Pathogenesis of primary chronic venous disease: Insights from animal models of venous hypertension

John J. Bergan, MD, FACS,[a] **Luigi Pascarella, MD,**[b] and **Geert W. Schmid-Schönbein, PhD,**[b] *LaJolla, Calif*

Background: **Reflux of blood through incompetent venous valves is a major cause of the venous hypertension that underlies clinical manifestations of chronic venous disease, including varicose veins, lipodermatosclerosis, and venous ulcers.**

Objective: **To review published literature relating to animal models in which venous hypertension has been produced and which have yielded information on the mechanisms by which venous hypertension may trigger inflammation and cause changes in the skin and venous valves.**

Methods: **Medline searches, with additional papers identified from reference lists in published papers.**

Results: **At least three types of animal model were identified that have contributed to a better understanding of the trigger mechanisms and role of inflammatory processes in chronic venous disease. These models involve venous hypertension induced either by acute venular occlusion, placement of a chronic arteriovenous fistula, or ligation of several large veins. Model results suggest that elevated venous pressure and altered flow can trigger inflammatory cascades in the vein wall and venous valves which can cause progressive valvular incompetence and eventual valvular destruction, and which are also important in the skin changes associated with venous disease. Treatment with agents that reduce oxidative stress by scavenging free radicals and that inhibit the inflammatory cascade can prevent the progressive deterioration of function in valves exposed to elevated venous pressure and can prevent the development of reflux blood flow.**

Conclusions: **Understanding these processes suggests potential therapeutic targets that could be effective in slowing or preventing progression, and could help promote a more positive and proactive attitude towards treatment of the underlying disease process, rather than the later manifestations of chronic venous disease. (J Vasc Surg 2008;47: 183-92.)**

Venous reflux through incompetent venous valves is a major cause of the venous hypertension that underlies many, and possibly all, the clinical manifestations of primary chronic venous disease (CVD).[1,2] Examination of affected veins from patients with CVD has shown abnormalities of valve structure, including thinning, stretching, and tearing of valve leaflets.[3] Microscopic studies have reported leaflet hypotrophy with collagen abnormalities and endothelial changes.[4,5] The number of valves in a given venous segment is reduced,[6] and degenerated valve stumps have been identified,[7] in veins from patients with CVD.

Valves in veins removed from patients with CVD show clear signs of inflammation. Monocyte/macrophage infiltration of the valve leaflets and venous wall was seen in every specimen in one surgical series,[7] and expression of intercellular adhesion molecule-1 (ICAM-1) was elevated in the vein wall and valves in another series.[8] In patients with CVD, circulating leukocytes show greater levels of activation, and their plasma shows elevated hydrogen peroxide production and also contains an (unknown) activating factor for granulocytes.[9] Interestingly, varicose veins from individuals with a family history of CVD showed elevated levels of mast cell infiltration, suggesting that inflammation may be a cause rather than a consequence of venous disease.[10] However, studies in humans can generally give information only about processes operating in established disease, whereas the very early stages may be helpful in understanding pathogenesis and trigger mechanisms. Animal models have a role to play in this regard. The aim of this article is to review results that have been obtained using three different animal models of primary CVD that have enabled investigation of the mechanisms by which venous hypertension may trigger inflammation and cause changes in the skin and venous valves: a mesenteric venule occlusion model; an arteriovenous fistula model; and a large vein ligation model. Some of the literature was selected based on ongoing research activities in the authors' laboratories. Additional material was found by Medline searching. Although it is not certain that the triggering events in these models closely reflect those in human CVD, these findings may be useful in informing future research and therapeutic decision-making.

From the Department of Surgery, University of California San Diego,[a] and the Department of Bioengineering, The Whitaker Institute of Biomedical Engineering, University of California San Diego.[b]

Competition of interest: none

Additional material for this article may be found online at www.jvascsurg.org

Reprint requests: John J. Bergan MD, FACS, Department of Surgery, University of California San Diego, 9850 Genesee, Suite 410, La Jolla, CA 92037, USA (e-mail: jbergan@ucsd.edu).

0741-5214/$34.00

Copyright © 2008 by The Society for Vascular Surgery.

doi:10.1016/j.jvs.2007.09.028

MESENTERIC VENULE OCCLUSION MODEL

An acute rodent model in which a mesenteric venule is exteriorized and reversibly occluded has allowed direct

observation of events occurring early after the onset of venous hypertension, and during reperfusion and return to normal pressures.[11-13]

Methods

In anesthetized rats, the mesentery was exposed through a midline incision, draped on a transparent pedestal on a heated stage, and observed using an intravital microscope.[14] Fluid pressures within venules were measured with micropipettes using the servo-null technique.[15]

Single unbranched vessels, at least 350 μm long and 35 to 70 μm in diameter, were selected and observed for a 20-minute control period. Occlusion of the venule was performed using a glass micropipette with a smooth rounded tip, and a region approximately 100 μm upstream of the occlusion was selected for observation. Measurements were taken before occlusion, at 20-minute intervals during 1 hour of occlusion, and during 1 hour after reperfusion. Venules prepared in a similar way but without occlusion were used as controls.

Investigation of inflammation mechanisms. Levels of oxidative stress due to hydrogen peroxide (H_2O_2) were measured using dichlorofluorescein (DCF) fluorescence,[16-18] and oxidative stress due to hydroxyl radicals was assessed using the hydroxyl radical scavenger dimethylthiourea (DMTU). Flavonoids are naturally occurring antioxidant substances that can scavenge superoxide anions and lipid peroxy radicals, and can sequester metal ions.[19-21] Their effect was assessed using micronized purified flavonoid fraction (MPFF), which has been shown to reduce leukocyte adhesion and migration after arterial ischemia/reperfusion.[22-24] MPFF was administered orally for 7 days before experimentation at doses of 50 and 100 mg/kg body weight per day, while a control group received vehicle alone. Cell death in the tissue parenchyma adjacent to the occluded venule was evaluated by propidium iodide (PI) staining.[25]

Results

Venular micropressures upstream of the occlusion increased from 13.4 ± 3.8 mm Hg before occlusion to 44 ± 4.7 mm Hg during occlusion, and venular diameters increased by 10% to 20%. There was no change in pressure downstream of the occlusion.

The number of leucocytes migrating across the venule wall increased progressively during occlusion, from zero before occlusion to 5.5 per 100 μm length of venule at 1 hour, and increased further during reperfusion to 9.7 per 100 μm ($P < .05$). During reperfusion, the numbers of rolling and adherent leukocytes increased significantly relative to preocclusion levels.

Multiple microhemorrhages occurred upstream of the occlusion. Hemorrhages 20 to 30 μm in diameter were first detected after about 23 minutes of occlusion, and expanded and merged to cover an area of mesentery some 200 μm in diameter. No hemorrhages were seen at or downstream of the occlusion. Cell death in the tissue parenchyma adjacent to the venule was increased significantly relative to nonoccluded control venules, both during occlusion and subsequent reperfusion.

