BASIC RESEARCH STUDIES

Remodeling of experimental arteriovenous fistula with increased matrix metalloproteinase expression in rats

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Objective: Venous dilatation and wall thickening are part of the maturation of an arteriovenous fistula (AVF). However, the underlying mechanism of AVF remodeling remains unknown. We therefore studied whether matrix remodeling elicited by matrix metalloproteinases (MMPs) may contribute to AVF maturation.
Methods: A femoral AVF model in rats was established by invagination of the distal end of the left femoral artery into the femoral vein after venotomy (fistula group). In the sham group, the left femoral artery was cut, but venous invagination was not performed. Changes in the hemodynamics and the diameter of the iliac vein were studied on days 3, 14, and 28, then the iliac vein was removed and examined for changes in wall thickness and expression of MMP-2 and MMP-9, type 4 tissue inhibitor of metalloproteinases (TIMP-4), and collagen I and III by immunohistochemical staining or Western blotting.
Results: Femoral AVF resulted in a sixfold increase in blood flow in the fistula iliac vein and a gradual, but significant, increase in the thickness of the intima and media and marked up-regulation of MMP-2 and MMP-9, down-regulation of TIMP-4, as well as degradation of collagens I and III. The collagen I/III ratio was significantly higher in the 14-day fistula group (1.44 ± 0.32) than in the sham group (0.82 ± 0.15) and was even higher in the 28-day fistula group (1.76 ± 0.21).
Conclusion: The present results confirmed our hypothesis that a high blood flow rate in the fistula vein affects the expression of MMPs and TIMP-4, resulting in the remodeling or maturation of the AVF. Remodeling is associated with degradation of collagen, with an increase in the collagen I/III ratio. (J Vasc Surg 2007;45:804-11.)

Clinical Relevance: Understanding the mechanisms of fistula maturation will focus future studies of targeted interventions to improve the rate of fistula maturation and increase the number of dialysis patients with a functioning autogenous fistula.

Most patients with end-stage renal disease require hemodialysis. An arteriovenous fistula (AVF) is the preferred access for this. After its surgical creation, the fistular vein immediately faces a tremendous blood flow and the venous lumen is gradually dilated and the wall thickened, producing a fistular vein that can be routinely needled and deliver sufficient blood flow for dialysis.

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These AVF remodeling processes are termed the *maturation* of a fistula. Fistula maturation requires a compliant and responsive vasculature capable of dilating in response to the increased velocity of the blood flowing into the newly created low-resistance circuit. Successful maturation to a high-volume-flow circuit capable of sustaining hemodialysis typically occurs within the first few weeks after creation.[1]

A fistular vein faces a different environment from that faced by a vein graft created in an arterial system. The increase in the flow may be large, but the increase in pressure in the vein is less than that in a vein graft created in the arterial system. Nevertheless, the cellular and extracellular components of the venous wall of a fistula are still slightly stretched when the vein is distended by the arterial inflow.

Matrix metalloproteinases (MMPs) belong to a group of zinc-dependent proteases and degrade most extracellular matrix (ECM) proteins, such as collagen and elastin, this being a prerequisite for vascular remodeling. In the blood vessel,
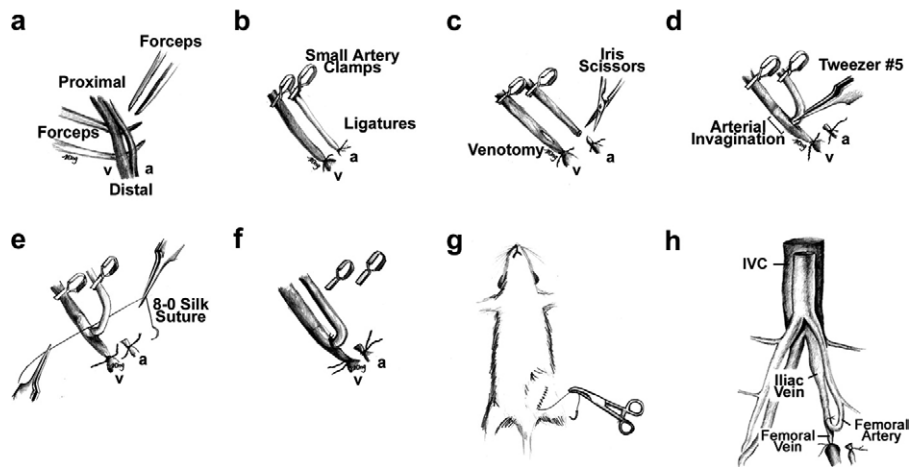
Fig 1. Schematic diagram of the arteriovenous fistula model. **(a)** After an incision was made in the left groin, the femoral artery *(a)* and vein *(v)* were carefully dissected. **(b)** The proximal ends of both vessels close to the abdomen were clamped and the distal ends ligated using 4-0 silk. **(c)** The distal end of the femoral artery was cut and a venotomy was created using iris scissors under a dissecting microscopy. **(d)** The anastomosis of the femoral artery and vein was constructed by invagination of the cut end of the artery into the vein. **(e)** Ligature using 8-0 silk was performed to prevent the artery from detaching after reflow. **(f)** The clamp on the proximal end of the artery was then released and the blood in the vein quickly turned bright red and the vein expanded. **(g)** The groin was then closed under aseptic conditions. **(h)** The vascular structures for the left arteriovenous shunt are shown.

