction images show the gene transcription of MMP-2, MMP-9 and β-actin (loading control). Densitometric measurement of MMP-2 transcript (normalized to the loading control). **B,** Bar graphs show the percentage of active MMP-2 and MMP-9 relative to the total protein expression (active + latent form) from 10 μg of sample protein extract. **Insets,** Representative loading control and immunoblots of the MMP-related immunoreactivity determined from Western blotting. * $P < .05$, paired *t* test; n = 7 to 8. *MMP,* Matrix metalloproteinase.

tation with respect to morphologic properties and regulation of MMPs. We found the short-term impact of distention had persistent effects, such as overstretching of the vein (Fig 1, *A* and *B*; Appendix Fig E3 and E4, online only) and disruption of the elastin fibril structure (Fig 1, *C* and *D*). The arterial hemodynamics during 2 weeks of grafting resulted in striking changes in structure and composition of the vein segments. The thickness of the intimal-medial layer was increased several times, and a profuse neointima was developed. Proteoglycans, practically absent in veins, became abundant in grafts, especially in the neointima.

Nevertheless, 2 weeks of exposure to the arterial circulation did not mask the damaging effect caused by pressure distention. The distended grafts had a higher degree of vessel wall thickening. The ratio of intima-to-media area was higher in the distended grafts, which also demonstrated more modifications in ECM composition than the drug-treated grafts. The neointima mainly consisted of radially oriented proteoglycan fibers and SMCs. Proliferation and migration of medial SMCs to neointima requires the loosening of the ECM around cells. An increase in the proteolytic activity of MMPs, resulting in degradation of ECM, could facilitate SMC migration and proliferation. Indeed, we found MMP activation was associated with distention.

We showed that the activities of both MMP-2 and MMP-9 increased markedly after distention. This is the first report of acute activation of MMP by distention in vein grafts. The instantaneous increase in MMPs activities, induced by distention (Fig 4), suggested that pressure distention triggers cellular responses. Two weeks of grafting augmented MMP-9 activity in both types of graft, but a greater increase in MMP-2 activity was observed in the distended grafts than in the drug-treated ones. The activities of both forms of MMP-2 remained higher in the distended grafts than in those pharmacologically treated. We can therefore suggest that the MMP activation stimulated by distention, which remains increased during grafting, contributes to more pronounced graft remodeling.

MMP-2 and MMP-9 have both been implicated in atherosclerosis and restenosis[13,15-19] as well as neointima formation in human saphenous vein in organ culture.[23,24] Increased secretion of MMP-2 and MMP-9 in a pig venous graft model has been shown,[20] and activation of these MMPs was demonstrated in human saphenous vein after surgical preparation that included manual distention.[25] However in the these experiments, in contrast to our design, the effect of distention on MMP activity could not be segregated from the effects of prolonged ischemia and other surgical procedures.

The initial response to MMP activation could be mediated through integrins,[26] which serve as a transmembrane link between the ECM and intracellular signaling and thus could be first affected by distention. Several mechanisms regulate MMP activity: gene transcription, levels of endogenous TIMPs, and activation of the latent enzyme.[13,15,21] In this study, we showed that arterial grafting upregulated MMP-2 at both messenger RNA (mRNA) and protein levels, while MMP-9 expression was increased only at protein levels, suggesting a differential regulation of these MMPs after grafting. Factors leading to remodeling and

