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An animal model of venous hypertension: The role of inflammation in venous valve failure

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Background: **Clinical observation suggests that chronic venous insufficiency is related to failure of venous valves. Duplex ultrasound studies of lower extremity superficial veins regularly show valve failure and venous reflux. Gross morphologic observation of venous valves in surgical specimens shows tearing, splitting, scarring, and disappearance of valves.**
Hypothesis: **Venous valve damage is acquired, linked with venous hypertension, and affected by inflammation.**
Objective: **The objective of this study was to investigate the inflammatory process in valve remodeling associated with acute and chronic venous hypertension.**
Methods: **A femoral arteriovenous fistula was created in study animals (Wistar rats, n = 60), and animals without an arteriovenous fistula were studied as controls (n = 5). At 1, 7, 21, and 42 days animals with the femoral arteriovenous fistula were anesthetized, and systemic pressure, the pressure in the femoral vein distal to fistula, and the pressure of the femoral vein in the contralateral hind limb were measured. Timed collection of blood backflow after division of the femoral vein distal to the fistula and in the alive, anesthetized animal was collected, measured, and calculated per unit time to be used as an indicator of valve insufficiency. The femoral vein distal to the fistula was harvested; valvular structures were examined and measured. Specimens were processed, and longitudinal sections were made and challenged with immunostaining antibodies against matrix metalloprotease (MMP)-2 and MMP-9. Sections were examined, and expression of molecular markers was determined by light absorption measurements after image digitization.**
Results: **One week after the procedure, all animals exhibited some degree of hind limb edema ipsilateral to the arteriovenous fistula. Pressure in the femoral vein distal to the fistula was markedly increased on average to 96 ± 9 mm Hg. Reflux was increased in a time-dependent manner, with the 21-day and 42-day groups showing the highest values. Valves just distal to the fistula showed an increased diameter of the valvular annulus and a shortening of the annular height. Venous wall findings included fibrosis and fusion of the media and adventitia and scarring and disappearance of valves principally in the 21- and 42-day specimens. Immunolabeling for MMP-2 showed an increased level in the 21- and 42-day groups. MMP-9 showed an increased level at 1 day, followed by a more marked level in the 21- and 42-day groups.**
Conclusions: **In this animal model of venous hypertension the findings of limb edema, increasing valvular reflux, and morphologic changes of increased annulus diameter and valve height are seen. Histologic changes included massive fibrosis of media and fusion with adventitia. Inflammatory markers MMP-2 and MMP-9 are strongly represented, and valve disappearance occurs after these markers are present. The gross morphologic changes seen are quite similar to those observed in human surgical specimens removed in treatment of venous insufficiency. (J Vasc Surg 2005;41:303-11.)**

Clinical relevance: **When observed angioscopically at the time of vein stripping, saphenous vein valves show severe deformities including shortening, scarring, and tearing. The current model of induced venous hypertension demonstrates early venous valve changes that replicate those observed in humans. This observation provides a link from venous hypertension to an induced inflammatory reaction that stimulates the valve damage. Thus the model could be useful for defining the fundamental mechanisms that cause venous valve failure and varicose veins and in pharmacologic testing to prevent or treat venous insufficiency.**

Clinical observation suggests that chronic venous insufficiency is related to failure of superficial and deep venous valves. Although there is a large body of literature addressing deep venous incompetence, it is the gross morphologic changes in superficial valves that have attracted careful study. Duplex ultrasound studies of lower extremity superficial veins regularly show valve failure and venous reflux.[1] Careful angioscopic preoperative study of 116 patients by Yamaki et al[2] showed 28% with elongated and atrophic valve cusps, 48% with expanded and depressed valve commissures, and even 33% with absent valves.[2]

In a morphologic study of 65 superficial venous valves Corcos et al[3] found changes of both thickening and thin-