MMPs are important for maintaining the integrity of the vessels by breaking down the ECM while new matrix is being synthesized.[2] These processes require coordinated changes in mitogenic signaling and the degradation and reorganization of the ECM by MMPs. MMP-2 and MMP-9 (gelatinase A and B, respectively) are especially important regulators of vascular architecture[3] because of their specificity for elastin and collagen.

Changes in protein level or activity of MMP-2 and MMP-9 have been suggested to be the main changes in MMPs contributing to vascular remodeling.[4,5] Flow increase, mechanical distention and stretch, vascular injury, oxidative stress, and inflammation are potent stimuli for their activation.[6,7] Mechanical stretch and vascular injury of an artery[8,9] or a venous graft in the arterial system[10] are known to activate MMPs and are associated with vascular smooth muscle cell migration and vascular remodeling.[9,11]

MMP activity can be regulated at several levels, including the interactions of MMPs with endogenous tissue inhibitors of MMPs (TIMPs).[12] TIMP-4 was recently cloned and characterized with a suggested role in the rat carotid artery damaged by balloon inflation and in mice arteries treated with nonradioactive coils.[13,14] It inhibits MMP-9 and shows a particular interaction with MMP-2, although unlike TIMP-2, it does not promote pro-MMP-2 activation.[15-17] TIMP-4 transcripts are abundant in the heart, but its role in cessation of smooth muscle cell migration and onset of collagen degradation suggest a possible cardiovascular specificity in regulating vascular remodeling.

Because the molecular mechanisms involved in the maturation of a venous graft in the AVF are unknown, we studied the vein in a fistula system in a rat model rather than a graft in an arterial system. We hypothesized that the pressure or flow increase in the AVF, or both, in the absence of any contribution from surgical injury and healing, might regulate MMP function, which may contribute to the changes in venous wall thickening and the maturation of the AVF.

MATERIAL AND METHODS

Female Wistar rats (200 to 250 grams) were housed at a constant temperature and on a light/dark cycle (light from 0700 to 1800). All animal experiments and animal care were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council of the National Academy of Sciences, 1997). Two groups of animals were used. Both groups underwent one side femoral artery ligation. The fistula group then underwent femoral arteriovenous connection in the left limb, but the sham group did not. Temporal changes in vessel thickness and biochemical changes were studied in the two groups at days 3, 14, and 28.

Surgical procedure for arteriovenous fistula. A schematic diagram of the femoral vascular shunting is shown in Fig 1. AVF was performed on rats anesthetized by intraperitoneal injection of a combination of ketamine hydrochloride (60 mg/kg) and pentobarbital sodium (15 mg/kg), 5 mg/kg of pentobarbital being given subsequently if needed. To prevent blood coagulation, heparin (500 U/kg of body weight) was administered intraperitoneally before the vessel was clamped.