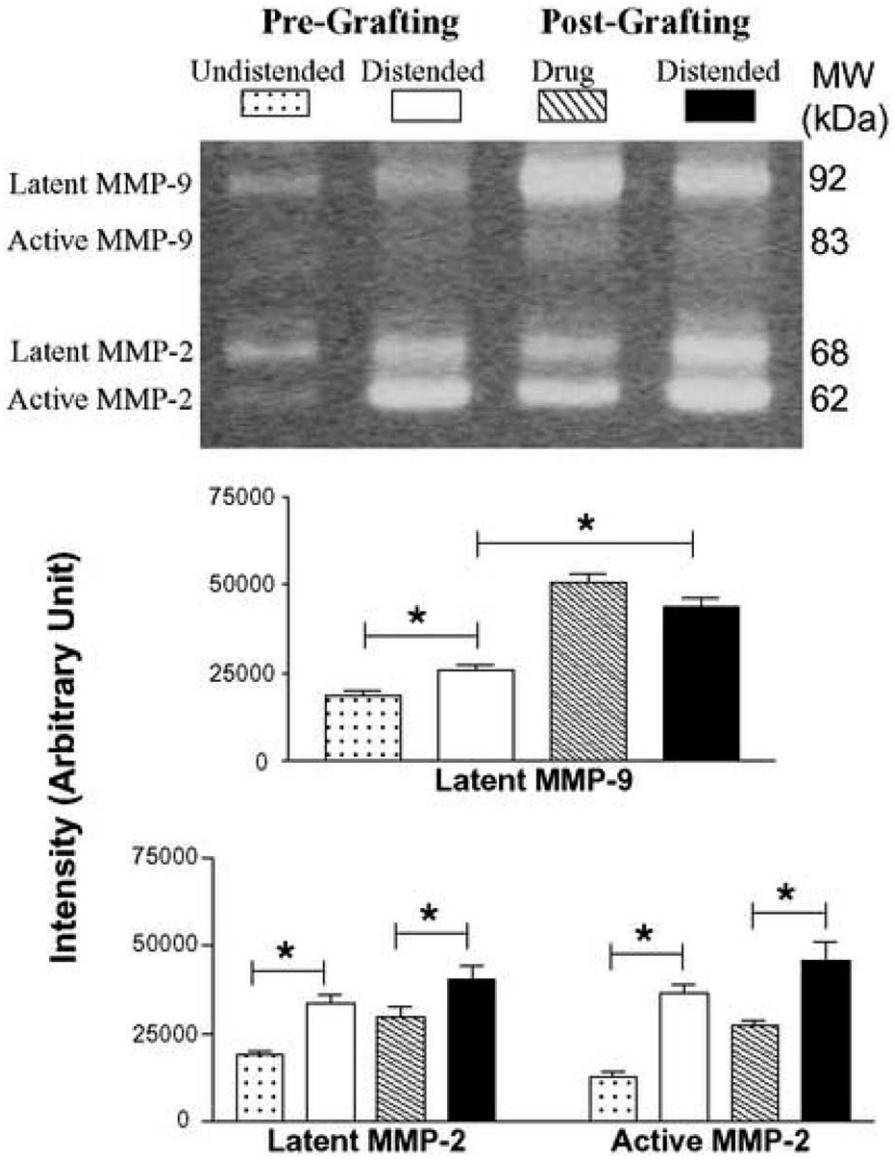

Fig 4. Proteolytic activities of MMP-2 and MMP-9 in veins and grafts subjected to pressure distention or drug treatment. Protein extract (2 μg) was electrophoresed in 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis copolymerized with gelatin (2 mg/mL). Gelatinolytic activities were identified as transparent bands against the blue background. Representative zymogram *(inset)* shows activities of latent MMP-9 (92 kDa), active MMP-9 (83 kDa), latent MMP-2 (68 kDa), and active MMP-2 (62 kDa). The densitometric measurement *(bar graphs)* shows the activities of latent MMP-9 and latent and active MMP-2. *$P < .05$, n = 5 to 8. *MMP*, Matrix metalloproteinase.

proliferation, such as cytokines, growth factors, inflammation and hemodynamic forces, have been suggested to induce MMP expression.[13,20,25]

TIMPs control MMP activity by inhibiting the active forms of MMPs directly and by inhibiting the transition of the proenzymes into the active form.[21] Additionally, TIMP-2 has been shown to regulate MMP-2 activity in a biphasic manner dependent on the stoichiometric ratio of TIMP-2 to pro-MMP-2. High concentrations of TIMP-2 inhibit MMP-2 activity, but a low level of TIMP-2 actually activates pro-MMP-2 in the cell surface MMP-14/TIMP-2/pro-MMP-2 trimeric complex.[27]

We showed decreased levels of TIMPs after grafting, which could contribute to the higher levels of MMP activities in the graft (Figs 4 and 5). Drug-prepared grafts expressed a significantly higher level of TIMP-1 protein (36.8% ± 4.1%) compared with distended grafts. A similar result was not observed with TIMP-2. This might be explained by the effects of peroxynitrite, which is generated during distention and grafting[28] and degrades TIMP-1[29].

