Hypertension-induced venous valve remodeling

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Introduction: **In human beings, chronic venous insufficiency is linked to venous hypertension. This in turn is associated with venous valve incompetence. This study was designed to test the hypothesis that venous hypertension serves to initiate a process that results in the venous valve and venous wall damage observed in venous insufficiency.**

Material and methods: **Acute venous hypertension was produced by creation of an arteriovenous (AV) fistula between the femoral artery and vein in Wistar rats. At specified intervals pressure in the veins was recorded. The proximal valve containing saphenous vein was exposed, and reflux was measured from reverse blood flow through the first proximal valve. The vein was excised, valve parameters were measured, a portion was taken for morphologic investigation, and the remaining specimen was frozen in liquid nitrogen for investigation of leukocyte infiltration, expression of adhesion molecules, matrix metalloproteinase (MMP) levels, and apoptotic markers. Contralateral nonpressurized saphenous veins were used as control specimens.**

Results: **The saphenous and femoral veins were immediately distended by pulsatile blood flow from the arterial system. Pressure was significantly increased from 11 ± 2 mm Hg to 94 ± 9 mm Hg. At 2 days no reflux was detected in the saphenous veins. At 1 week, one of four rats exhibited reflux; at 2 weeks, two of four rats had reflux; and at 3 weeks, three of four rats showed reflux. Contralateral saphenous veins were uniformly competent. Compared with control specimens, the veins were dilated; leaflet length and leaflet width were significantly reduced. Granulocytes, monocytes, and macrophages were identified in all regions of the vein wall, and the number was increased by the presence of the AV fistula. The number of T-lymphocytes was increased, and B-lymphocytes were present. P-selectin was upregulated in the saphenous vein walls, as was intercellular adhesion molecules. MMP-2 and MMP-9 expression in the veins was not enhanced. In the nuclear factor κβ family, Iκβ was not increased in any hypertensive veins. The number of apoptotic cells in the vein wall was increased in the presence of the AV fistula.**

Conclusion: **This study indicates that acute venous hypertension is accompanied by significant venous distention and some valve damage as early as 3 weeks after fistula creation. There is development of inflammatory markers, with leukocyte infiltration and increased adhesion molecule expression. We could not detect significant enhancement of MMP levels or nuclear transcription factors. It is uncertain whether this lack of evidence may be partially due to enhanced apoptosis in venous valves and vein walls. A detailed definition of the inflammatory reaction produced by venous hypertension should be the subject of further study. (J Vasc Surg 2004;39:1329-34.)**

Clinical Relevance: **Saphenous vein valves when observed at the time of vein stripping show deformities of shortening, scarring, and tearing. The current model of induced venous hypertension demonstrates early venous valve changes similar to those observed in human beings and links them to a venous hypertension–induced inflammatory reaction. Thus the model could be useful in pharmacologic testing to prevent or treat venous insufficiency and for defining the fundamental mechanisms that cause varicose veins.**

Valves are key regulators of blood flow and pressure in veins. Profound alterations in venous valves have been seen in surgical specimens and during angioscopy of the great saphenous vein during vein surgery.[1-3] Such alterations include absence of the subterminal valve,[3] thinning, elongation, stretching, splitting, or tearing, and thickening, retraction, and adhesion of valve leaflets.[2] At the time of surgery long valveless segments of the saphenous vein suggest complete remodeling of the valves that are usually present in normal saphenous veins.[3] Some investigators have noted a decreased number of valves in the saphenous vein in patients with varicose veins.[4,5]

Our recent intraoperative angioscopic observations and gross morphologic studies in a selected small number of postsurgical specimens have confirmed these changes in venous valves.[6] We found virtual disappearance of leaflets in three of 10 specimens. Evidence of degenerated valve stumps was present. Histologic investigation confirmed proliferation of smooth muscle cells in three specimens, and all tissue specimens showed clearly demarcated monocytes and macrophages in the leaflet and the venous wall.