Determination of blood flow or pressure changes in the vein. On the day of the experiment, the rats were anesthetized with intraperitoneal pentobarbital sodium (50

Table. Temporal changes in the body data and the width and hemodynamics of the iliac vein in the sham and fistula groups

	Day 3		Day 14		Day 28	
	Sham	*Fistula*	*Sham*	*Fistula*	*Sham*	*Fistula*
Rats (n)	8	8	11	11	8	8
Body weight (g)	175 ± 4	171 ± 2	210 ± 5	209 ± 4	240 ± 5	234 ± 7
Width (mm)*	ND	ND	1.38 ± 0.04	1.76 ± 0.08[†]	1.37 ± 0.05	2.32 ± 0.10[†]
Flow (ml/min)[†]	ND	ND	2.8 ± 0.7	18.9 ± 5.3[†]	2.4 ± 0.3	17.6 ± 3.5[†]
Pressure (mm Hg)[‡]	ND	ND	0.9 ± 0.5	2.3 ± 0.3	−0.6 ± 0.5	0.1 ± 0.5

ND, Not determined.
*The vessel width of iliac vein.
[†]$P < .05$, compared to the corresponding sham group.
[‡]Blood flow rate and intravascular pressure were measured in the iliac vein.

mg/kg). After tracheostomy and carotid arterial cannulation for spontaneous ventilation and measurement of the systemic blood pressure, respectively, a midline laparotomy was performed to expose the lower abdomen vessels. The external diameter of the left iliac vein was measured using a ruler. The iliac veins (at least 2 cm cephalad) and inferior vena cava (IVC) were then isolated from the surrounding tissue to measure the blood flow using a flowmeter (Transonic, Ithaca, NY). The pressure in the veins was measured by direct puncture with a 30# needle connected to a polyethylene tubing (PE)-10 tube and pressure transducer. The pressure and flow were found to be similar at the sites of the AV shunt in the femoral vein and 2 cm away from the anastomosis in the iliac vein (data not shown).

Sampling of vein segments. After the in vivo measurements, the iliac veins were removed after transcardiac perfusion at 4°C with 800 mL of phosphate-buffered saline (PBS), pH 7.4, as described previously.[18] Half of the vein was post-fixed in 4% paraformaldehyde in PBS for histologic examination or immunostaining. The remaining tissue was frozen at −80°C until used for Western blot analysis.

Histologic examination and immunohistochemistry. After post-fixation for 24 hours, the specimens were immersed overnight at 4°C in a paraformaldehyde solution containing 20% sucrose, then the tissue was embedded in optimal cutting temperature medium, and 5-μm-thick sections prepared as described previously.[18] Some of the tissue slices were prepared for staining with hematoxylin and eosin to determine the thickness of the intimal and medial layers using imaging software with a microruler (Spot, Diagnostic Instruments, Sterling Heights, Mich).

For immunohistochemical staining, sections were treated as described previously.[18] In brief, slides were incubated at room temperature with 5% skim milk. After washing, they were incubated overnight at 4°C with monoclonal mouse antibodies against MMP-2 (1:250, BioVision, Mountain View, Calif) or MMP-9 (1:100, BioVision). After washes, the sections were incubated at room temperature for 1 hour with peroxidase-conjugated antimouse immunoglobulin (Ig) G antibodies (BioVision). The bound antibodies were visualized by incubating with a commercial DAB kit (Vector, Burlingame, Calif), and the dark brown reaction product was examined under light microscopy at original magnification ×400 and an image analytic system (Diagnostic Instruments).

Western blot analysis. The venous tissue was homogenized and a total protein sample prepared, as described previously.[19] Two vein segments pooled as one protein sample were prepared and measured using a commercial assay kit (Bio-Rad, Hercules, Calif), then separated by sodium dodecyl (lauryl) sulfate gel electrophoresis, and electrophoretically transferred to nitrocellulose membranes (Amersham Bioscience, Piscataway, NJ). After blocking with 5% skim milk, the membranes were incubated overnight at 4°C with rabbit antibodies against MMP-2 (1:1000) or MMP-9 (1:500; both from BioVision) or TIMP-4 (1:2000, Santa Cruz), or mouse antibodies against collagen I or collagen III (both 1:1000; Sigma, St. Louis, Mo). The membranes were washed and incubated for 1 hour at room temperature with horseradish peroxidase–conjugated antirabbit IgG antibodies (Vector) or goat antimouse IgG antibodies (Lenico, St. Louis, Mo) at an appropriate dilution, washed, and bound antibody was detected using a commercial electrochemiluminescence (ECL) kit (Amersham Bioscience). The densities of the bands with appropriate molecular masses (72 kDa for MMP-2, 92 kDa for MMP-9, 23 kDa for TIMP-4, and 70 kDa for collagen I and III) were determined semiquantitatively using a densitometer.