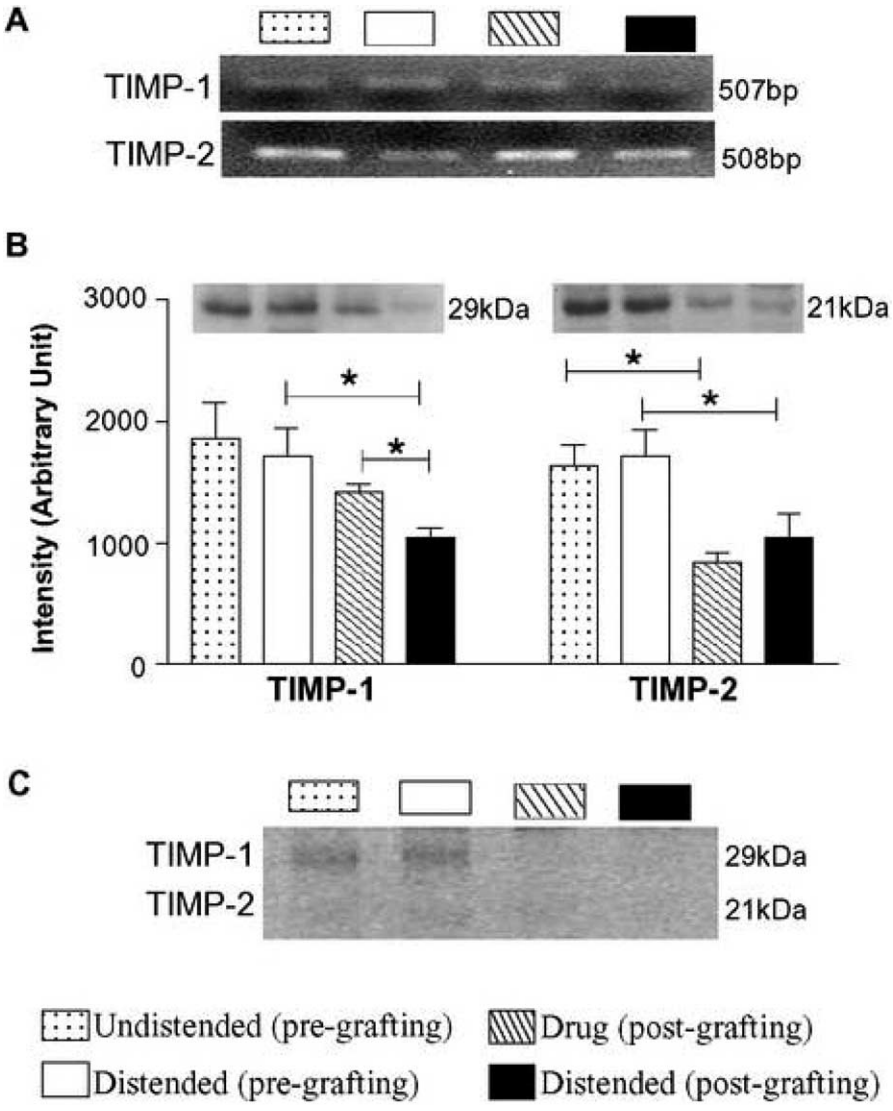

Fig 5. TIMP-1 and TIMP-2 gene transcription, protein expression, and activity in veins and grafts subjected to pressure distention and drug treatment. **A,** Representative polymerase chain reaction images show gene transcription of TIMPs. **B,** Bar graphs represent the densitometric measurement of TIMPs expression in 10 μg of sample protein. Representative immunoblots *(insets)* show the TIMP-related immunoreactivity. C, A representative reverse zymogram shows activity of TIMPs, which was visualized as dark bands against a clear background. $*P < .05$, n = 5 to 8. *TIMP*, Tissue inhibitor of matrix metalloproteinases.