In addition to changes in valve anatomy, evidence of inflammation was also observed. The number of monocytes in the valve sinus and proximal vein wall was greater than in the distal surface of the valves and the venous wall distal to (upstream of) the valves. This localization of inflammatory

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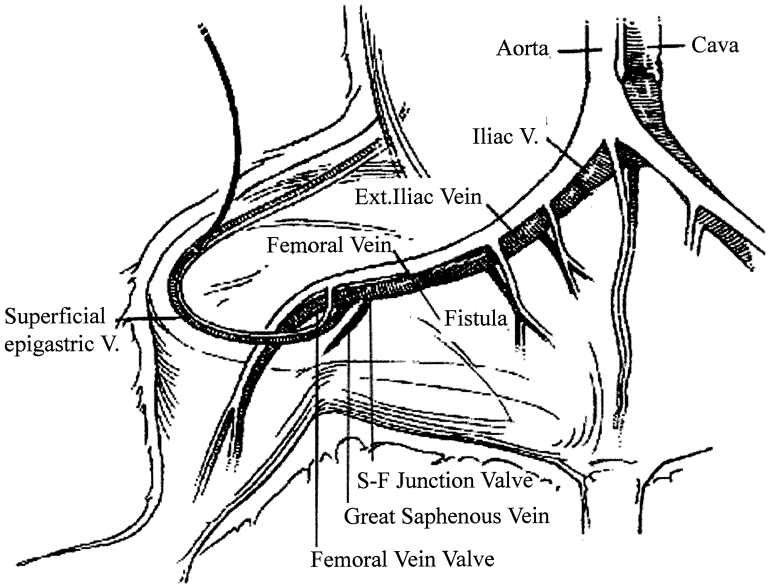

Fig 1. Diagram of femoral vascular anatomy in vicinity of arteriovenous fistula.

cells may be the consequence of venous hypertension on the valve leaflets and vein wall.

Accordingly, we investigated in an experimental model the distribution of inflammatory markers in the vicinity of a venous valve subjected to hypertension. We adopted a model by van Bemmelen et al[7] in the rat by creating an arteriovenous (AV) fistula, and observed valvular incompetence as well as destruction of commissures and the valve sinus. In a related rat model of venous hypertension, Hahn et al[8] demonstrated an increase in leukocytes in areas of venous hypertension. Thus the link between venous hypertension, leukocyte infiltration, and tissue damage requires further investigation.

This study was designed to test the hypothesis that acute venous hypertension may produce morphologic changes and inflammatory reactions in venous walls and valves, including endothelial cell activation and attachment of leukocytes.

MATERIAL AND METHODS

Animals and surgery. We produced venous hypertension by creating a fistula between the femoral artery and vein in Wistar rats (Fig 1) under general anesthesia (pentobarbital sodium, 50 mg/kg intramuscularly). Monofilament sutures (9-0 to 12-0; Ethicon) were used to create the fistula (0.5-mm inner diameter) between the femoral artery and vein proximal to the saphenofemoral junction. The size of the fistula was confirmed at tissue collection.

Blood pressure was measured in the vein distal to the fistula. To avert cardiac failure after placement of the AV fistula, tributaries to the femoral vein, including the superficial epigastric vein near the anastomosis, were ligated. To prevent blood coagulation, heparin was administered (1000 U/kg body weight). The skin incision was closed, and the rats were given intensive postsurgical care. The rats were housed in a light-cycle controlled flow hood, and maintained with standard pellet diet and water ad libitum. The rats with the AV fistula were maintained for up to 3 weeks. The animal protocol was reviewed and approved by the Animal Subjects Committee of the University of California San Diego.

Valve reflux. At the end of selected observation periods the animals were given general anesthesia (pentobarbital sodium, 50 mg/kg intramuscularly). The contralateral nonpressurized femoral vein and its enclosed valve was collected as a control specimen and was subjected to the same analyses as the pressurized vein and valve. Each femoral vein was sectioned distal to its proximal valve. Reflux through the valve was determined in situ for each rat with timed collection of the backflow in the presence of the native femoral pressure.