Statistics. Numerical data are presented as the mean ± standard error of the mean. Differences between groups were analyzed using an unpaired *t* test or one-way analysis of variance, with a post-test using Duncan's multiple-range test. Differences were regarded as significant at $P < .05$.

RESULTS

Dilatation of the fistular vein. As summarized in the Table, the two groups had similar body weight and weight gain. On day 14 after creation of the AVF, the mean external diameter of the iliac vein on the fistular side was significantly increased compared to that on the ipsilateral side in sham rats at the same time point. On day 28, the dilatation of the fistular vein was more pronounced, and the
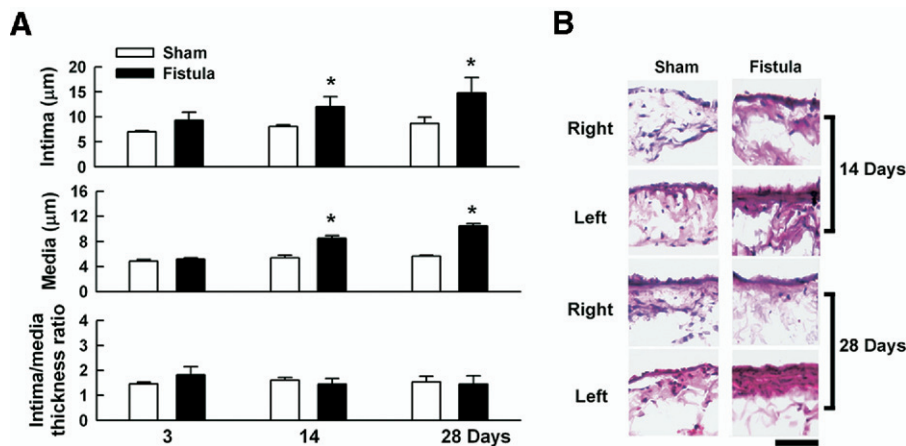
Fig 2. Changes in the thickness of the layers in the iliac vein. **A,** Temporal changes in the mean thickness of the intima and media and the intima/media thickness ratio of the left iliac vein in the sham and fistula groups. *$P < .05$ compared with the corresponding sham group. **B,** Representative vessel segments of the right and left iliac veins from sham and fistula rats at day 14 or 28. Note that the vessel walls on days 14 and 28 are thicker than at the same time point on either the contralateral right side in the same animal or on the same side in sham rats. Reduced from original magnification ×400. The *horizontal bar* indicates 20 μm.

diameter of the vein was significantly greater than in day 28 sham rats or on day 14 fistula rats. The iliac vein in some animals in the fistula group was enlarged to a size similar to that of the IVC.

The blood flow in the iliac vein in the sham group at 2 cm cephalad (same level as in the fistula rats) was <3 mL/min on days 14 and 28. However, the femoral arterio-venous connection resulted in a significant increase in flow on days 14 and 28 to 18.9 ± 5.3 and 17.6 ± 3.5 mL/min, with no significant difference between days 14 and 28. In the fistula rats, the mean arterial pressure was similar on days 14 (118 ± 6 mm Hg) and 28 (122 ± 8 mm Hg), but there was a tendency (not statistically significant) to an increase in intravascular pressure in the iliac vein on the fistular side compared with the contralateral iliac vein. In the fistula group, the intravascular pressure in the IVC was −0.4 ± 0.7 mm Hg on day 14 and −1.8 ± 0.9 mm Hg on day 28 (data not shown).

Increase in wall thickness. In the fistula group, a gradual increase in the wall thickness of the fistular iliac vein was seen (Fig 2, *A*). As shown in the upper panel, the mean thickness of the intima was 9.34 ± 1.62 μm on day 3, 12.83±1.95 μm on day 14, and r 14.79 ± 1.83 μm on day 28, all significantly different when compared with sham rats at the same time-points (day 3, 6.99 ± 0.21 μm; day 14, 8.22 ± 0.34 μm; day 28, 8.68 ± 0.75 μm). The thickness of the media (middle panel) was also significantly increased on days 14 (5.38 ± 0.44 vs 8.51 ± 0.36 μm) and 28 (5.67 ± 0.15 μm vs 10.42 ± 0.41 μm).

