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Matrix Metalloproteinases in Remodeling of Lower Extremity Veins and Chronic Venous Disease

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Abstract

The veins of the lower extremity are equipped with efficient wall, contractile venous smooth muscle (VSM) and competent valves in order to withstand the high venous hydrostatic pressure in the lower limb and allow unidirectional movement of deoxygenated blood towards the heart. The vein wall structure and function are in part regulated by matrix metalloproteinases (MMPs). MMPs are zinc-dependent endopeptidases that are secreted as inactive proMMPs by different cells in the venous wall including fibroblasts, VSM and leukocytes. ProMMPs are activated by other MMPs, proteinases, and other endogenous and exogenous activators. MMPs degrades various extracellular matrix (ECM) proteins including collagen and elastin, and could affect other cellular processes including endothelium-mediated dilation, VSM cell migration and proliferation as well as modulation of Ca^{2+} signaling and contraction in VSM. It is thought that increased lower limb venous hydrostatic pressure increases hypoxia inducible factors and other MMP inducers such as EMMPRIN, leading to increased MMP expression/activity, ECM protein degradation, vein wall relaxation, and venous dilation. Vein wall inflammation and leukocyte infiltration cause additional increases in MMPs, and further vein wall dilation and valve degradation, that could lead to chronic venous disease and varicose veins (VVs). VVs are often presented as vein wall dilation and tortuosity, incompetent venous valves and venous reflux. Different regions of VVs show different MMP levels and ECM proteins with atrophic regions showing high MMP levels/activity and little ECM compared to hypertrophic regions with little or inactive MMPs and abundant ECM. Treatment of VVs includes compression stockings, venotonics, sclerotherapy or surgical removal. However, these approaches do not treat the cause of VVs, and other lines of treatment may be needed. Modulation of endogenous tissue inhibitors of metalloproteinases (TIMPs), and exogenous synthetic MMP inhibitors may provide new approaches in the management of VVs.

Keywords

Cell Signaling; Collagen; Extracellular Matrix; Proteinases; Protein Degradation; Remodeling

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CONFLICT OF INTEREST

None

1. INTRODUCTION

Veins are a complex network of blood vessels that help transfer deoxygenated blood from various tissues and organs toward the heart. In the lower extremities, an intricate network of superficial, perforator and deep veins accounts for the transfer of blood towards the heart against venous hydrostatic pressure (Fig. 1). Superficial veins carry blood from the skin and subcutaneous tissue and include the great saphenous vein and small saphenous vein. The great saphenous vein is located in the medial side of the lower limb and runs from the ankle upward until it joins the common femoral vein at the saphenofemoral junction. The small saphenous vein is located in the back of the lower limb and runs from the ankle upward until it joins the popliteal vein at the saphenopopliteal junction. In addition, the anterior and posterior accessory saphenous veins run in the thigh and leg. The deep veins are embedded in the muscles and carry blood from all other parts of the lower extremity. The deep veins include the common femoral, deep femoral, femoral, popliteal, and tibial veins.¹ In all parts of the lower extremity, blood flows from the superficial veins to the deep veins.^{1,2} An exception is the foot, where the blood flow is bidirectional. Connecting the superficial and deep venous system are the perforator veins, with inward direction of blood flow to the deep veins. The lower limb veins are equipped with bicuspid valves that protrude from the inner wall. The vein valves ensure the flow of blood in one direction from the superficial to the deep veins and towards the heart. In addition, contraction of skeletal muscle in the calf, foot and thigh helps to drive blood flow towards the heart, and against gravity and the venous hydrostatic pressure. In the standing position, the venous hydrostatic pressure could reach as high as 90–100 mmHg at the ankle.^{1,3}

When compared to the arteries, the veins are relatively thin. However, the structural integrity of the veins is still maintained and presented in three histological layers. The innermost layer or the tunica intima comprises mainly endothelial cells which line the venous wall and therefore are in direct contact with the changes in venous blood flow. The tunica media is separated from the tunica intima by the internal elastic lamina, and contains several layers of vascular smooth muscle (VSM). The tunica adventitia is the outermost layer in the venous wall and mainly contains fibroblasts that are embedded in an extracellular matrix (ECM) of several proteins including collagen, elastin and other proteins.⁴

The vein wall structure and function are regulated by a host of ions, signaling molecules, and enzymes. Matrix metalloproteinases (MMPs) are Zn²⁺-dependent endopeptidases that are largely known for their ability to degrade various ECM proteins. MMPs could play a major role in venous tissue remodeling by degrading various components of ECM. In addition to their effects on ECM, MMPs may interact with bioactive molecules on the cell membrane and could regulate G-protein coupled receptors (GPCRs) and cell signaling. MMPs could play a role in various physiological processes and could affect cell proliferation, migration, and differentiation. MMPs could also be involved in cell apoptosis, immune response, tissue repair, and angiogenesis. MMPs are regulated at the mRNA expression and enzymatic activity levels. MMPs expression and activity could be altered in uteroplacental and vascular tissues and could play a role in the uteroplacental and vascular remodeling during normal pregnancy. MMPs are also regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs). MMP/TIMP imbalance has been implicated in

various vascular diseases including atherosclerosis, hypertension and aortic aneurysm. MMPs may also play an important role in the regulation of venous structure and function, MMP imbalance has been implicated in venous dysfunction and the pathogenesis of chronic venous disease (CVD).³ One of the manifestations of CVD is varicose veins (VVs). VVs are a common health problem manifested as large, unsightly dilated and tortuous veins of the lower extremities. If untreated, VVs may lead to chronic venous insufficiency (CVI) with skin changes and venous leg ulcers. VVs may also lead to other venous complications including thrombophlebitis and deep venous thrombosis. Therefore, it is imperative to carefully examine the mechanisms involved in CVD in order to develop better treatment approaches.