Valve anatomy. The femoral vein was mobilized and opened longitudinally to permit examination of the valve leaflet structure and shape. Morphologic measurements obtained included length of vein half circle, valve height, leaflet height, valve sinus length, edge length, and leaflet width (Fig 2). The excised tissue was examined after hematoxylin-eosin staining.

Leukocyte infiltration and membrane adhesion molecule expression. Valve specimens not subjected to hematoxylin-eosin staining were freshly frozen in liquid nitrogen and stored at −70°C until examination. Longitudinal sections (6-μm thick) were cut across the valve leaflets with a cryostat at −23°C, air dried for 30 minutes, and fixed in purified acetone for 9 minutes. Leukocyte infiltration, expression of endothelial adhesion molecules, metallopro-

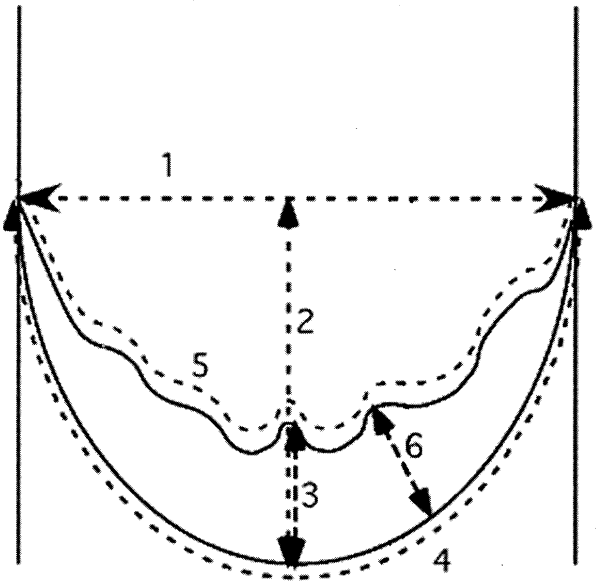

Fig 2. Diagram with designation of geometric parameters on valve leaflets examined. *1,* Half-circle; *2,* valve height; *3,* leaflet height; *4,* sinus length; *5,* edge length; *6,* leaflet width (maximum leaflet height).

teinase (MMP), and apoptotic markers were labeled on the frozen sections with antibodies against macrophages (monoclonal Ab HIS36), T-lymphocytes (OX-52), B-lymphocytes (OX-33), granulocytes (HIS 48), intercellular adhesion molecule-1 (ICAM-1 [1A29]), the nuclear transcription factor IκBα (Ab-2; Oncogene Research Product), NFκB proteins p50 (NFκB1, Ab-1; Oncogene Research Product), p65 (RelA, Ab-1; Oncogene Research Product), MMP-2 (42-5D11; Oncogene) and MMP-9 (56-2A4, Oncogene), and terminal deoxynucleotidyl transferase–mediated (dUTP) nick end labeling; Trevigen kit TACS 2). Secondary biotinylated antibodies were applied with peroxidase-conjugated avidin to yield a dark brown reaction product. The sections were examined with light microscopy at several magnifications up to ×100 objective (numerical aperture, 1.4). In addition to direct microscopic observations, the expression of molecular markers was determined with direct light absorption measurements after image digitization on a laboratory computer (Power Mac; Macintosh Computer) and expressed in terms of grade 0 (unstained), grade 1 (slightly stained), grade 2 (moderately stained), and grade 3 (strongly stained).

Statistical analysis. All values are expressed as mean ± SD. Differences between the pressurized valves and control valves were analyzed with repeated measures analysis of variance combined with a Scheffe-type multiple comparison test. $P < .05$ was considered significant. The analysis was performed with Statview software (version 4.5 for Macintosh; Abacus Concepts).