It is possible that the observed increase in parenchymal cell death could have been caused by toxic effects of escaped red blood cells in the microhemorrhages. However, this seems unlikely for two reasons. First, there was no colocalisation between the PI-positive cells and the microhemorrhages. Second, in a separate series of experiments, it was found that puncturing the venule with micropipette, allowing escape of blood cells and plasma, did not produce an increase in parenchymal cell death.[26]

Comparison of upstream and downstream sites. All the above observations were made at sites upstream of the occlusion, subjected to both reduced flow (to near zero) and increased pressure. In order to distinguish between the effects of these two factors, in a subsequent series of experiments,[13] upstream sites were compared with sites downstream of the occlusion (which experienced only the reduction in flow). The number of leukocytes rolling and adhering during reperfusion, the number migrating into the interstitial space, and the amount of parenchymal cell death were all elevated at both sites, and the kinetics of the changes were similar. However, the degree of elevation was consistently greater at the upstream site (Fig 1). This suggests that the reduction in flow was sufficient to trigger the leukocyte cascade at both sites, but the elevated pressure at the upstream site enhanced the magnitude of the inflammatory reactions.

Mechanisms of inflammation. In most experiments, DCF fluorescence from the endothelium and the mesentery parenchyma increased over time at the same rate in both control (nonoccluded) and occluded venules. By contrast, DMTU and MPFF treatments attenuated the increases in leukocyte rolling, adherence, and migration, and reduced parenchymal cell death associated with occlusion (Fig 2, online only, Fig 3). MPFF also significantly increased the delay between occlusion and the first detection of microhemorrhage, from 23 ± 13 minutes in the vehicle group to 50 ± 30 minutes and 48 ± 8 minutes in the 50 and 100 mg/kg/day MPFF groups, respectively ($P < .05$ for both doses). MPFF treatment had no significant effect on DCF fluorescence intensity.

ARTERIO-VENOUS FISTULA MODEL

In order to investigate the effects of venous hypertension over a longer time scale and in larger veins we have used a model originally developed by van Bemmelen[27] in which a fistula is created surgically between the femoral artery and vein in the groin of the rat.[28-31]

Methods

Under pentobarbital anesthesia, the femoral artery and vein were clamped, and a fistula of approximately 0.5 mm internal diameter was created between them, proximal to the saphenofemoral junction, using monofilament sutures. Heparin (1000 U/kg body weight) was administered to prevent blood coagulation, and the incision was closed.

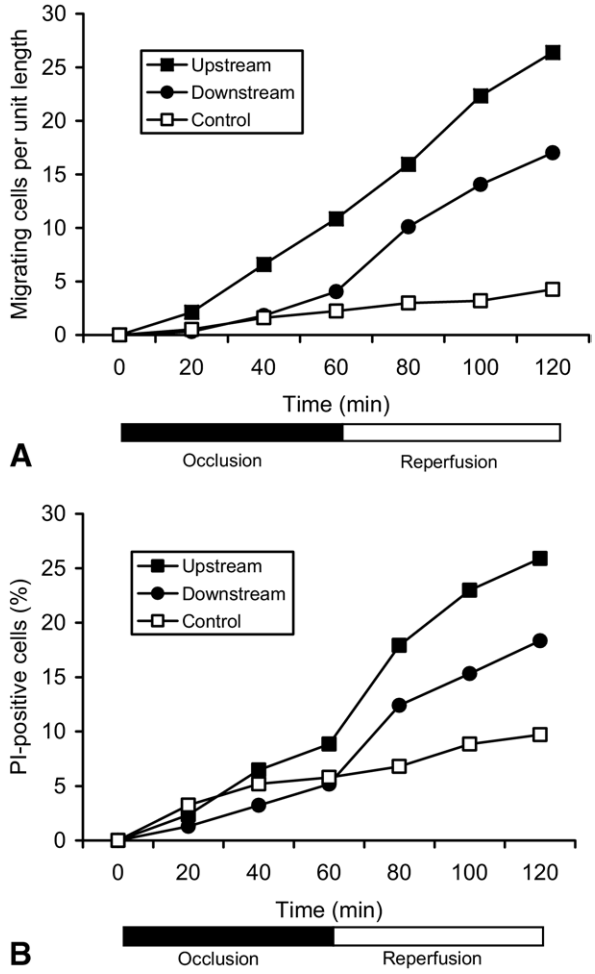

Fig 1. Comparison of upstream and downstream sites in the venular occlusion model. **A,** number of migrating leukocytes per 100 μm length of venule. **B,** proportion of propidium iodide (PI)-positive cells, a measure of parenchymal cell death, in a tissue region close to the experimental venule. From Takase et al.[13]

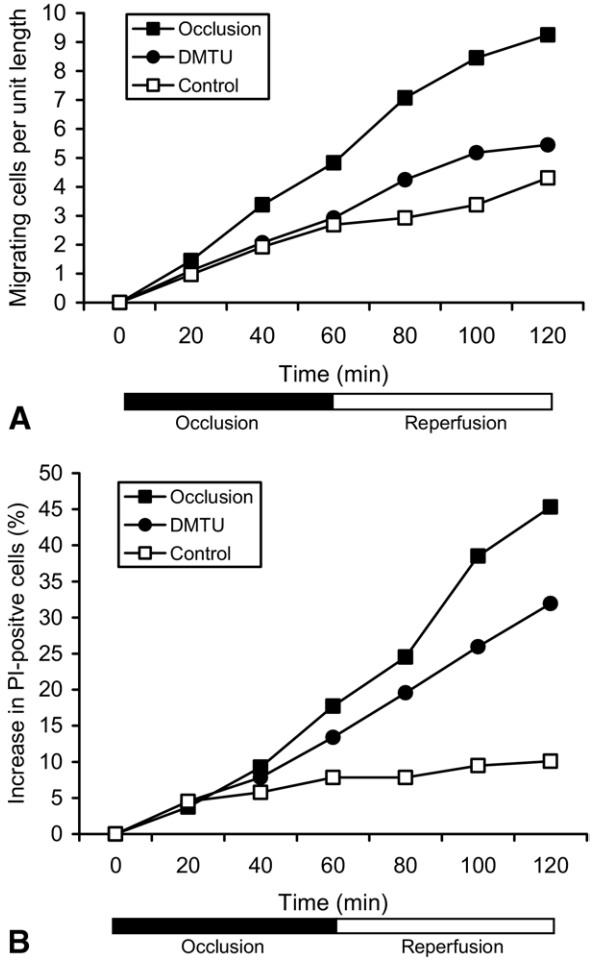

Fig 2. Effects of the hydroxyl radical scavenger DMTU (2 mM) at sites upstream of the venular occlusion. **A,** number of migrating leukocytes per 100 μm length of venule. **B,** net increase (%) of propidium iodide (PI)-positive cells, a measure of parenchymal cell death. From Takase et al.[13]

The rats were given intensive postsurgical care and maintained for up to 6 weeks.

To assess the effects of the fistula, rats were anesthetized once more and pressures were measured by catheterisation in the femoral vein 3 cm distal to the fistula, in the contralateral femoral vein, and in the contralateral femoral artery. Each femoral vein was then sectioned distal to its proximal valve, and blood reflux through the valve was collected over a timed period. The femoral vein distal to the fistula was then removed for morphometric assessment. Valve specimens were processed for histology and immunohistochemistry for macrophages, granulocytes and T-lymphocytes, the matrix metalloproteinases (MMP)-2 and MMP-9, and the endothelial adhesion molecules P-selectin and ICAM-1. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) staining for apoptotic cells was also performed. In some experiments, matrix metalloproteinase (MMP) activity was determined by gelatin zymography as described by Rosario et al.[32]

MPFF treatment. The effects of MPFF were investigated in another series of experiments. MPFF (50 and 100 mg/kg/day) or vehicle alone were administered orally for 4 days before fistula placement and continued for 21 days afterwards, at which time the second surgery and evaluations were performed.