In this chapter, we will review data reported in PubMed and other scientific databases as well as data from our laboratory to provide insights on the role of MMPs in the regulation of vein structure and function, the remodeling of lower extremity veins, and the pathogenesis of CVD. We will describe the structural and functional abnormalities observed in VVs, and the changes in MMP expression/activity associated with VVs. We will discuss the potential factors that could drive the changes in venous tissue MMPs including increases in the lower limb venous hydrostatic pressure, the inflammatory response, hypoxia, and the various endogenous and exogenous MMP activators and inducers. We will also discuss the mechanisms of action of MMPs and how they could cause increases in ECM turnover as well as endothelial cell and VSM dysfunction leading to progressive venous dilation and VVs formation. We will conclude the chapter by summarizing some of the current medical and surgical approaches used for treatment of VVs, and explore the potential benefits of overexpression of endogenous TIMPs or exogenous application of synthetic MMP inhibitors as novel tools in the management of VVs.

2. CHRONIC VENOUS DISEASE (CVD)

CVD is a common disorder of the lower extremity venous system with major social and economic implications. According to the clinical-etiology-anatomy-pathophysiology (CEAP) classification, CVD could have several clinical stages, C0–6. The C0 stage shows no visible signs of CVD. C1 is manifested as telangiectasies or spider veins. C2 is presented as VVs. C3 is associated with edema. C4a shows skin pigmentation or eczema and C4b shows lipodermatosclerosis or atrophie blanche. The C5 stage shows healed ulcer, and the C6 stage shows active ulcer. The advanced stages C4–6 of CVD are often described as chronic venous insufficiency (CVI).⁵

VVs is a common venous disorder affecting approximately 25 million adults in the United States.⁶ VVs are presented as abnormally distended and tortuous superficial veins of the lower extremity. In addition to vein wall dilation and tortuosity, VVs often show incompetent venous valves and measurable venous reflux (Fig. 2). While VVs are often detected in the lower extremity, the vein pathology may not be confined to the lower limb veins. It is possible that VVs is a sign of a generalized pathology in the venous system that is mainly manifested in the lower limb veins because of the high venous hydrostatic pressure. In support of this paradigm, patients with VVs also show increased distensibility in their arm veins, suggesting a generalized disorder in the venous system.⁷ In addition to their

socioeconomical impact and unsightly cosmetic appearance, VVs can lead to major complications such as thrombophlebitis, deep venous thrombosis, and venous leg ulcers.⁵

Several risk factors may lead to VVs, e.g. advanced age, female gender, contraceptive pills and estrogen therapy, pregnancy, overweight and obesity, prior leg injury, vein inflammation and phlebitis. Estrogen may activate estrogen receptors in the vein wall leading to venous dilation, and females show enhanced estrogen receptor-mediated venous dilation and more distended veins when compared with males.⁸ Estrogen is markedly increased and may contribute to the increased venous dilation during pregnancy. Also, during pregnancy the progressive increases in uterine size and maternal body weight along with the changes in hemodynamics and cardiac output could lead to increased hydrostatic pressure in the lower extremity veins and the development of VVs. Behavioral factors such as sedentary lifestyle and prolonged standing could also increase the risk for CVD.^{9–11}

Family history and hereditary and genetic factors may represent potential risk factors for VVs.¹² Primary lymphedema-distichiasis is a rare syndrome involving a mutation in the FOXC2 region of chromosome 16, and is associated with VVs in early age.¹³ A study on nine families have shown a link between VVs and the D16S520 marker on chromosome 16q24 near the FOXC2 region, providing evidence that VVs could be linked to FOXC2, and that CVD could be inherited in an autosomal dominant mode with incomplete penetrance.¹⁴ Patients with Klippel-Trenaunay Syndrome also have VVs, supporting heritability of VVs.¹⁵ The lower limb venous dynamic and vein wall elasticity may also be reduced in children of VVs patients.¹⁶ Evidence also suggests a genetic component of VVs. A heterozygous mutation in the Notch3 gene has been identified in the cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) pedigree with VVs.¹⁷ Microarray analysis of 3,063 human cDNAs from VVs showed upregulation of 82 genes, particularly those associated with the regulation of ECM, cytoskeletal proteins, and myofibroblasts.¹² Subjects with Ehlers-Danlos syndrome type-IV are prone to developing vascular pathology and VVs.¹⁸ A single nucleotide polymorphism in the promoter region of MMP-9 gene has been identified in Chinese individuals with VVs, and a significant correlation has been found between patients with VVs and controls at -1562C/T in the gene promoter of MMP-9.¹⁹