Hyperplasia of both the intimal and medial layers contributed to the thickening of the venous wall. The intima-to-media thickness ratio (bottom panel) was unchanged at any time-point compared with the sham group. Fig 2, *B* shows representative pictures of the wall thickness on days

14 and 28. In the left iliac vein of the fistula group (right panels), the intima and media thickened simultaneously and in a time-dependent manner compared with the sham group (left panels). The wall thickness of the right iliac vein in sham and fistula rats was similar.

Upregulation of matrix metalloproteinase 2 and 9 in fistular veins. Because MMPs are responsible for vascular remodeling, we measured the levels of MMP-2 and MMP-9 in the iliac vein grafts to see whether changes in their expression correlated with AVF maturation. Western blot analysis showed that levels of MMP-2 (Fig 3, *A*) and MMP-9 (Fig 3, *B*) increased with time after AVF compared with the sham group ($P < .05$). MMP-2 and MMP-9 expression was negligible in sham rats, with no significant difference between the two iliac veins at the same time point or between the values for the left iliac vein at the time points of 14 and 28 days (data not shown); therefore, pooled day 14 and 28 samples were used as controls.

Linear regression analysis showed significant correlations between the levels of the MMPs and the wall thickness of the iliac vein ($R = 0.67$ for MMP-2 and $R = 0.32$ for MMP-9; Fig 3, *C*). We localized the MMPs in situ in the venous wall of sham and fistula rats by immunohistochemistry. Fig 3, *D* shows representative staining for both MMP-2 and MMP-9 in representative sham and fistula rats at day 28. Little MMP-2 expression was seen in the sham rat (left upper panel), but levels were markedly increased in the fistular vein in the thickened intima and media (right upper panel). MMP-9 expression was seen in the intimal and subintimal tissues in the sham rat (left lower panel), but was greatly increased in the fistular rat (right lower panel).