Results

Some investigations and measurements were performed in more than one series of experiments. To avoid repetition, when results were consistent across experimental series, a single representative set of results will be presented.

Reflux rates and valve morphology. Immediately after fistula creation, the distal femoral and saphenous veins were visibly distended with pulsatile blood flow through

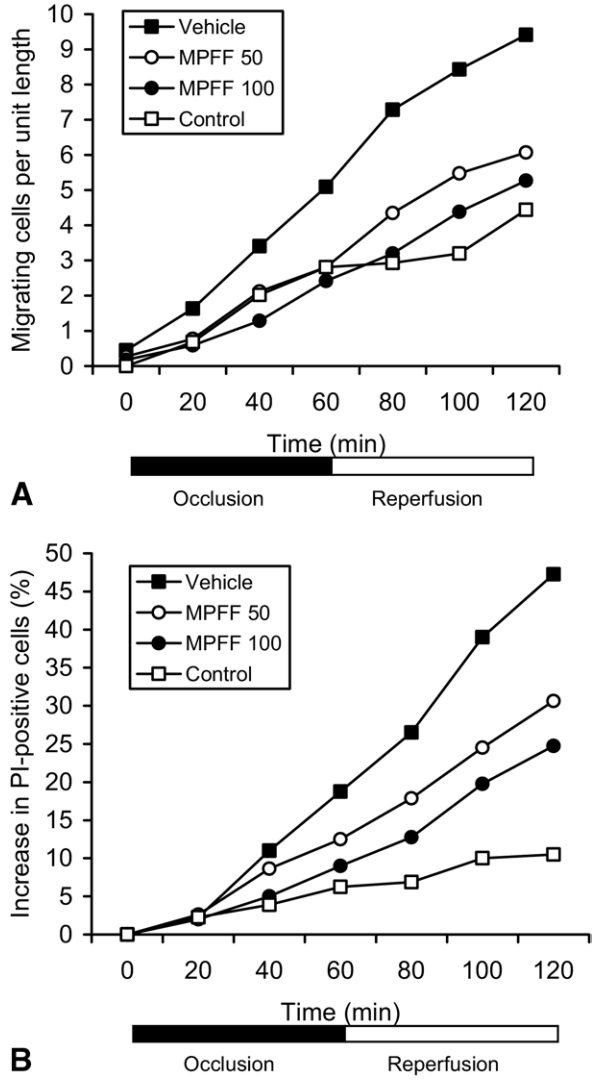

Fig 3. Effect of MPFF (50 mg/kg/day and 100 mg/kg/day) at sites upstream of the venular occlusion. **A,** number of migrating leukocytes per 100 μm length of venule. **B,** net increase (%) of propidium iodide (PI)-positive cells, a measure of parenchymal cell death. From Takase et al.[12]

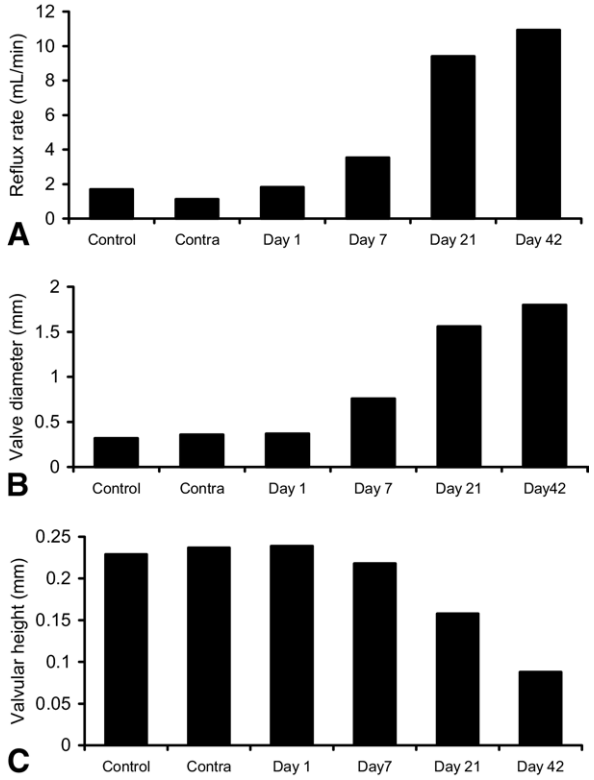

Fig 4. Time course of development of reflux and changes in venous valve morphology after placement of an arteriovenous fistula (at day 0). **A,** reflux rate. **B,** valve diameter. **C,** valvular height. Control: unoperated rats; contra: femoral vein contralateral to the fistula (n = 15 in each group). From Pascarella et al.[30]

the fistula. One week after the procedure, all rats showed some degree of edema of the hind limb ipsilateral to the fistula. During the second surgery, at the end of the experimental period, pressure in the femoral vein distal to the fistula was markedly elevated to 94 ± 9 mm Hg, compared with 11 ± 2 mm Hg in the contralateral femoral vein. Mean arterial pressure was relatively unchanged in animals with the fistula (97 ± 8 mm Hg) compared with controls (92 ± 4 mm Hg).

No reflux occurred at 1 or 2 days after fistula creation. By 7 days, the reflux rate was significantly higher than in controls ($P < .05$), and the rate was markedly increased at 21 and 42 days ($P < .01$ for both times) (Fig 4, online only), reflecting progressively greater valvular incompetence over time. Macroscopic examination of the terminal valve in the saphenous vein and the valve just distal to the fistula in the femoral vein showed significant increases in valve diameter (to 1.8 mm at day 42 compared with <0.4 mm in controls, ($P < .01$) and reductions in valve height. The time course of these morphological changes closely mirrored the development of reflux flow (Fig 4, online only). Macroscopic disappearance of valves was noted in the 21- and 42-day groups, and histology confirmed the complete disappearance of valvular structures at 42 days.

Immunohistochemistry. The numbers of granulocytes, monocytes/macrophages, and T-lymphocytes were increased in pressurized valves compared with control valves at 21 days. Expression of the inflammatory markers P-selectin and ICAM-1 was also greater on the endothelium of saphenous venous valve leaflets.