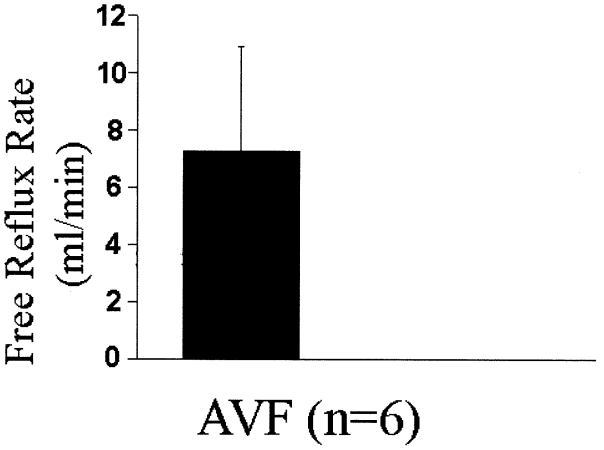

Fig 3. Reflux rates (mean ± SD) in pressurized valves at 3 weeks after arteriovenous fistula placement.

RESULTS

On completion of the AV fistula, the saphenous vein and femoral vein were immediately distended with pulsatile blood flow from the arterial system. Pressure as measured in the vein just distal to the fistula at creation was significantly increased, from 11 ± 2 mm Hg to 94 ± 9 mm Hg.

Reflux. At 2 days no reflux was detected in the explanted veins (n = 3). At 1 week one of four veins exhibited reflux; at 2 weeks two of four veins tested showed reflux; and at 3 weeks three of four veins showed reflux (Fig 3). Contralateral control saphenous veins were uniformly normal in anatomy, and were competent.

Vein anatomy. Observations were made on explanted specimens at 3 weeks after creation of the AV fistulas. Compared with control veins, the pressurized veins were dilated. The length of the half-circle circumference was increased approximately 25%. Leaflet height was significantly reduced, as was leaflet width (Fig 4, *A* to *E*).

Leukocyte infiltration. Granulocytes were identified in all regions of the vein wall, and the number was increased by the presence of the AV fistula (Fig 5, *A*). The number of monocytes and macrophages on pressurized valves was greater than on control valves (Fig 5, *B*). The number of T-lymphocytes was increased, and B-lymphocytes were present. T-lymphocytes were markedly increased in the valves subjected to high pressure (Fig 5, *C*).

Inflammatory markers. P-selectin was upregulated in the saphenous vein walls (Fig 6, *A*), as was ICAM-1 (Fig 6, *B*). MMP-2 and MMP-9 expression in the veins was not enhanced.

In the NFκB family, p50 and p65 were not increased in any hypertensive veins. The number of apoptotic cells in the vein wall was increased in the AV fistula group.

DISCUSSION

Venous hypertension and the inflammatory reaction associated with valve remodeling are inextricably linked.

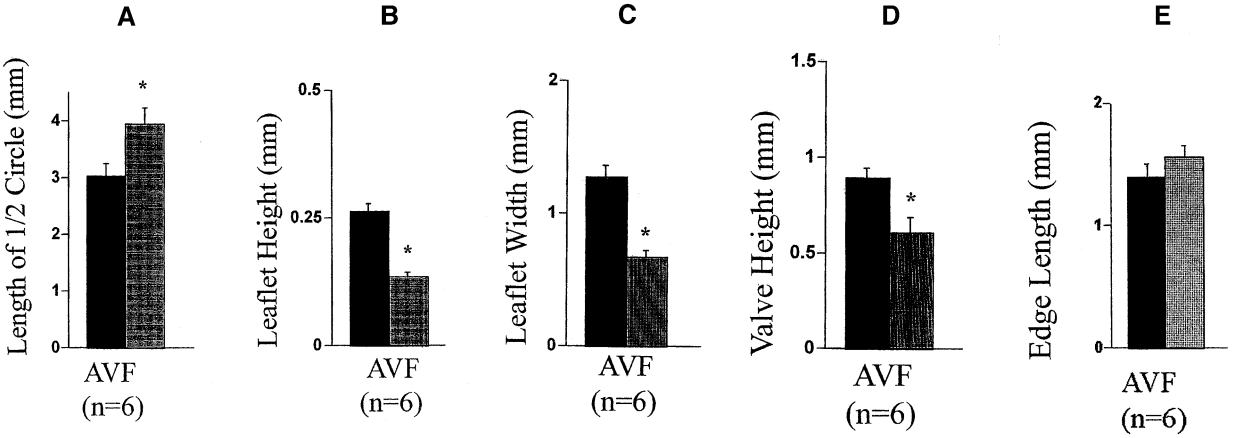

Fig 4. A, Valve half-circle length. **B,** Leaflet height. **C,** Leaflet width. **D,** Valve height. **E,** Valve edge length of control and pressurized valves. *Solid bars,* Control valves. For definition of geometric parameters see Fig 2. $P < .05$ vs control was considered significant.