Desmuslin is an intermediate filament protein important in smooth muscle function, and mutations or nucleotide polymorphic variant may be associated with VVs. In support, human saphenous vein smooth muscle cells (SMCs) treated with desmuslin siRNA showed increased collagen synthesis and MMP-2 expression and decreased expression of the phenotype and differentiation markers SM α -actin, SM-myosin heavy chain, and smoothelin and exhibited disassembly of actin stress fibers when compared with the control cells. These observations have suggested that desmuslin is required for maintaining the VSMC phenotype, and that decreased desmuslin expression may affect differentiation of VSMCs and ultimately contribute to the development of VVs. Other genetic defects have been implicated in advanced stages of CVD especially the venous leg ulcer risk and delayed healing, and include the genes for hemochromatosis, ferroportin, factor XIII, fibroblast growth factor receptor-2, and MMP-12.²⁰

3. STRUCTURAL AND FUNCTIONAL ABNORMALITIES IN VVS

VVs often appear as dilated, engorged, and tortuous veins, giving the impression that the lower limb veins may be undergoing marked hypertrophic remodeling. However, structural and histological evidence suggest that VVs may have not only hypertrophic, but also atrophic regions (Fig. 2).²¹ The hypertrophic regions of VVs often show abnormal VSMC shape and orientation and ECM accumulation. In contrast, the atrophic regions show ECM degradation and an increase in inflammatory cell infiltration.²² Histological examination of tissue sections of VVs does not show distinct vascular layers, and there are no clear boundaries between the tunica intima, media, and adventitia. Tissue sections of VVs, show focal intimal thickening, increased medial thickening and fragmentation of elastin fibers.²³ In tissue sections of VVs, VSMCs appear disorganized in the tunica media and the adjacent intima, with abundant ill-defined unstructured material. Also, collagen fibers appear disorganized making it difficult to delineate the tunica media from the adventitia, and the elastic fibers appear thick and fragmented in the tunica intima and adventitia.⁴

VVs show imbalance in the protein components of ECM mainly due to changes in collagen and/or elastin content. Collagen measurements in the walls of VVs show marked variability ranging from an increase,²⁴ to a decrease,²⁵ or no change.²⁶ Experiments on cultured VSMCs from VVs and cultured dermal fibroblasts from patients with CVD have shown an increase in the synthesis of type-I collagen and a decreased in the synthesis of type-III collagen, with no apparent change in gene transcription. These observations suggest that patients with VVs have post-translational inhibition of type-III collagen synthesis and may have a systemic abnormality in collagen production in various tissues. Type-III collagen is a critical factor in determining the elasticity and distensibility of blood vessels, and alterations in collagen synthesis and the collagen type-I/type-III ratio could cause marked changes in the vein wall integrity, leading to structural weakness in the vein wall, venous dilation, and formation of VVs.⁴ Some studies have suggested that a decrease in the elastin content could play a role in the pathogenesis of VVs, as it may cause a decrease in the vein wall elasticity and lead to vein wall dilation.²⁷ However, other studies have suggested that the elastin network may be increased in VVs.⁴

In addition to the changes in the vein wall, VVs also show incompetent venous valves. However, whether valve dysfunction is a primary event that leads to vein wall dilation, or vice versa, the changes in VVs wall lead to valve dysfunction is debatable. It has been suggested that valve dysfunction could lead to venous reflux and high venous hydrostatic pressure, and the excessive and prolonged pressure could lead to progressive damage and dilation of the vein wall. The dilation of the vein segments in close proximity to the vein valves would then cause further distortion and disruption of the valves leading to further increases in venous reflux, venous hydrostatic pressure and progressive vein wall dilation. This view has been supported by the observations that VVs show hypertrophy of the vein valves, increased width of the valvular annulus,²⁸ decreased collagen content and viscoelasticity,²⁹ and increased inflammation and monocyte and macrophage infiltration in the valvular sinuses compared to distal VVs walls.³⁰ However, this view has been challenged by the observation that vein wall dilation and VVs may sometimes be seen below competent venous valves.²¹ Also, increased collagen and decreased elastin have been

observed not only in VVs segments but also in competent saphenous vein segments in close proximity to the varices, suggesting that imbalance in ECM proteins may occur in the vein wall prior to the vein valve insufficiency.²⁴ Also, VVs do not always develop in a descending direction i.e. from the thigh, to the calf, and ankle, and antegrade progression in VVs in the normal direction of venous flow from the ankle, to the calf and the thigh may be caused by primary changes in the vein wall, which could lead to dysfunction of the venous valves.²¹ These observations lend support to the suggestion that vein wall dilation could be a primary pathological event that could cause distortion and dysfunction of the venous valves, and lead to venous reflux and higher venous hydrostatic pressure, and ultimately causing progressive venous dilation and VVs.³¹ Regardless of which one is the primary pathological event, both vein wall dilation and venous valve dysfunction appear to contribute to the pathogenesis of VVs and abnormal venous blood flow. This is typically manifested as a venous reflux or backflow of blood away from the heart that lasts longer than 0.5 second in the superficial VVs.³¹