Matrix metalloproteinases. Results for MMP levels in vein walls were less consistent across experimental series. In one series,[28] MMP-2 and MMP-9 expression levels, assayed by immunohistochemistry, were not increased in the pressurized veins. However, in a later series,[31] immunohistochemistry showed a significant increase in MMP-2 expression at 21 and 42 days, with no elevation at 1 and 7

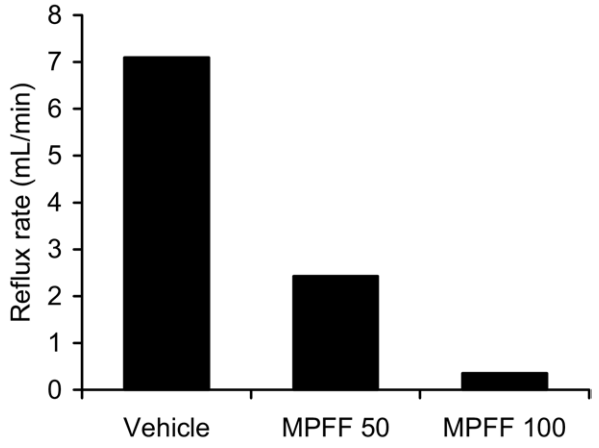

Fig 5. Effect of treatment with MPFF 50 mg/kg/day and 100 mg/kg/day, or vehicle alone on reflux flow rates 21 days after placement of an arteriovenous fistula (n = 6 in each group). From Takase et al.[29]

days. MMP-9 showed a bimodal pattern of expression, with significant elevations at day 1 and again at days 21 and 42. In a further series of experiments,[30] gelatin zymography showed elevated MMP-2 activity at 21 and 42 days and elevated MMP-9 activity at 7 days.

MPFF treatment. MPFF treatment significantly attenuated the reduction in valve height in pressurized veins, although it did not affect the increase in valve diameter. Importantly, the rate of reflux blood flow at 3 weeks was markedly reduced by MPFF ($P < .05$), in a dose-dependent manner (Fig 5).

With MPFF treatment, the numbers of T-lymphocytes in pressurized valves were reduced markedly compared with vehicle treatment, and were the same as in control valves in the contralateral, nonpressurized limb (Fig 6, online only). The numbers of granulocytes and monocytes/macrophages in the valve leaflets were all less in MPFF-treated rats, but the differences did not reach significance. Levels of P-selectin and ICAM-1 expression in endothelial cells, and the number of apoptotic, TUNEL positive cells in the vein wall were all lower with MPFF than in vehicle-treated rats, but the differences were not significant (Fig 6, online only).

LARGE VEIN LIGATION MODEL

Another rodent model, in which venous hypertension was produced by ligation of several large veins,[33-35] has been used to investigate the link between venous hypertension and the skin changes that are important manifestations of severe clinical CVD.[2]

Methods

Under pentobarbital anesthesia, the distal vena cava was ligated and the common femoral veins and common iliac veins were ligated bilaterally using cotton ties. In acute experiments, anesthesia was maintained and experimental investigations began directly.[33] In the chronic model, the

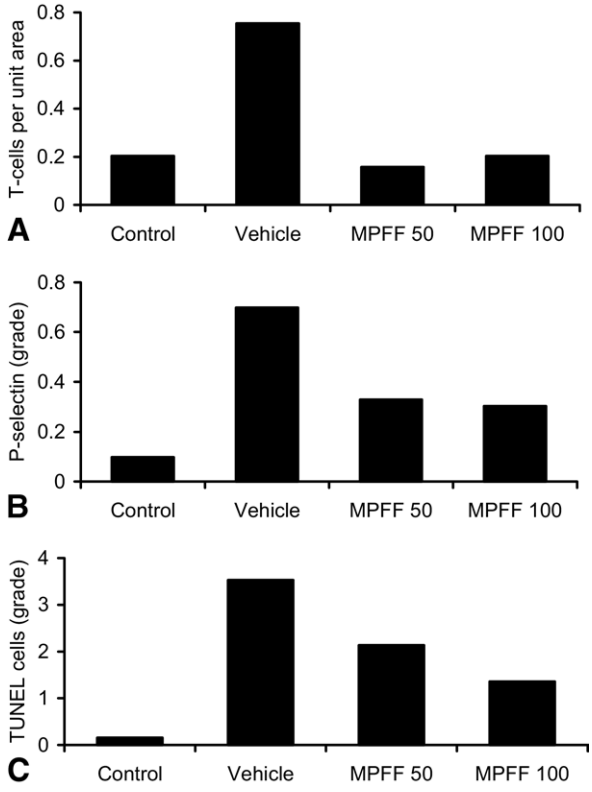

Fig 6. Effects of fistula placement and MPFF treatment on numbers of T-lymphocytes (**A**), P-selectin expression (**B**), and apoptotic TUNEL-positive cells (**C**) in saphenous venous valves. Control: unpressurized, contralateral valves; vehicle, pressurized valves treated with vehicle alone; MPFF 50 and MPFF 100: pressurized valves treated with MPFF 50 and 100 mg/kg/day. From Takase et al.[29]

incisions were closed and the rats were maintained for 7 or 14 days before a second anesthesia and investigation.[34]

Arterial and venous pressure measurements were obtained via cannulation of the right carotid artery and the right superficial epigastric vein. Tissue distribution of leukocytes was assessed by tissue myeloperoxidase (MPO) activity.[36] One unit of MPO activity is equivalent to approximately 10^6 leukocytes in tissues.[37]

Results

Ligation produced elevation of pressure in the superficial epigastric vein of the hindlimb in both the acute and chronic models, from 3.3 ± 1.2 mm Hg in sham-operated controls to 12.6 ± 3.2 mm Hg in ligated rats at 7 days in the chronic model.

Tissue MPO activity was significantly increased in the skin of the hind limb after 135 minutes in acute rats and 7 days in chronic rats compared with skin from the forelimb or sham-operated hind limb (Fig 7), indicating increased numbers of leukocytes in skin from the limb exposed to elevated venous pressure.[33,34] Pretreatment with anti-ICAM-1 monoclonal antibody significantly reduced skin

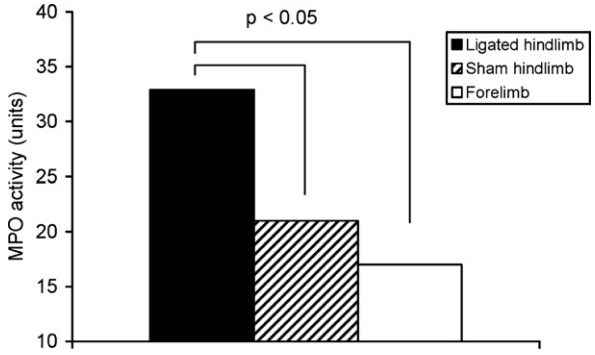

Fig 7. Myeloperoxidase (MPO) activity, indicative of tissue leukocyte numbers, in skin samples from the forelimb, sham-operated hindlimb, and ligated (hypertensive) hindlimb in the rat large vein ligation model, 7 days after ligation. From Hahn et al.[34]

MPO activity in acute rats, from 19.8 ±1.54 units to 6.71 ± 2.46 units ($P < .001$). There was no difference in endothelial expression of ICAM-1 between normotensive and hypertensive limbs 2 weeks after ligation.[35]

DISCUSSION

Venular occlusion model

The venular occlusion experiments showed that reduced flow can rapidly set in motion an inflammatory cascade, including hallmarks like leukocyte adhesion to the endothelium, migration into the interstitium, free radical production, and parenchymal cell death that begins soon after occlusion and continues for at least 1 hour after the occlusion is removed and reperfusion occurs. Normal blood flow, producing physiological levels of fluid shear stress transduced by endothelial cells, can inhibit inflammation by several mechanisms,[38] including inhibition of mitogen-activated kinase inflammatory pathways,[39,40] reducing expression of adhesion molecules,[41] and activating protective antioxidant mechanisms.[42] Shear stress also acts on leukocytes themselves, causing retraction of pseudopods, down-regulation of CD18 adhesion molecules, and detachment from the endothelial surface.[43-45] Thus, conditions of low or zero flow and shear stress are likely to promote a shift towards an inflammatory state.