Temporal changes in the amounts of tissue inhibitor of metalloproteinase 4 and collagens. We further analyzed TIMP-4 expression because TIMP-4 selectively
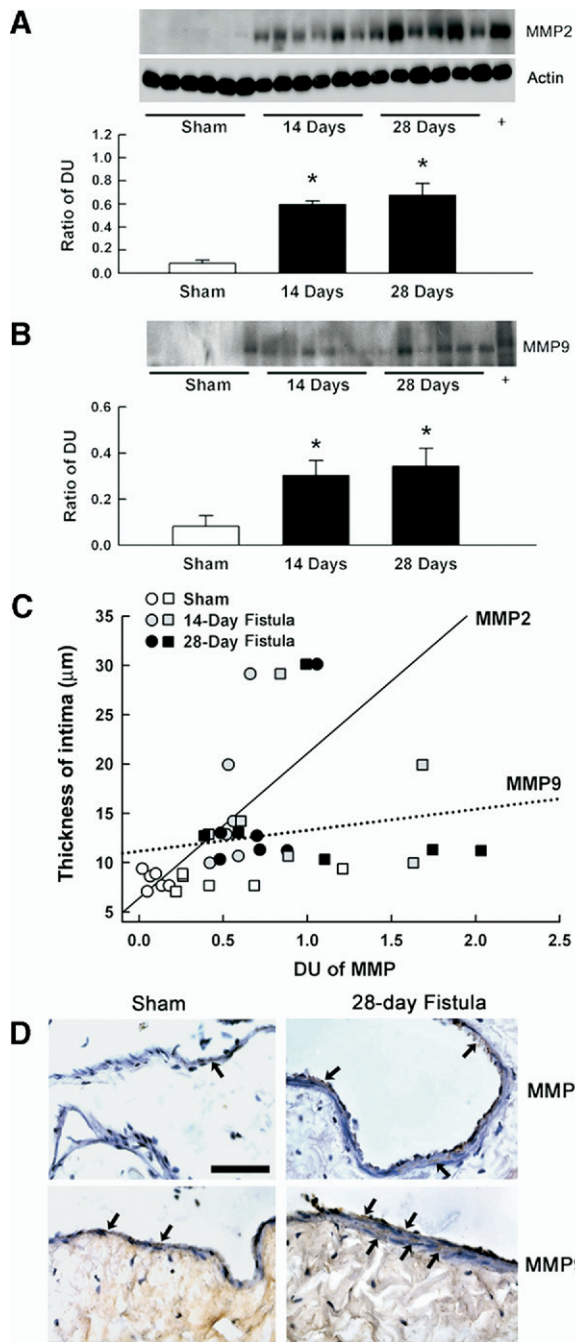
Fig 3. Upregulation of matrix metalloproteinase *(MMP)* 2 and 9. **A,** Changes in the expressions of MMP2 and **B,** MMP9 in six rats examined by Western blotting. The bar graphs show that the change to MMP2 or MMP9/actin density unit *(DU)* ratio. *$P < .05$ compared with the sham group (days 14 and 28 pooled). **C,** Linear regression shows that the amounts of MMP2 and 9 are positively correlated with the thickness of the intima. **D,** Representative micrographs show the location of MMP2 and MMP9 in situ by immunohistochemical staining at 28 days in sham rat and a fistula rat. Reduced from original magnification ×400. The *black arrows* and horizontal bar indicate positive signals and 100 μm.
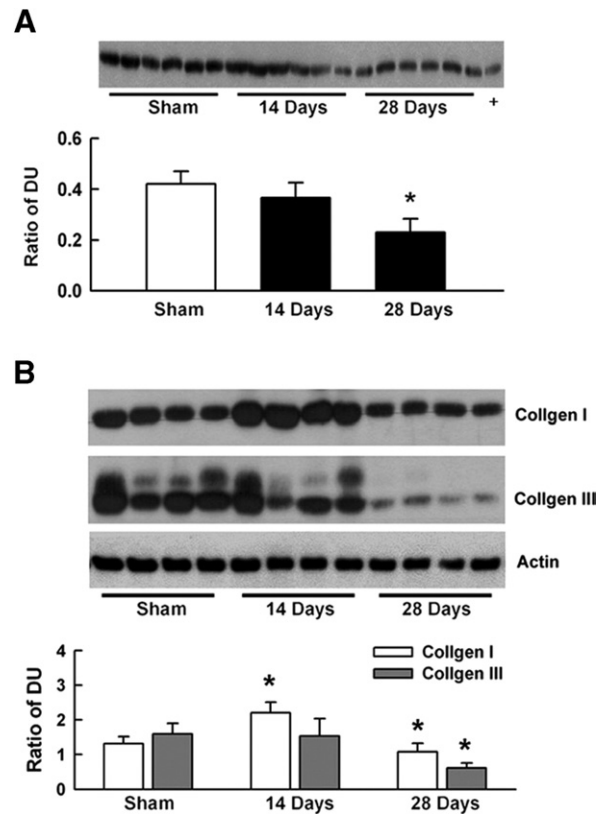
Fig 4. A, Decreased levels of tissue inhibitor of matrix metalloproteinase-4 and **B,** collagen I/III. Representative blots from six (TIMP-4) and four (collagen I and III) rats. The bar graphs show that the change to TIMP-4/actin density unit *(DU)* ratio and collagen I or III/actin DU ratio. *$P < .05$ compared with the sham group (pooled day 14 and 28 samples).

inhibits the activity of both MMP-2 and MMP-9, probably via its *N*-terminal region.[3] As shown in Fig 4, *A,* compared with the sham group (day 14 and 28 samples pooled), TIMP-4 levels in the fistula group were gradually down-regulated, a significant difference being seen at day 28.

The above results clearly showed that MMP expression was increased and TIMP-4 expression decreased, suggesting that these changes may contribute to the thickening of the fistular vein subjected to a high blood flow. Since MMPs are the principal matrix enzymes cleaving fibrillar or nonfibrillar structures, we investigated whether the observed the changes in collagens were associated with increased MMP activity. As shown in Fig 4, *B,* the amount of collagen I in the fistula group was significantly higher on day 14, but significantly lower on day 28 than in the sham group (pooled samples from days 14 and 28). Collagen III expression in the fistula group was unchanged on day 14 but showed a significant decrease on day 28. Of interest, the collagen I/III ratio was 0.82 ± 0.15 in the sham group and was significantly increased (1.44 ± 0.32) in the fistula group at day 14, and further increased (1.76 ± 0.21) in the fistula group at day 28.

DISCUSSION

The present study showed the effect of arteriovenous hemodynamics on fistular vein remodeling and enhanced MMP in a rat AVF model. The rat model presented here has several novel features. First, it showed native fistula hemodynamics, with a high flow and low pressure resembling those seen in a clinically constructed AVF for hemodialysis. There was both outward geometric remodeling (dilatation) and inward remodeling (shrinkage) of the vein that correlated well with the clinical maturation of an AVF.