4. MMP LEVELS IN VVS

VVs may show significant changes in MMP expression/activity.³ Studies have shown an increase in the levels of MMP-1, -2, -3, and -7 with a prominent increase in MMP-2 activity in VVs.⁴ Increased plasma levels of MMP-10 and the hemostatic markers d-dimers, prothrombin fragments 1 and 2, von Willebrand factor, and activity of plasminogen activator inhibitor (PAI-1) have also been observed in patients with primary VVs, suggesting a prothrombotic and proinflammatory state.³² Other studies have shown an increase in MMP-1 protein level in the great saphenous vein, and an increase in the levels of MMP-1 and -13 in the proximal versus distal segments of VVs, with no change in MMPs mRNA expression, suggesting that the increased MMPs levels are related to changes in MMP post-transcriptional modification or protein degradation.³³ The levels of MMPs may also vary within the different tissue layers and cellular components of the VVs wall. Immunohistochemical analysis in tissue sections of VVs showed prominent localization of MMP-1 in fibroblasts, VSMCs, and endothelial cells, MMP-9 in endothelial cells, medial VSMCs and adventitial microvessels, and MMP-12 in VSMCs and fibroblasts.³⁴ Other studies have shown increased MMP-1 expression in all layers of VVs and MMP-9 expression in the intimal and adventitial layers of VVs.²³ The localization of MMPs in the tunica adventitia and fibroblasts is consistent with the role of MMPs degradation of ECM proteins. Interestingly, studies also showed increased levels of MMP-2 levels in all layers of the vein wall, and of MMP-1, -3 and -7 in the tunica intima and media of VVs,⁴ suggesting additional effects of MMPs on the endothelium and VSMCs.

Although several studies have shown increases in the levels of certain MMPs in VVs, some studies have shown no change or even a decrease in the levels of MMPs. One study has shown that the levels of active MMP-1 and both pro and active forms of MMP-2 are decreased in VVs.³⁵ The variability in the levels of MMPs may explain the variability in the measurements of collagen content in VVs wall which ranged from a decrease.²⁵ to no change,²⁶ or even an increase.²⁴ The variability in the levels of MMPs may be due to examining different vein segments from different regions of VVs, i.e. hypertrophic versus

atrophic regions, or examining vein specimens from patients at different stages of CVD, or inability to distinguish between pro- and active forms of MMPs.

Changes in MMP expression/activity have also been associated with the progression of CVD and advanced stages of CVI. Studies have shown elevated serum levels of MMP-2, ADAMTS-1 and ADAMTS-7 in the initial stages of CVD, whereas the serum levels of MMP-1, -8, -9, neutrophil gelatinase-associated lipocalin (NGAL), ADAM-10 and -17 and ADAMTS-4 were particularly elevated during CVD complications and skin changes.³⁶ The collagenases MMP-1 and -8 are overexpressed in the fluids and tissues of long lasting non-healing chronic venous ulcers.³⁷ The levels of MMP-1 and -8 were even higher in patients with infected ulcers than those with uninfected ulcers.³⁸

5. POTENTIAL MMP INDUCERS/ACTIVATORS IN VVs

Multiple factors can induce or activate MMPs *in vitro*, *ex vivo* and *in vivo*. Many factors could modulate the expression/activity of MMPs in VVs including increases in lower extremity venous hydrostatic pressure, inflammation of the vein wall, hypoxia, and other factors.

5.1 Venous Hydrostatic Pressure and MMPs in VVs

Increased lower extremity venous hydrostatic pressure is a major factor that could lead to increased expression/activity of MMPs in VVs (Fig. 3). Studies have suggested that mechanical stretch may lead to increases in the expression of MMPs in endothelial cells, VSMCs and fibroblasts.³⁹ We have also shown that prolonged increases in mechanical tension or wall stretch of isolated rings of rat inferior vena cava (IVC) are associated with increased expression of MMP-2 and -9 in the tunica intima and increased MMP-9 in the tunica media of the vein wall. Prolonged IVC stretch was also associated with decreased vein contraction to the α -adrenergic agonist phenylephrine. Importantly, in IVC pretreated with specific MMP inhibitors, prolonged mechanical stretch did not cause decreases in IVC contraction. These observations suggested that prolonged increases in venous pressure/wall tension may cause changes in MMP expression/activity, which in turn cause decreases in vein contraction, and thereby increase venous dilation.⁴⁰ The factors linking the increased venous pressure to increased MMP expression in the vein wall are not clearly understood but may involve intermediary factors such as inflammation or hypoxia inducible factors (HIFs).⁴¹

5.2 Inflammation and MMPs in VVs

Endothelial cells are exposed to marked fluctuations in blood flow, and increases in venous pressure could cause endothelial cell injury, increased permeability, activation of adhesion molecules, leukocyte infiltration of the vein wall, and collectively these factors could contribute to inflammation of the vein wall.⁴² Rat models of increased lower extremity venous pressure have been produced by induction of femoral arterio-venous fistula. These rat models show increased venous pressure in the saphenous vein, and the prolonged increases in venous pressure are associated with leukocyte infiltration, increased expression of intercellular adhesion molecule-1 (ICAM-1) and P-selectin, and inflammation of the vein

wall.⁴³ Leukocytes are a major source of MMPs.⁴⁴ Accumulation of adhesion molecules facilitates leukocyte adhesion and infiltration of the vein wall, and leads to further inflammation and increased expression/activity of MMPs. MMPs in turn cause degradation of ECM proteins, weakening of the vein wall, vein wall dilation, vein valve dysfunction, further increases in lower limb venous hydrostatic pressure, and advanced stages of CVD (Fig. 3).⁴⁵ The link between increased lower extremity venous hydrostatic pressure, vein wall inflammation and increased MMP expression/activity may be well-presented in the atrophic regions of VVs, where increased degradation of ECM proteins often occurs.