The lesser decrease in TIMP-1, which has preferential inhibitory capability against MMP-9,[13,21] is consistent with less expression of the active MMP-9 in the drug-treated grafts than in distended grafts (Fig 3, *B*; Fig 5). However, the activity of latent MMP-9 from both postgrafts was not substantially different (Fig 4). The equal reduction in TIMP-2 in both grafts, which has a higher affinity for MMP-2,[13,21] correlates with the same increased level of active MMP-2 expression after grafting (Fig 3, *B*; Fig 5).

Nevertheless, alterations in TIMPs could not explain the significant difference in the activity of MMP-2 observed between distended and drug-treated veins and grafts (Fig 4), without significant changes in their protein levels (Fig 3, *B*). These discrepancies raise the possibility that additional mechanisms regulate MMP activity related to the activation of latent proteinases. MMPs are secreted as proenzymes in which the prodomain shields the catalytic site by a thiol linkage. The proenzyme transforms into the active form by the cleavage of the prodomain or the disruption of the thiol linkage, which leads to autoactivation.[30]

It has been shown in SMCs that reactive oxygen species (ROS) and peroxynitrite, the product of their reaction with nitric oxide, react with thiol groups and stimulate the transition to active forms.[31] ROS may be increased within

minutes after mechanical stretch,[31,32] and enhanced superoxide production has been demonstrated in vein-carotid artery bypass grafting.[28] Increased MMP expression and activity under cyclic mechanical stretch in a mouse model is dependent on ROS production.[32] The activation of MMPs by ROS/peroxynitrite could therefore participate in the pathogenesis of distention and grafting of the veins. However, peroxynitrite causes tyrosine nitration, which is accompanied by decreased immunosensitivity of MMPs to their antibodies,[31] and consequently decreased the corresponding band in Western blotting. Thus, the discrepancy between MMP activation after distention revealed by zymography (Fig 4) and the protein expression (Fig 3, *B*) could be explained by tyrosine nitration of the MMP due to the increased ROS level, which reduces immunosensitivity of the MMP.

Although we believe that most of the differences between the distended and pharmacologically treated grafts are contributed by the detrimental effects of distention, we cannot rule out the possible beneficial effects of the application of vasodilators before grafting. Vessels were exposed to drugs only for 30 minutes, but the effect lasted for at least 24 hours after the drugs were removed.[7] This period of time was crucial for the vessels, as the absence of circulation during surgery causes hypoxia and accumulation of cytokines that leads to extensive proliferative and inflammatory responses.

The drugs in the pharmacologic combination we used are known not only as vasodilators but also as regulators of inflammation and proliferation. Fasudil is effective in suppressing vascular remodeling and reducing intimal hyperplasia through inhibiting migration and proliferation of SMCs and enhancing cell apoptosis.[33] Nicardipine treatment in a rabbit venous graft model resulted in reduction of intimal and medial thickness.[34] A role for α-adrenergic stimulation in DNA synthesis and SMC proliferation has also been suggested.[35] It is therefore possible that the drug treatment by itself contributed to the reduction of vascular remodeling and neointima formation in the vein grafts.

CONCLUSION

We demonstrated that pressure distention and arterial hemodynamics synergistically affect ECM integrity and vascular remodeling in vein grafts. Compared with pharmacologic relaxation of veins during preparation for grafting, pressure distension accelerated vessel wall thickening, neointima development, and modification of ECM composition. This vascular remodeling is associated with the elevated activities of MMP-2 and MMP-9 induced by distention.

The increase in MMP activities was observed even after a 2-week implantation of the distended grafts and could in part be responsible for the detrimental effects of pressure distention compared with pharmacologic vasorelaxation. Appropriate pharmacologic treatment of the vein during surgical preparation with a combination of vasodilatatory agents could therefore be an alternative to damaging pressure distention, not only to overcome vasospasm but also to preserve vein grafts before implantation, attenuate graft remodeling, and eventually prolong graft patency.

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