Second, we showed that a great increase in flow and minor pressure distension of the vein increased neointima formation in the fistular vein ≤28 days, the period often required for the AVF maturation in human hemodialysis.[20]

Third, the vascular remodeling was associated with collagen degradation and an increase in the collagen I/III ratio. This rapid (<28 days) upregulation of MMPs, associated with a decrease in TIMP-4, might account for the modification of the ECM seen in the venous segment of the AVF.

Several mechanical factors can influence vessel remodeling. The surgical stresses of dissection, distension, and anastomosis increase MMP activity and stimulate neointimal formation, which predisposes the vein graft to extensive remodeling.[21-24] Wound healing processes, inflammation, and possible later infection also have a profound effect on the vessels under the wound.[25] Our model showed direct evidence of the effect of simple hemodynamic forces on levels of MMPs and TIMP-4, collagen deposition, and remodeling of the fistular vein in the absence of any effect caused by surgical wounding. However, our present animal model of AVF involves the iliac vein, which belongs to the deep venous system rather than the superficial system used in the clinic for uremic patients, and these two systems might have different phenotype presentations.

High blood flow affects matrix metalloproteinases in arteriovenous fistula maturation. Significant venous dilatation and increased flow in the fistula were seen in the present study. The increase in fistula blood flow was seen immediately after surgical anastomosis (data not shown) and was followed by a gradual increase to maximal blood flow on day 14. These findings are consistent with clinical findings of the human fistula, which continues to dilate for many months.[20,26] Wong et al[26] showed that the average luminal diameter is increased by 56% on day 1 after creation and increases to 123% of control at 12 weeks. Similarly, Corpataux et al[20] found that the vein lumen of an AVF increased by 86% at 1 week and 179% at 12 weeks. Our AVFs also exhibited continuous dilatation, but the extent was less than in the human studies, only increasing by about 28% and 68% of sham levels on day 14 and 28. This difference can be explained by the fact that the human superficial AVF dilates more easily than the deep AVF created in our rat model.

Venous histologic changes, characterized by varying degrees of neointimal hyperplasia and thickening of the venous wall, were seen in the AVF group at days 14 and 28.

In a study of aortocaval fistula, Nath et al[27] showed venous thickening, evidenced by smooth muscle cell proliferation and ECM deposition. In the clinic, no change in the intima-media thickness of the venous wall was detected by ultrasound imaging after fistular creation, but a marked increase in the calculated venous wall cross-sectional area was observed by Corpataux et al.[20] These authors suggested that the venous remodeling of the forearm takes the form of an eccentric hypertrophy, resulting in an increase in vascular mass.

In the present study, high blood flow through the fistular vein was correlated with upregulation of MMP-2 and MMP-9 and an increase in wall thickness and luminal diameter. Blood flow in the fistular iliac vein was increased sixfold in the day 14 fistula, and this was maintained to day 28 (Table), whereas levels of MMP-2 increased tremendously and those of MMP-9 significantly at the same time points (Fig 3, *A* and *B*). Hemodynamic changes, mechanical injury, or inflammation may trigger vascular remodeling by regulation of the levels and activation of MMPs.[1,9]

The study of Pasterkamp et al[22] showing increases in MMP-2 and MMP-9 messenger RNA (mRNA) levels and enzymatic activity have been performed on the fistular artery rather than the vein. In an AVF model in which a side-to-side shunt was created between the carotid artery and the jugular vein for 7 days in rabbits, Sho et al[28] showed that the flow increase in the artery correlated with proliferation of the neointima and media and a marked and prolonged increase in MMP-2 mRNA levels, whereas MMP-9 mRNA levels were high on day 2, then decreased.