Studies have shown increased monocyte/macrophage infiltration in the walls and valves of saphenous vein specimens from patients with CVD.^{30,46} In addition to the increased inflammatory cell infiltration, VVs specimens show increased endothelial cell expression of ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1).⁴⁷ Also, the plasma levels of endothelial cell and leukocyte activation markers such as ICAM-1, VCAM-1, angiotensin converting enzyme, and L-selectin are increased in patients with VVs, and the increases in inflammatory markers are associated with increases in the plasma levels of proMMP-9, lending support to a potential relation between postural blood stasis and increase in lower extremity venous hydrostatic pressure, polymorphonuclear leukocyte infiltration and activation and increased release of MMPs in VVs.⁴⁸

Inflammatory cytokines could play a role linking pressure-induced leukocyte infiltration, vein wall inflammation and increases in MMP expression/activity. Urokinase could contribute to the inflammatory response by increasing the expression of tumor necrosis factor- α (TNF- α) in damaged vessels. TNF- α could in turn increase the activity of MMP-9 gene promoter partly through activation of activator protein-1 (AP-1), specificity protein-1 (Sp-1), or nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B).⁴⁹ Other cytokines including interleukins IL-17 and -18 may induce MMP-9 expression via activation of AP-1 and NF- κ B dependent pathways.⁵⁰ Interestingly, studies have shown that higher levels of MMP-1 and -8 are associated with higher levels of IL-1, -6, -8, vascular endothelial growth factor (VEGF) and TNF- α in patients with infected venous leg ulcers compared to those with uninfected ulcers, documenting a possible association between infection, MMP activation, cytokine secretions and CVD symptoms.³⁸

Cytokines are known to increase reactive oxygen species (ROS), which could in turn affect MMP expression/activity. Studies in fibroblasts have suggested that the levels of MMP expression may be influenced by the levels of NADPH oxidase-1 (Nox-1).⁵¹ Urokinase may affect MMP-9 expression partly through increasing the generation of ROS.⁵² While leukocytes are a major source of MMPs, they also generate ROS that can influence MMP activity. For instance, ROS may activate MMPs via oxidation of the MMP prodomain thiol followed by autolytic cleavage. On the other hand, ROS may inactivate MMPs by modifying the amino acids critical for catalytic activity, thus providing a feedback-mechanism that could control any bursts in MMP proteolytic activity.⁵³

5.3 Hypoxia and MMPs in VVs

Hypoxia inducible factors (HIFs) could provide a potential mechanism linking increases in lower extremity venous hydrostatic pressure, to the increases in MMP expression/activity

and reduced venous contraction (Fig. 4). HIFs are major nuclear transcriptional factors that typically regulate most of the genes involved in oxygen homeostasis. Interestingly, mechanical stretch may influence the expression/activity of HIFs. Studies have shown that exposure of rat skeletal muscle fibers to prolonged mechanical stretch is associated with increased mRNA expression and protein levels of HIF-1 α and -2 α in the skeletal muscle capillary endothelial cells.⁵⁴ Also, experiments on the rat ventricle have suggested that mechanical stretch of the ventricular wall is associated with upregulation of HIF-1 α .⁵⁵ We have tested the role of HIFs in mechanical stretch-induced reduction of vein contraction in rat IVC. We have found that prolonged increases in wall tension in isolated segments of rat IVC are associated with increases in the mRNA expression and protein levels of not only MMP-2 and -9, but also HIF-1 α and -2 α . The increases in MMP and HIF expression and protein levels were associated with a decrease in the magnitude of IVC contraction to phenylephrine. Interestingly, vein contraction was further reduced in IVC pretreated with the HIF stabilizer dimethylxaloylglycine (DMOG), which prevents inactivation of HIF by HIF-prolyl hydroxylase. On the other hand, the reduction in IVC contraction was reversed in tissues pretreated with the HIF inhibitors U0126 and echinomycin, supporting a role of HIF as a linking mechanism between increased venous hydrostatic pressure and reduced venous contraction (Fig. 4).⁴¹ An important question is how mechanical stretch could affect HIFs. One possibility is that mechanical stretch could activate Ca²⁺ influx through transient receptor potential (TRP) channels such as TRPV4, and the increases in Ca²⁺ could activate phosphoinositide 3-kinase (PI₃K), which could in turn affect HIF.⁵⁶ Another possibility is that mechanical stretch could interact with the cell membrane integrins which could activate a cascade of intracellular signaling pathways that ultimately activate mitogen-activated protein kinase (MAPK) and affect HIF expression. As a form of biomechanical stress, mechanical stretch could activate G protein-coupled receptors (GPCRs) or receptor tyrosine kinases or increase the formation of ROS, which could lead to activation of MAPK. We have found that the increase in HIF mRNA expression and the reduction in IVC contraction associated with prolonged vein wall stretch are reversed in IVC treated with MAPK inhibitors, supporting a role of MAPK in transducing the effects of mechanical stretch on HIF.⁴¹