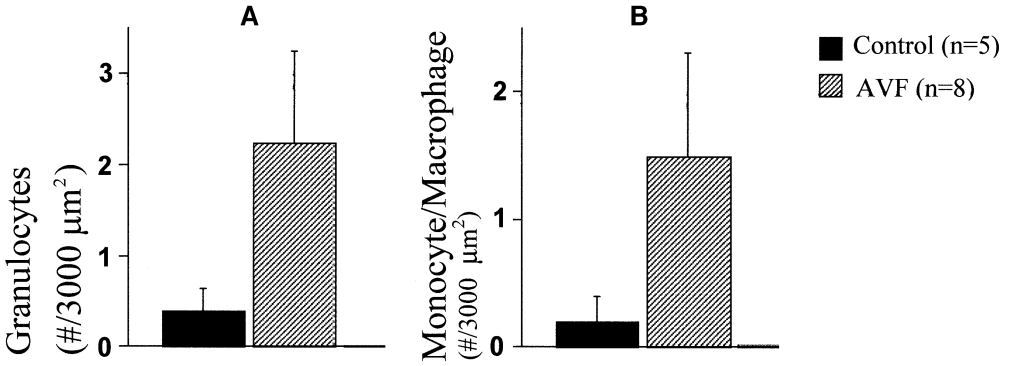

Fig 5. Number of granulocytes **(A)**, monocytes and macrophages **(B)**, and T-lymphocytes **(C)** on control and pressurized valves. *Solid bars,* Control valves. $P < .05$ vs control was considered significant.

Control venous valves, unaffected by the pressure from the AV fistula, remained normal over the observation period of this study. In contrast, marked changes in valve anatomy occur in veins with elevated pressure. Under observation at low magnification these changes are similar to those noted in incompetent saphenous veins.[2] The length of the half-circle vein arc is dilated. Leaflet height is significantly reduced. The edge length of the valve is increased. These changes were also described by van Bemmelen et al,[7] who stated that "separation and leakage of the cusps were seen along the entire free border." In the present experimental preparation no differences in valve sinus length were observed between the control valves and valves exposed to hypertension.

Granulocytes were seen in all regions of the venous wall affected by the fistula. The number of granulocytes infiltrating the venous wall was enhanced, as was the number of T-lymphocytes, B-lymphocytes, and monocytes and macrophages.

In surgical specimens removed at saphenous vein surgery, only monocytes and macrophages were seen infiltrating the valves and the vein wall.[6] Thus the cellular infiltrate in the chronic state encountered in human beings differs from that in the acute experimental preparation. In human beings monocytes can be attracted by chemotactic factors that do not necessarily attract neutrophils, including oxidized products (eg, low-density lipoprotein) and tissue breakdown products. The issue needs to be examined further.

Several mechanisms of venous pressure elevation exist in veins of the lower extremities in human beings. The hydrostatic pressure generated by the weight of the blood column from the right atrium transmitted through valveless iliocaval veins and the venous pressure generated by abdominal compression press directly on the terminal (most proximal) saphenous valve. This valve, in turn, as it becomes incompetent, enables reflux and raises the pressure more distally. The combined action of these mechanisms