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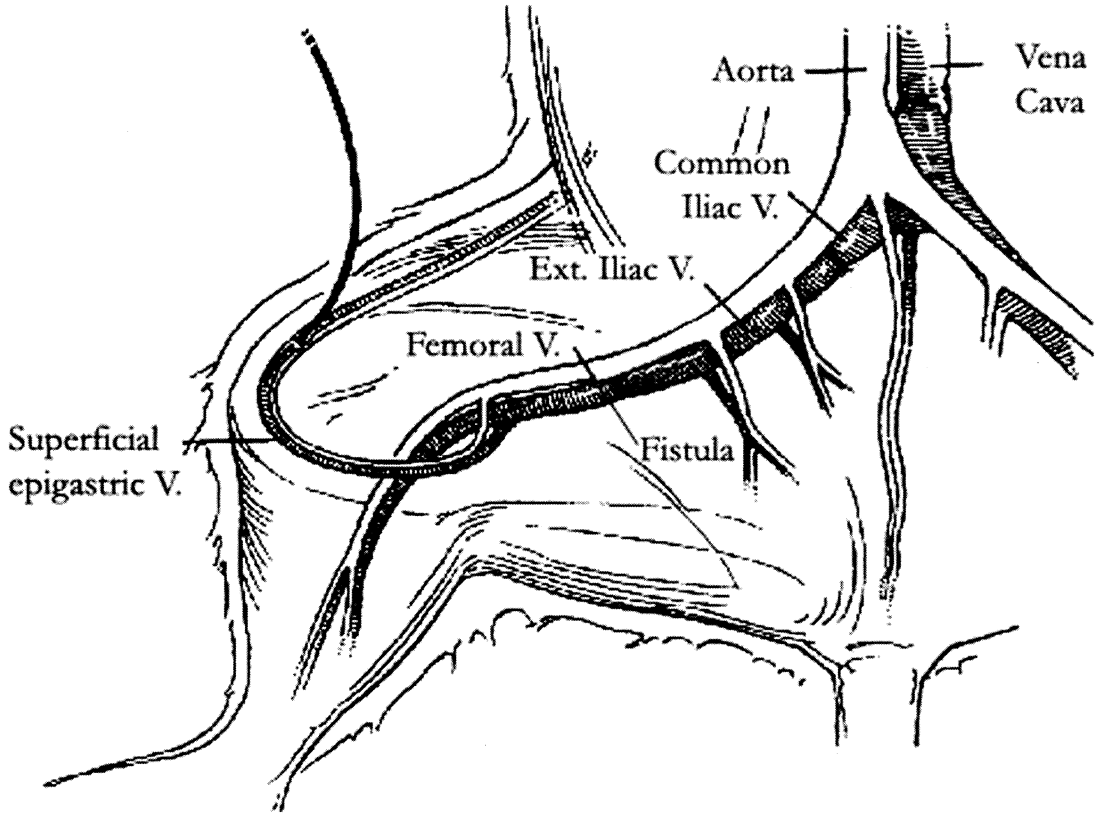

Fig 1. This diagram shows the location of the arteriovenous fistula and the femoral vein.

ning. Thickening of the distal portion of the cusp was related to the collagen component that shortened and crumpled the valve. Thinning in the commissural regions led to dilation (aneurysm formation). Forty years earlier Cotton[4] found dilation of the vein wall projecting beneath the cornua and widening the cornual angle. He said, ". . . of 156 valves which were examined, 123 were macroscopically normal; only 33 were sclerosed, The sclerosis was often so marked that only the rim of the valve was left at its attached border."

Proximal veins in the lower extremities are subject to pressure fluctuations produced by intra-abdominal compression and abdominal wall stresses. Kistner[5] has suggested that primary valve incompetence develops from stress factors such as straining and coughing. Surgical specimens of saphenous veins with reflux confirmed by duplex ultrasound scanning have more extensive leukocyte infiltration and membrane adhesion molecules more numerous on proximal surfaces of venous valves and vein walls than on distal surfaces.[6]

Observations of histologic sections from varicose veins harvested during therapeutic surgical procedures done for chronic venous insufficiency and study of venous blood samples from patients with chronic venous insufficiency suggest that in venous disease significant tissue inflammation occurs, and multiple indicators of cell activation can be found.[7] Inflammatory indicators include attachment of leukocytes to the endothelium, infiltration of monocytes, lymphocytes, and mast cells into connective tissue, and development of fibrotic tissue infiltrates.[8]

This study was done to investigate the inflammatory process associated with acute and chronic venous hypertension. The working hypothesis was that venous valve damage is acquired, linked with venous hypertension, and affected by the inflammatory process associated with acute and chronic venous hypertension. Although the model requires arterialization of a vein segment, there is no evidence that aterializing a vein segment produces an inflammatory reaction or venous valve damage.

MATERIALS AND METHODS

The animal procedures in this study have already been reviewed and approved by the Animal Subjects Committee of the University of California San Diego. The animals were kept in an approved animal facility in accordance with University of California San Diego policies, provisions set forth by National Institutes of Health, and all federal, state, and local laws and regulations governing the use of animals in research. Postprocedural record keeping policies were followed according to policies established by the Animal Subjects Committee and Office of Campus Veterinary Services at University of California San Diego.