Studies in patients with VVs support a role of HIF in the pathogenesis of CVD. The expression of HIF-1 α and -2 α and HIF target genes is upregulated in VVs.⁵⁷ Studies also suggest that HIF-1 α may regulate the expression of MMP-2 and -9 in patients with hemodialysis polytetrafluoroethylene grafts or arteriovenous fistulas.⁵⁸ In addition to mechanical stretch, other factors such as low oxygen tension, low pH, cytokines, hormones and heat may influence HIF expression and, in turn, affect venous MMP expression/activity.

5.4 Other MMP Inducers/Activators in VVs

Other MMP inducers/activators have been identified in the veins and may promote MMP expression/activity in VVs. Extracellular MMP inducer (EMMPRIN, CD147, Basigin) is a widely expressed membrane protein of the immunoglobulin superfamily. EMMPRIN has been suggested to play a role in tissue remodeling and has been implicated in pathological conditions such as atherosclerosis, aneurysm, heart failure, rheumatoid arthritis, and cancer. Studies have shown that high volume mechanical ventilation causes acute lung injury, and is

associated with upregulation of MMP-2, MMP-9, and MT1-MMP as well as EMMPRIN.⁵⁹ Also, EMMPRIN along with MMP-2, MT1-MMP and MT2-MMP are overexpressed in dermal structures of venous leg ulcers, which could lead to unrestrained activation of MMPs and enhanced ECM turnover.⁶⁰

Prostanoids are bioactive lipids produced by many vascular cells and may interact with MMPs in the pathogenesis of VVs. Prostaglandin E2 (PGE2) through activation of EP1–4 receptor subtypes play a role in the regulation of vascular tone, inflammation and vascular wall remodeling.⁶¹ Activation of EP2/EP4 receptors by PGE2 is associated with increased MMP activity in human endometriotic epithelial and stromal cells.⁶² PGE2 synthesis may be decreased in VVs due to a compensatory increase in anti-inflammatory 15-deoxy-delta-12,14-PGJ2, a decrease in membrane-associated prostaglandin E synthase-1, and an increase in the degrading enzyme 15-hydroxyprostaglandin dehydrogenase. The overall decrease in PGE2 and its EP4 receptor activity may then cause a decrease in the activity of MMP-1 and -2 and lead to increased collagen deposition, which may explain the hypertrophic remodeling observed in some regions of VVs.³⁵

Chymase is a chymotrypsin-like serine protease purified from mast cell granules and mammalian cardiovascular tissues. Chymase has been implicated in the increased MMP-9 activity and the accumulation of monocytes and macrophages observed in the aorta of stroke-prone spontaneously hypertensive rats.⁶³ Also, gonadal hormones such as estrogen and progesterone may increase the expression/activity of MMP-2 and -9 in uteroplacental and vascular tissues.^{64,65} Some MMP modulators could prevent MMP degradation, and thereby increase MMP levels and activity. For instance, NGAL may form a complex with MMP-9, which would protect MMP-9 from proteolytic degradation and thereby increase its levels and activity.⁶⁶ Whether these MMP inducers/activators are increased in VVs needs to be further examined.

6. MECHANISMS OF MMP ACTIONS IN VVS

MMPs are widely recognized for their proteolytic effects on ECM proteins, and the effects of MMPs on ECM protein degradation and tissue remodeling could play an important role in the pathogenesis of VVs. However, MMPs could also affect other components of the vein wall and influence other cellular and molecular pathways in VSMCs and the endothelium. These additional signaling effects of MMPs could affect vein function, and may play a role at least in the initial vasodilatory stages of VVs.

6.1 MMPs and ECM Abnormalities in VVs

Changes in MMP activity could alter the composition of ECM and, in turn, contribute to the structural and functional abnormalities associated with in VVs. While it is widely believed that venous tissue levels of MMPs increase in VVs, decreases in MMP levels have also been observed,³⁵ and this may partially explain the different venous pathologies in the atrophic versus hypertrophic regions of VVs. An increase in MMP activity is predicted to degrade and decrease ECM proteins in the atrophic regions of VVs.²² On the other hand, a decrease in MMP activity may lead to ECM accumulation in the media of hypertrophic regions of

VVs, which could interfere with the contractile function of VSMCs, thus hindering venous contraction and leading to venous dilation and VVs.⁶⁷