Occlusion of a mesenteric venule also induced a significant elevation of pressure upstream of the occlusion. In many microvascular beds, including skeletal muscle, that have a high level of connectivity with relatively short venular interconnections, occlusion of any one venule does not cause a significant increase in pressure upstream of the occlusion. However, in the mesenteric circulation, there are microvascular domains that are connected to only a single, relatively long, venous outflow vessel. In such a situation, occlusion of one outflow vessel causes a significant increase in capillary pressure and pressure in the portion of the venule upstream of the occlusion. The addition of elevated micropressure enhanced the inflammatory reaction caused by flow reduction and produced microhemorrhages. Elevated pressure causes stretching of the endothelium, which can itself cause expression of cytokines and adhesion molecules,[46,47] activation of extracellular signal-related kinases 1 and 2,[48] and free radical production.[49] Elevated pressure can also cause the formation of transcellular gaps through endothelial cells,[50] which may be related to the development of microhemorrhages. It may be that these processes reinforce the pro-inflammatory signals generated by the reduction in flow.

The inflammatory responses seen with venular occlusion are similar to those seen after exposure to inflammatory mediators such as platelet activating factor and N-formyl-methionyl-leucyl-phenylalanine.[51,52] They also have features in common with reactions seen during arterial occlusion/reperfusion.[53-55] The effects of venular occlusion seem to be more severe than arterial occlusion, with greater parenchymal cell death and microhemorrhage formation. The greater severity may be related to elevated pressure in capillaries and postcapillary venules produced in the venular occlusion model.

Results from experiments with DMTU demonstrated the importance of oxidative stress and free radicals, particularly the hydroxyl ion, in the inflammatory process. Flavonoids have multiple actions, so MPFF may interfere with the inflammatory process at several points and so represents only a nonspecific probe of inflammatory mechanisms. However, being of low toxicity and active by oral administration, MPFF and other flavonoids are of considerable therapeutic interest.[56-58] MPFF is used clinically in some countries for the treatment of patients with the later manifestations of venous disease and their symptoms, such as varicose veins, hemorrhoids, and venous ulcers (for review, see Lyseng-Williamson and Perry[59]). The present animal model results suggest that MPFF is effective in reducing the initial components of the inflammatory cascade. It is therefore possible that MPFF (and potentially other anti-inflammatory treatments) could also be of benefit in the early stages of chronic venous disease and might inhibit inflammation-induced tissue damage and disease progression.

Arterio-venous fistula model

These experiments have demonstrated that formation of a femoral arteriovenous fistula produces an immediate increase in venous pressure and dilation of the distal veins. Detectable reversed flow did not begin immediately, but rather, the rate of reversed flow increased over the course of the next 21 days or more. The increasing reflux mirrored progressive changes in venous valve morphology that were consistent with those originally reported by van Bemmelen et al,[60] and led in some cases to complete disappearance of valvular structures at 42 days. Pressurized valves showed increased numbers of granulocytes, monocytes/macrophages, and T-lymphocytes, and elevated levels of inflammatory markers. Thickening and fibrosis of the vein wall were also seen at 21 and 42 days.

Recent studies on the operation of venous valves have shown that closure normally occurs with no or minimal reflux, and that blood flowing through the valve separates

into two separate streams: a proximally-directed axial jet, and also a vortical fluid motion into the sinus pocket behind the leaflet.[61] The vortical flow in the pocket of the valve prevents stagnation of blood in the valve pocket and ensures that all parts of the valvular endothelium are exposed to significant fluid shear stress. Elevated venous pressure causes dilation and distortion of the valve, possibly preventing proper closure of the leaflets, disturbing the flow pattern within the valve, and allowing reflux.

There is considerable evidence that unidirectional, laminar flow (including pulsatile flow) produces shear stress that is transduced by endothelial cells and causes an anti-inflammatory response, while zero or low flow is a pro-inflammatory stimulus.[37,60] It is now becoming clear that disturbed or turbulent flow, and in particular flow whose direction reverses (often referred to as oscillatory flow with reciprocating shear stress), also has a pro-inflammatory action.[62-65] Furthermore, elevated pressure itself can activate pro-inflammatory signalling pathways. Thus, a venous valve subjected to high pressure, causing structural distortion and disturbed and reversing flow, is likely to display an inflammatory reaction that may result in tissue damage and weakening of valvular structures.

The effects of MPFF treatment are important in understanding the pathogenetic sequence of events. We propose that, in the absence of MPFF, the increased venous pressure produced by fistula placement distorts the venous valves exposed to it, altering the flow pattern through the valves and possibly producing some reflux flow. The altered, possibly reversed flow and the increased pressure set in motion an inflammatory reaction that leads to damage of the valvular tissue, in turn leading to impaired valvular function and increased reflux flow. In this way, a vicious cycle of increasing valvular damage and greater reflux is produced, that may culminate in the complete destruction of valves. MPFF treatment may inhibit the inflammatory cascade, reducing valvular tissue damage and preventing the establishment of this vicious cycle, thereby accounting for the dose-dependent reduction of reflux flow that was observed.

It could be argued that placement of an arteriovenous fistula represents an extreme form of venous hypertension, in that parts of the venous system are exposed to pressures >90 mm Hg. However, the venous system of the lower limb in the human can also be exposed to high pressures. A mean pressure of 95.4 ± 5.5 mm Hg was recorded in the dorsal foot veins during quiet standing in a group of 20 healthy volunteers aged 22 to 34 years,[66] a value consistent with earlier studies.[67] Some vessels in the calf are exposed to the pressures generated by calf muscle contraction, and peak intramuscular pressures in the human soleus muscle have been recorded at 181 mm Hg during walking and 269 mm Hg during running.[68] Proximal venous valves may be exposed to high pressures caused by intra-abdominal pressure, which has recently been reported to reach 107 mm Hg during coughing and 171 mm Hg during jumping in healthy young adults.[69] Thus, parts of the human venous system are exposed to pressures at least as high as those produced by fistula placement in the rat. However, the rat model is severe in terms of the continuous nature of the pressure elevation. This may account for the fact that valve destruction can occur within 6 weeks in the model, but may progress over many years in human patients.