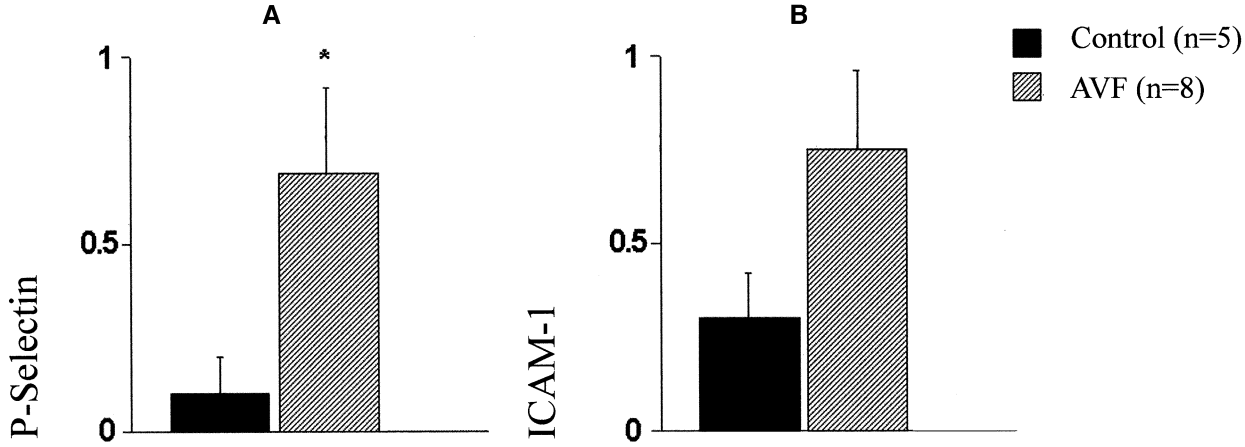

Fig 6. P-selectin **(A)** and ICAM-1 **(B)** levels in pressurized veins and valves as compared with control specimens. *Solid bars*, Control valves. $P < .05$ vs control was considered significant.

can lead to dramatic elevation of venous pressure. While measuring pressure in calf muscle and calf veins in human beings, Arnoldi[9] found that calf muscle pressure during contraction varies from 80 to 90 mm Hg. During relaxation these pressures dropped to 30 to 40 mmHg. He concluded that "the venous circulation of the lower extremities is not a low pressure system during walking."[9] Thus the blood pressure exerted on the most proximal femoral valve in the current experimental preparation is comparable to venous pressure exerted against calf perforating veins in human beings during walking.

The expression of P-selectin on the endothelial cells in the fistula-affected saphenous vein was elevated. However, we could not detect significant enhancement of MMP-2 or MMP-9 expression in the veins with fistulas at 3 weeks. This observation is at variance with changes in MMP-1 and MMP-2 expression in varicose saphenous veins in human beings,[10] and requires further investigation at other time points. While the elevated blood pressure will passively stretch the structures that make up a venous valve, the more chronic restructuring requires destruction and rearrangement of extracellular matrix components such as elastin, collagen, laminin, and fibronectin by release of oxygen free radicals and proteolytic MMPs.

Even though the venous valve is instantly stretched by pressure elevation, reflow does not occur until later. Once reflow starts, we expect a significant change in the flow pattern in the valve, with a shift of the fluid shear stress on the endothelial cells. Such shift in fluid shear stress can have a strong effect on the gene expression pattern in endothelial cells.[11] Thus it is uncertain whether the initial distention due to pressure or the following shift in fluid shear stress during reflow, or a combination of these factors, leads to endothelial cell activation, attachment and infiltration of the leukocytes into the venous wall and valve leaflets, and eventual apoptosis.

At angioscopic study of the saphenous veins at surgery, the greater saphenous vein is often valveless from the saphenofemoral junction to the upper calf, where the first normal valve appears. Clinical observations confirm this whenever stripping instruments pass unimpeded from groin to knee. Clinically, such veins are subjected to many years of chronic venous hypertension, which allows time for extensive valve remodeling to the point of total destruction.

CONCLUSION

This study indicates that acute venous hypertension is accompanied by significant venous distention and some valve damage as early as 3 weeks after fistula creation. Inflammatory markers appear, along with leukocyte infiltration and increased adhesion molecule expression. We could not detect significant enhancement of MMP levels or nuclear transcription factors NFκB. It is uncertain whether this lack of evidence may be partially due to the enhanced apoptosis in venous valves and vein wall. Further details of the inflammatory cascade during venous hypertension need to be studied to improve current interventions.

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