Increased MMP-2 and MMP-9 protein levels have also been reported in a pig model of vein graft exposure to the arterial circulation.[29] Southgate et al[10] used saphenous vein into carotid artery interposition grafting in a pig model and showed increased production of MMP-2 and MMP-9 proteins during the period of neointima formation and smooth muscle cell proliferation in the experimental vein grafts. Sharony et al[30] found that both inflammatory mediators and extracellular signal-regulated kinases affected MMP-2 and MMP-9 protein expression in the arterialized vein graft in a canine model. Similar findings of neointimal hyperplasia were obtained in a study of a human aortocoronary vein graft.[31]

Taken together, these studies suggest that a high flow and shear stress can cause endothelial cells and vascular smooth muscle cells in an artery or a vein graft to express MMP-2 and MMP-9, resulting in neointimal hyperplasia and vessel enlargement. Our study showed that exposure of a fistular vein to a high blood flow without the substantial injury of grafting or surgical dissection also resulted in increased levels of MMP-2 and MMP-9, similar to the previous findings on arteries or vein grafts. We therefore suggest that a high blood flow causes fistular venous dilatation and wall thickening, probably by the mechanism associated with MMP upregulation and TIMP downregulation.

Effect of pressure changes on MMP expression. In a human AVF, the venous pressure increases to about 30%

of that seen in the immediate upstream artery, and it typically attains 40% to 60% of the maximal blood flow within days and maximal blood flow is reached within weeks.[20,26] We did find a marked increase in blood flow in the rat model fistular vein ≤28 days, but not an increase in the intravascular pressure in the segment of vein 2 cm away from the anastomosis (Table).

Elevation of the transmural and pulsatile pressure has been proposed as a factor up-regulating the matrix-degrading activity of MMPs.[10,24,32] However, our study in an animal model of native fistula circulation showed insignificant changes in the intravascular pressure in the venous segment of the AVF, and we were unable to find any correlation between transmural pressure and MMP expression (data not shown). This low-pressure circuit in the rat iliac vein is consistent with findings in the human wrist AVF that the venous pressure increase is limited and appears not to be a contributing factor in the process of venous hypertrophy.[20]

Because the fistular vein in the present study was a low-pressure circuit, the metabolism of ECM proteins will be different from that seen in studies on arterial remodeling in human subjects with hypertension. Several studies showed increased TIMP-1 activity and lower degradation of collagen I in hypertensive patients with left ventricular hypertrophy or in patients with essential hypertension and concluded that systemic extracellular degradation of collagen I is depressed.[23,33,34] The present study did not measure the activity of any of the proteins or measure collagen production or degradation.

In our AVF model, levels of MMPs increased, levels of TIMP-4 decreased gradually (Fig 4, *A*), and collagens I and III were degraded after 28 days, suggesting that a high blood flow and a low pressure produces less tensile stress and that collagens were degraded in the venous segment for the purpose of vascular dilatation. However, increased MMP expression was seen at 14-day remodeling associated with only a minor change in TIMP-4 and probably no collagen degradation was seen at the same time point, suggesting that factors other than TIMP may contribute to the changed MMP levels.

Effect of collagen degradation. An altered collagen I/III ratio, with an increase in collagen type III, has been claimed to reduce the mechanical strength of connective tissues.[25] Rosch et al[35] also found that the type I procollagen mRNA/type III procollagen mRNA ratio is decreased in patients with primary hernia. In the sham group in the present study, we showed that the collagen I/III ratio was <1.0 in a normal thin-walled vein in the low-pressure circulation, whereas the collagen content changed and the collagen I/III ratio increased in the fistular vein facing a high flow on days 14 and 28 (Fig 4, *B*).

Although the absolute amounts of collagen I and III in the fistular vein decreased at day 28, levels of collagen III decreased more quickly than those of collagen I (second panel in Fig 4, *B*), and the collagen I/III ratio therefore remained elevated. This change in the phenotype of collagens in the ECM, together with the dilatation of the venous lumen and the thickening of venous wall, might associate with the AVF maturation.

CONCLUSION

The results of the present study confirmed our hypothesis that up-regulation of MMP-2 and MMP-9 and down-regulation of TIMP-4 are involved in the remodeling or maturation of an AVF, affecting both the outward dilatation and vessel wall thickening. This also suggests that MMP is one of the biochemical mechanisms involved in the flow-induced fistular dilatation and cell proliferation in veins. The increased collagen I/III ratio in the ECM and the thickening of the venous wall strengthen the vessel for hemodialysis access.

AUTHOR CONTRIBUTIONS

Conception and design: CY, MC, YS, CF
Analysis and interpretation: CY, MC, YS, CF
Data collection: CY, MC
Writing the article: CY, MC
Critical revision of the article: CY, MC
Final approval of the article: CY, MC, YS, CF
Statistical analysis: CY, MC
Obtained funding: CY, MC, YS
Overall responsibility: CY, MC

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