As well as in primary venous disease, venous valves may become incompetent secondary to lower limb trauma or venous thrombosis. Although beyond the main scope of this article, animal models have been important in showing that an inflammatory response is also involved in thrombus formation and the deleterious changes in venous valves and vein walls that accompany it.[70-73] In rodent models of venous thrombosis, thrombus initiation was associated with a rapid vein wall inflammatory reaction involving early neutrophil infiltration,[74] and later vein wall remodelling associated with increased MMP-9 and MMP-2 expression.[75] Inhibition of the pro-inflammatory adhesion molecule P-selectin had no effect on thrombus mass, but reduced vein wall injury.[73]

Large vein ligation model

Skin changes in the lower limb, including hyperpigmentation, lipodermatosclerosis and, most seriously, venous ulcer, are frequent manifestations of long-standing or severe CVD.[2] Studies in humans have shown that blood returning from feet experiencing venous hypertension due to calf muscle inactivity is relatively depleted of leukocytes.[76,77] Consistent with this phenomenon, the numbers of macrophages, lymphocytes, and mast cells are elevated in skin biopsies from patients with CVD.[78,79] Results such as these have led to the "leukocyte trapping hypothesis", according to which an inflammatory reaction, involving leukocyte activation and entrapment in the microcirculation (including capillaries), migration from the microvasculature into skin tissue, and accompanied by release of inflammatory mediators, is important in provoking skin changes.[80,81]

Research using the large vein ligation model of venous hypertension in the rat has shown that a relatively modest elevation of venous pressure can provoke leukocyte migration leading to elevated tissue leukocyte levels that are detectable within approximately 2 hours of ligation and which persist for at least a week with chronic pressure elevation.[33,34] Leukocyte accumulation was largely prevented by prior injection of an anti-ICAM-1 monoclonal antibody,[35] confirming the key role of ICAM-1 in leukocyte adherence and migration in skin tissue as well as in the walls and valves of large veins. Interestingly, endothelial expression of ICAM-1 was not increased after 14 days of venous hypertension. This may mean that basal levels of endothelial ICAM-1 expression are sufficient to support significant leukocyte migration.

Implications for the pathogenesis of primary CVD in humans

Events that would be predicted, on the basis of animal model results, to trigger inflammatory reactions are likely to occur during everyday human activities. Indeed, plasma

levels of adhesion molecules including ICAM-1 are elevated and leukocyte "trapping" occurs in normal subjects in response to the venous hypertension produced by 30 minutes of quiet standing.[82] However, in most individuals such events do not lead to the development of CVD. The inflammatory cascade is basically a repair mechanism, and there is a balance between inflammation-induced tissue injury and the processes of healing and repair. The overall level of injury may depend on the intensity and frequency of triggering events, and a combination of inflammatory stimuli are likely more injurious than just a single stimulus. Risk factors such as prolonged standing, greater body height, and physical inactivity, together with genetic susceptibility, may tip the balance in favour of injury and eventually lead to the clinical manifestations of CVD. In women, the action of progesterone may be important,[83] possibly by reducing smooth muscle tone and allowing greater venous distension, compromising the action of venous valves. Once initiated, venous valve damage will be self-reinforcing, exacerbating venous hypertension and disturbance of venous flow, and causing further inflammation.

Animal models differ from patients in terms of their genomes, hemodynamic pressures and flow, and disease risk factors, so mechanisms identified in animal models require testing in patients. Nonetheless, animal models have highlighted the central role of inflammation and elucidated some of the key processes involved. The results obtained with MPFF and free radical scavengers suggest that inhibition of the early stages of the inflammatory cascade offers potential targets for therapeutic intervention. It may be that existing anti-inflammatory agents warrant wider use and more detailed study, and there may be opportunities for the development of novel agents acting in this area. These findings also suggest that the optimal and earliest use of those treatment options that are currently available, including lifestyle changes, aimed at reducing venous hypertension and inhibiting inflammation, could minimise valve damage and prevent the development or worsening of venous reflux. It is to be hoped that future clinical research will confirm or deny these suggestions before too long.[62]

AUTHOR CONTRIBUTIONS

Conception and design: JB
Analysis and interpretation: JB
Data collection: JB
Writing the article: JB
Critical revision of the article: JB
Final approval of the article: JB
Statistical analysis: JB
Obtained funding: JB
Overall responsibility: JB

REFERENCES

1. Eberhardt RT, Raffetto JD. Chronic venous insufficiency. Circulation 2005;111:2398-409.
2. Bergan JJ, Schmid-Schönbein GW, Coleridge Smith PD, Nicolaides AN, Boisseau MR, Eklof B. Chronic venous disease. N Engl J Med 2006;355:488-98.
3. Van Cleef JF, Hugentobler JP, Desvaux P, Griton P, Cloarec M. Endoscopic study of reflux of the saphenous valve. J Mal Vasc 1992: 17(Suppl B):113-6. [Article in French]
4. Budd TW, Meenaghan MA, Wirth J, Taheri SA. Histopathology of veins and venous valves of patients with venous insufficiency syndrome: ultrastructure. J Med 1990;21:181-99.
5. Corcos L, De Anna D, Dini M, Macchi C, Ferrari PA, Dini S. Proximal long saphenous vein valves in primary venous insufficiency. J Mal Vasc 2000;25:27-36.
6. Sales CM, Rosenthal D, Petrillo KA, Jerivs HS, Matsuura J, Clark MD, et al. The valvular apparatus in venous insufficiency: a problem of quantity? Ann Vasc Surg 1998;12:153-5.
7. Ono T, Bergan JJ, Schmid-Schönbein GW, Takase S. Monocyte infiltration into venous valves. J Vasc Surg 1998;27:158–6.
8. Takase S, Bergan JJ, Schmid-Schönbein GW. Expression of adhesion molecules and cytokines on saphenous veins in chronic venous insufficiency. Ann Vasc Surg 2000;14:427-35.
9. Takase S, Schmid-Schönbein GW, Bergan JJ. Leukocyte activation in patients with venous insufficiency. J Vasc Surg 1999;30:148-56.
10. Kakkos SK, Zolota VG, Peristeropoulou P. Apostolopoulou A, Geroukalos G, Tsolakis IA. Increased mast dell infiltration in familial varicose veins: pathogenetic implications? Int Angiol 2003;22:43-9.
11. Takase S, DeLano FA, Lerond L, Bergan JJ, Schmid-Schönbein GW. Inflammation in chronic venous insufficiency: is the problem insurmountable? J Vasc Res 1999;36(Suppl 1):3-10.
12. Takase S, Lerond L, Bergan JJ, Schmid-Schönbein GW. The inflammatory reaction during venous hypertension in the rat. Microcirculation 2000;7:41-52.
13. Takase S, Lerond L, Bergan JJ, Schmid-Schönbein GW. Enhancement of reperfusion injury by elevation of microvascular pressures. Am J Physiol Heart Circ Physiol 2002;282:H1387-94.
14. DeLano FA, Forrest MJ, Schmid-Schönbein GW. Attenuation of oxygen free radical formation and tissue injury during experimental inflammation by P-selectin blockade. Microcirculation 1997;4:349-57.
15. DeLano FA, Schmid-Schönbein GW, Skalak TC, Zweifach BW. Penetration of the systemic blood pressure into the microvasculature of rat skeletal muscle. Microvasc Res 1991;41:92-110.
16. Cathcart R, Schwiers E, Ames BN. Detection of picomole levels of hydroperoxides using a fluorescent dichlorofluorescein assay. Anal Biochem 1983;134:111-6.
17. Suematsu M, Schmid-Schönbein GW, Chavez-Chavez RH, Yee TT, Tamatani T, Miyasaka M, et al. In vivo visualization of oxidative changes in microvessels during neutrophil activation. Am J Physiol 1993;264:H881-91.
18. Carter WO, Narayanan PK, Robinson JP. Intracellular hydrogen peroxide and superoxide anion detection in endothelial cells. J Leukoc Biol 1994;55:253-8.
19. Sorata Y, Takahama U, Kimura M. Protective effect of quercetin and rutin on photosensitized lysis of human erythrocytes in the presence of hematoporphyrin. Biochim Biophys Acta 1984;799:313-7.
20. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem Pharmacol 1988;37:837-41.
21. Afanas'ev IB, Dorozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem Pharmacol 1989; 38:1763-9.
22. Friesenecker B, Tsai AG, Allegra C, Intaglietta M. Oral administration of purified micronized flavonoid fraction suppresses leukocyte adhesion in ischemia-reperfusion injury: in vivo observations in the hamster skin fold. Int J Microcirc Clin Exp 1994;14:50-5.
23. Bouskela E, Donyo KA. Effects of oral administration of purified micronized flavonoid fraction on increased microvascular permeability induced by various agents and on ischemia/reperfusion in the hamster cheek pouch. Angiology 1997;48391-9.
24. Pickelman S, Nolte D, Leiderer R, Mollmann M, Schutze E, Messmer K. Effects of the phlebotropic drug Daflon 500 mg on postischemic