The content of ECM could be determined by MMP-induced degradation of major ECM proteins such as collagen and elastin. Collagen content varies in VVs compared to normal veins, with an increase in collagen type I and a decrease in collagen type III.^{68–70} In cultured VSMCs from VVs, collagen type III and fibronectin are decreased likely due to post-transcriptional degradation by MMP-3.⁷⁰ Elastin levels may also show a decrease in VVs possibly due to increased elastolytic activity of MMPs or other elastases produced by macrophages, monocytes, platelets, and fibroblasts.²⁷ The changes in collagen and elastin content in VVs are dynamic processes that may depend on the stage of CVD. For instance, increased collagen content may compensate for the decreased elastin levels at early stages of VVs. On the other hand, collagen levels may decrease at later stages of CVD. This may partly explain the divergent reports regarding the collagen content in VVs, with some studies showing a decrease,²⁵ while other studies showing hardly any change^{26,27} or even an increase.²⁴ Other ECM proteins may show changes in VVs, e.g. increases tenascin and decreases in laminin.^{68–70}

6.2 MMPs and VSM Dysfunction in VVs

In addition to changes in venous tissue remodeling and ECM proteins, VVs may show alterations in VSMC growth, migration, and contractile function (Fig. 4). When compared with contractile VSMCs from normal veins, VSMCs from VVs are more dedifferentiated and show increased migration, and MMP-2 levels, which may contribute to vein wall remodeling and weakening against increased venous hydrostatic pressure.⁷¹ MMP-1 and -9 have also been shown to increase human aortic SMC migration.^{72,73} MMP-induced ECM proteolysis may modulate cell-matrix adhesion either by removal of sites of adhesion or by exposing a binding site and in turn facilitate VSMC migration. MMPs can also facilitate VSMC growth by promoting permissive interactions between VSMCs and components of ECM, possibly via integrin-mediated pathways.⁷⁴ MT1-MMP may stimulate the release of transforming growth factor- β (TGF- β) and promote the maturation of osteoblasts.⁷⁵ Also, upregulation of MMP-2 increases the expression of VEGFa, while downregulation of MMP-2 decreases VEGFa expression in human gastric cancer cell line SNU-5.⁷⁶ MMPs may also promote the release of growth factors by cleaving the growth factor-binding proteins or matrix molecules, and this may partly explain the VSMC hypertrophy observed in some parts of the hypertrophic regions of VVs.⁷⁷

While MMPs could stimulate growth factor release, MMPs can be regulated by growth factors.⁷⁸ For example, overexpression of VEGFa in SNU-5 cells increases MMP-2 expression, while downregulation of VEGFa decreases MMP-2 expression.⁷⁹ Also, platelet derived growth factor-BB (PDGF-BB) increases MMP-2 expression in rat VSMCs, possibly via Rho-associated protein kinase, extracellular signal-regulated kinases, and p38 MAPK phosphorylation.⁸⁰ Also, in a study on carotid plaques, EGF upregulated MMP-9 activity and increased MMP-1, -9, and EGFR mRNA transcripts in VSMCs.⁸¹ Other studies have shown that TGF- β 1 could induce the expression of MMP-9 and -12 and TIMP-1 and -2 in

the great saphenous vein, and suggested the involvement of TGF- β 1 in the vein wall pathology.²³

Because synthetic VSMCs do not contract, MMP and growth factor-mediated VSMC dedifferentiation and migration are likely to decrease venous contraction and promote dilation (Fig. 4). MMPs may also affect VSM contraction mechanisms. VSM contraction is triggered by increases in Ca^{2+} release from the intracellular stores and Ca^{2+} entry from the extracellular space. MMPs do not inhibit phenylephrine-induced aortic contraction in Ca^{2+} -free medium, suggesting that they do not inhibit the Ca^{2+} release mechanism in the sarcoplasmic reticulum.⁸² On the other hand, MMP-2 and -9 cause aortic relaxation by inhibiting Ca^{2+} influx,⁸² and MMP-2 inhibits Ca^{2+} -dependent contraction in rat IVC.⁸³ It has been proposed that during substrate degradation MMPs may produce Arg-Gly-Asp (RGD)-containing peptides, which could bind to $\alpha_v\beta_3$ integrin receptors and inhibit Ca^{2+} entry into VSM.⁸⁴ This is an unlikely mechanism as our data have shown that RGD peptides do not affect contraction of IVC segments.⁸³ The mechanism by which MMPs inhibit Ca^{2+} entry may likely involve direct effects on plasma membrane Ca^{2+} or K^+ channels. In rat IVC, MMP-2 induced relaxation is abolished in high KCl depolarizing solution, which prevents K^+ ion from moving out of the cell via K^+ channels. Importantly, blockade of large conductance Ca^{2+} -activated K^+ channels (BK_{Ca}) by iberiotoxin inhibited MMP-2 induced IVC relaxation, suggesting that MMP-2 actions may involve hyperpolarization and activation of BK_{Ca} , which in turn lead to decreased Ca^{2+} influx through voltage-gated Ca^{2+} channels (Fig. 4).⁸⁵ Sustained MMP-induced inhibition of venous tissue Ca^{2+} influx and contraction mechanisms may lead to progressive venous dilation and VVs.

6.3 MMPs and Endothelium-Dependent Relaxation

The endothelium controls vascular tone by releasing relaxing factors such as nitric oxide (NO) and prostacyclin (PGI_2) and through hyperpolarization of the underlying VSMCs by endothelium-derived hyperpolarizing factor (EDHF).⁸⁶ MMPs may stimulate protease activated receptors (PARs), which are GPCRs that may play a role in venous dilation in VVs (Fig. 4). PARs 1–4 have been identified in humans, and MMP-1 has been shown to activate PAR-1.⁸⁷ PAR-1 is expressed in VSMCs,⁸⁸ endothelial cells, and platelets⁸⁹ and is coupled to increased NO production,⁹⁰ which could contribute to progressive venous dilation and the formation of VVs.