reperfusion injury in striated muscle: a histomorphologic study in the hamster. J Lab Clin Med 1999;134:536-45.
25. Suematsu M, Suzuki H, Ishii H, Kato S, Hamamatsu H, Miura S, et al. Topographic dissociation between mitochondrial dysfunction and cell death during low-flow hypoxia in perfused rat liver. Lab Invest 1992; 67:434-42.
26. Wilms H, DeLano FA, Schmid-Schönbein GW. Mechanisms of parenchymal cell death in-vivo after microvascular hemorrhage. Microcirculation 2000;7:1-11.
27. van Bemmelen SP. Venous valvular incompetence: and experimental study in the rat. University of Amsterdam MD Thesis, 1984.
28. Takase S, Pascarella L, Bergan JJ, Schmid-Schönbein GW. Hypertension-induced venous valve remodeling. J Vasc Surg 2004;39:1329-34.
29. Takase S, Pascarella L, Lerond L, Bergan JJ, Schmid-Schönbein GW. Venous hypertension, inflammation and valve remodeling. Eur J Vasc Endovasc Surg 2004;28:484-93.
30. Pascarella L, Penn A, Schmid-Schönbein GW. Venous hypertension and the inflammatory cascade: major manifestations and trigger mechanisms. Angiology 2005;56(Suppl 1):S3-S10.
31. Pascarella L, Schmid-Schönbein GW, Bergan J. An animal model of venous hypertension: the role of inflammation in venous valve failure. J Vasc Surg 2005;41:303-11.
32. Rosario HS, Waldo SW, Becker SA, Schmid-Schönbein GW. Pancreatic trypsin increases matrix metalloproteinase-9 accumulation and activation during acute intestinal ischemia-reperfusion in the rat. Am J Pathol 2004;164:1707-16.
33. Lalka SG, Unthank JL, Nixon JC. Elevated cutaneous leukocyte concentration in a rodent model of acute venous hypertension. J Surg Res 1998;74:59-63.
34. Hahn TL, Unthank JL, Lalka SG. Increased hindlimb leukocyte concentration in a chronic rodent model of venous hypertension. J Surg Res 1999;81:38-41.
35. Hahn TL, Whitfield R, Salter J, Granger DN, Unthank JL, Lalka SG. Evaluation of the role of intercellular adhesion molecule 1 in a rodent model of chronic venous hypertension. J Surg Res 2000;88:150-4.
36. Arndt H, Smith CW, Granger DN. Leukocyte-endothelial cell adhesion in spontaneously hypertensive and normotensive rats. Hypertension 1993;21:667-73.
37. Granger DN, Zimmerman BJ, Sekizuka E, Grisham MB. Intestinal microvascular exchange in the rat during luminal perfusion with formyl-methionyl-leucyl-phenylalanine. Gastroenterology 1988;94:673-81.
38. World CJ, Garin G, Berk B. Vascular shear stress and activation of inflammatory genes. Curr Atheroscler Rep 2006;8:240-4.
39. Yamawaki H, Lehoux S, Berk BC. Chronic physiological shear stress inhibits tumor necrosis factor-induced proinflammatory responses in rabbit aorta perfused ex vivo. Circulation 2003;108:1619-25.
40. Yamawaki H, Pan S, Lee RT, Berk BC. Fluid shear stress inhibits vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells. J Clin Invest 2005; 115:733-8.
41. Chiu J-J, Chen L-J, Lee P-L, Lee C-I, Lo L-W, Usami S, et al. Shear stress inhibits adhesion molecule expression in vascular endothelial cells induced by co-culture with smooth muscle cells. Blood 2003;1012: 2667-74.
42. Hojo Y, Saito Y, Tanimoto T, Hoefen RJ, Baines CP, Yamamoto K, et al. Fluid shear stress attenuates hydrogen peroxide-induced c-Jun NH_2-terminal kinase activation via a glutathione reductase-mediated mechanism. Circ Res 2002;91:712-8.
43. Moazzam F, DeLano FA, Zweifach BW, Schmid-Schönbein GW. The leukocyte response to fluid stress. Proc Natl Acad Sci USA 1997;94: 5338-43.
44. Fukuda S, Schmid-Schönbein GW. Regulation of CD18 expression on neutrophils in response to fluid shear stress. Proc Natl Acad Sci USA 2003;100:13152-7.
45. Kim MB, Sarelius IH. Role of shear forces and adhesion molecule distribution on P-selectin-mediated leukocyte rolling in postcapillary venules. Am J Physiol Heart Circ Physiol 2004;287:H2705-11.
46. Wang DL, Wung BS, Shyy YJ, Chao YJ, Usami S, Chien S. Mechanical strain induces monocyte chemotactic preotein-1 gene expression in endothelial cells. Effects of mechanical strain on monocyte adhesion to endothelial cells. Circ Res 1995;77:294-302.
47. Yun JK, Anderson JM, Ziats NP. Cyclic-strain-induced endothelial cell expression of adhesion molecules and their roles in monocyte-endothelial interaction. J Biomed Mater Res 1999;44:87-97.
48. Cheng JJ, Wung BS, Chao YJ, Wang DL. Sequential activation of protein kinase C (PKC)-alpha and PKC-epsilon contributes to sustained Raf/ERK1/2 activation in endothelial cells under mechanical strain. J Biol Chem 2001;276:31368-75.
49. Cheng JJ, Wung BS, Chao YJ, Wang DL. Cyclic strain-induced reactive oxygen species involved in ICAM-1 gene induction in endothelial cells. Hypertension 1998;31:125-30.
50. Neal CR, Michel CC. Openings in from microvascular endothelium induced by high intravascular pressures. J Physiol (Lond) 1996;492:39-52.
51. Ohashi KL, Tung DK, Wilson J, Zweifach BW, Schmid-Schönbein GW. Transvascular and interstitial migration of neutrophils in rat mesentery. Microcirculation 1996;3:199-210.
52. Tung DK, Bjursten LM, Zweifach BW, Schmid-Schönbein GW. Leukocyte contribution to parenchymal cell death in an experimental model of inflammation. J Leukoc Biol 1997;62:163-75.
53. Suematsu M, DeLano FA, Poole D, Engler RL, Miyasaka M, Zweifach BW, et al. Spatial and temporal correlation between leukocyte behavior and cell injury in post-ischemic rat skeletal muscle microcirculation. Lab Invest 1994;70:684-95.
54. Harris AG, Costa JJ, DeLano FA, Zweifach BW, Schmid-Schönbein GW. Mechanisms of cell injury in rat mesentery and cremaster muscle. Am J Physiol 1998;274:H1009-15.
55. Carden DL, Granger DN. Pathophysiology of ischemia-reperfusion injury. J Pathol 2000;190:255-66.
56. Frick RW. Three treatment for chronic venous insufficiency: escin, hydroxyethylrutoside, and Daflon. Angiology 2000;51:197-205.
57. Bergan JJ, Schmid-Schönbein GW, Takase S. Therapeutic approach to chronic venous insufficiency and its complications: place of Daflon 500 mg. Angiology 2001;52(Suppl 1):S43-7.
58. Di Carlo A, Passi S, Ippolito F, Candiani C. Free radical scavenging activity of rutosides. Minerva Cardioangiol 2002;50:701-7.
59. Lyseng-Williamson KA, Perry CM. Micronised purified flavonoid fraction: a review of its use in chronic venous insufficiency, venous ulcers and haemorrhoids. Drugs 2003;63:71-100.
60. van Bemmelen SP, Hoynck van Papendrecht AA, Hodde KC, Klopper PJ. A study of valve incompetence that developed in an experimental model of venous hypertension. Arch Surg 1986;121:1048-52.
61. Lurie F, Kistner RL, Eklof B, Kessler D. Mechanism of venous valve closure and role of the valve in circulation: a new concept. J Vasc Surg 2003;38:955-61.
62. Li YS, Haga JH, Chien S. Molecular basis of the effects of shear stress on vascular endothelial cells. J Biomech 2005;38:1949-71.
63. Hwang J, Saha A, Boo YC, Sorescu GP, McNally JS, Holland SM, et al. Oscillatory shear stress stimulates endothelial production of O2- from p47phox-dependent NAD(P)H oxidases, leading to monocyte adhesion. J Biol Chem 2003;278:47291-8.
64. Sorescu GP, Sykes M, Weiss D, Platt MO, Saha A, Hwang J, et al. Bone morphogenic protein 4 produced in endothelial cells by oscillatory shear stress stimulates an inflammatory response. J Biol Chem 2003; 278:31128-35.
65. Hosoya T, Maruyama A, Kang MI, Kawatani Y, Shibata T, Uchida K, et al. Differential responses of the Nrf2-Keap1 system to laminar and oscillatory shear stresses in endothelial cells. J Biol Chem 2005;280: 27244-50.
66. Kügler C, Strunk M, Rudofsky G. Venous pressure dynamics of the healthy human leg. J Vasc Res 2001;38:20-29.
67. Stick C, Jaeger H, Witzleb E. Measurements of volume changes and venous pressure in the human lower leg during walking and running. J Appl Physiol 1992;72:2063-8.
68. Ballard RE, Watenpaugh DE, Breit GA, Murthy G, Holley DC, Hargens AR. Leg intramuscular pressures during locomotion in humans. J Appl Physiol 1998;84:1976-81.
69. Cobb WS, Burns JM, Kercher KW, Matthews BD, Norton HJ, Heniford BT. Normal intra-abdominal pressure in healthy adults. J Surg Res 2005;129:231-5.

70. Wakefield TW, Strieter RM, Wilke CA, Kadell AM, Wrobleski SK, Burdick MD, et al. Venous thrombosis-associated inflammation and attenuation with neutralizing antibodies to cytokines and adhesion molecules. Arterioscler Thromb Vasc Biol 1995; 15:258-68.
71. Myers DD, Hawley AE, Farris DM, Wrobleski SK, Thanaporn P, Schaub RG, et al. P-selectin and leukocyte microparticles are associated with venous thrombogenesis. J Vasc Surg 2003;38:1075-89.
72. Myers DD, Wakefield TW. Inflammation-dependent thrombosis. Front Biosci 2005;10:2750-7.
73. Myers DD Jr, Henke PK, Bedard PW, Wrobleski SK, Kaila N, Shaw G, et al. Treatment with an oral small molecule inhibitor of P-selectin (PSI-6797) decreases vein wall injury in a rat stenosis model of venous thrombosis. J Vasc Surg 2006;44:625-32.
74. Downing LJ, Strieter RM, Kadell AM, Wilke CA, Brown SL, Wrobleski SK, et al. Neutrophils are the initial cell type identified in deep venous thrombosis induced vein wall inflammation. ASAIO J 1996;42:M677-82.
75. Deatrick KB, Eliason JL, Lynch EM, Moore AJ, Dewyer NA, Varma MR, et al. Vein wall remodeling after deep vein thrombosis involves matrix metalloproteinases and late fibrosis in a mouse model. J Vasc Surg 2005;42:140-8.
76. Moyses C, Cederholm-Williams SA, Michel CC. Hemoconcentration and accumulation of white cells in the feet during venous stasis. Int J Microcirc Clin Exp 1987;5:311-20.
77. Thomas PR, Nash GB, Dormandy JA. White cell accumulation in dependent legs of patients with venous hypertension: a possible mechanism for trophic changes in the skin. BMJ 1988;296:1693-5.
78. Wilkinson LS, Bunker C, Edwards JC, Scurr JH. Leukocytes: their role in the etiopathogenesis of skin damage in venous disease. J Vasc Surg 1993;17:669-75.
79. Pappas PJ, DeFouw DO, Venezio LM, Gorti R, Padberg FT Jr, Silva MB Jr, et al. Morphometric assessment of the dermal microcirculation in patients with chronic venous insufficiency. J Vasc Surg 1997;26:784-95.
80. Cheatle TR, Sarin S, Coleridge Smith PD, Scurr JH. The pathogenesis of skin damage in venous disease: a review. Eur J Vasc Surg 1991;5: 115-23.
81. Coleridge Smith PD. The microcirculation in venous hypertension. Vasc Med 1997;2:203-13.
82. Saharay M, Shields DA, Georgiannos SN, Porter JB, Scurr JH, Coleridge Smith PD. Endothelial activation in patients with chronic venous disease. Eur J Vasc Endovasc Surg 1998;15:342-9.
83. Perrot-Applanat M, Cohen-Solal K, Milgrom E, Finet M. Progesterone receptor expression in human saphenous veins. Circulation 1995;92: 2975-83.

Submitted Jun 15, 2007; accepted Sep 3, 2007.

Additional material for this article may be found online at www.jvascsurg.org.

ON THE MOVE?

Send us your new address at least six weeks ahead

Don't miss a single issue of the journal! To ensure prompt service when you change your address, please photocopy and complete the form below.

Please send your change of address notification at least six weeks before your move to ensure continued service. We regret we cannot guarantee replacement of issues missed due to late notification.

JOURNAL TITLE:
Fill in the title of the journal here. _____

OLD ADDRESS:
Affix the address label from a recent issue of the journal here.

NEW ADDRESS:
Clearly print your new address here.

Name _____

Address _____

City/State/ZIP _____

COPY AND MAIL THIS FORM TO:
Elsevier Inc.
Subscription Customer Service
6277 Sea Harbor Dr
Orlando, FL, 32887

OR FAX TO:
407-363-9661

OR E-MAIL:
elspcs@elsevier.com

OR PHONE:
800-654-2452
Outside the US, call
407-345-4000