EDHF-mediated relaxation may involve the opening of small and intermediate conductance Ca^{2+} -activated K^+ channels and hyperpolarization of endothelial cells. Endothelial cell hyperpolarization may spread via myoendothelial gap junctions causing relaxation of VSMCs. Although the exact nature of EDHF is unclear, studies suggest that EDHF-mediated responses could involve epoxyeicosatrienoic acids, which are epoxides of arachidonic acid generated by cytochrome P450 epoxygenases. Other possible EDHFs include hydrogen peroxide or even the potassium ion.⁸⁵ EDHF could in turn cause vascular hyperpolarization through opening of BK_{Ca} in VSMCs.⁸⁶ MMP-2 may increase EDHF release and enhance K^+ efflux via BK_{Ca} , leading to venous tissue hyperpolarization and relaxation.⁸⁵ In contrast, MMP-3 may be associated with impaired endothelium-dependent

vasodilation,⁹¹ making it important to further examine the effects of MMPs on endothelium-derived relaxing factors.

7. MANAGEMENT OF VVS

Management of VVs comprises physical approaches such as graduated compression stockings. Graduated elastic compression stockings enhance venous emptying, reduce the pain and edema, and may slow the progression of VVs to the more advanced forms of CVI manifested with skin changes and venous leg ulcer.⁹² Compression stockings could also decrease the incidence of venous thromboembolism following VVs surgery, and improve hemodynamic performance in post-thrombotic syndrome.⁹³ Pharmacological treatment of VVs includes venotonic drugs that improve venous tone and capillary permeability, and reduce leukocyte infiltration. Venotonic drugs include naturally occurring plant extracts and glycosides such as α -benzopyrones (coumarins), γ -benzopyrones (flavonoids), plant extracts (blueberry and grape seed, ergots, Ginkgo biloba), and saponosides (Centella asiatica, escin, horse chestnut seed extract, ruscus extract).⁴⁵ Benzopyrones include catechin (Green tea), dicoumarols, diosmin (Daflon-500), escletin, flavonoic acid, hesperitin, hesperidine, oxerutin, quercetin, rutosides, troxerutin, umbelliferone, and venoruton.⁹⁴ Diosmin is a flavonoid and an active ingredient in Daflon-500 that may improve venous tone, microvascular permeability, lymphatic activity, and microcirculatory flow.⁹⁵ Rutosides enhance endothelial function in patients with CVI.⁹⁶ Flavonoids may affect the endothelium and leukocytes and reduce edema and inflammation. Saponosides, such as escin (horse chestnut seed extract), may limit venous wall distensibility and morphologic changes. We have shown that escin promotes Ca^{2+} -dependent venous contraction.⁹⁷ Other compounds have been tested in advanced CVI and include pentoxifylline, a xanthine derivative with beneficial anti-inflammatory and hemorheologic properties including inhibition of $\text{TNF-}\alpha$ and leukotriene synthesis and improved red blood cell deformability,⁹⁸ and red vine leaves (AS 195) and PGE1, which may increase microcirculatory blood flow and transcutaneous oxygen tension and alleviate edema.⁴⁵

Other approaches have been developed to obliterate dilated VVs and improve venous hemodynamics. Sclerotherapy under Duplex ultrasound guidance entails injection of concentrated sclerosing agents such as hypertonic saline, sodium morrhuate, and ethanolamine oleate in the dilated veins. Some of the FDA-approved sclerosing agents for treatment of VVs include sodium tetradecyl sulfate (STS), which is a liquid detergent, sodium morrhuate, and polidocanol.⁹⁹ STS and polidocanol are also used in a foam state to displace blood and reduce thrombosis in VVs. Proprietary polidocanol endovenous microfoam has recently been approved by the FDA, and has shown excellent results and improved quality of life in patients with VVs.¹⁰⁰

Management of VVs may also include surgical approaches such as endovenous ablation with a radiofrequency or infrared laser typically at wavelengths 810 to 1320 nm, but as high as 1470 and 1550 nm. The high endoluminal thermal heat causes denaturation of endothelial proteins and leads to vein occlusion.¹⁰¹ Ablation therapy shows relatively good outcome and vein occlusion rates with a 2% vein recanalization rate 4 years after radiofrequency therapy¹⁰² and 3–7% VVs recurrence rate 2–3 years after infrared laser therapy.¹⁰³ Surgical

stripping of the saphenous vein with high ligation of the saphenofemoral junction is another surgical approach with low recurrence rates. “Stab phlebectomy” is another surgical approach that entails avulsion of large VVs clusters that communicate with the incompetent saphenous vein. Transilluminated power phlebectomy is an alternative to open phlebectomy that removes clusters of VVs using fewer incisions and shorter operation time.¹⁰⁴

In addition to ultrasound guided foam sclerotherapy, innovative technologies for endovenous treatment of VVs are emerging and involve the use of non-thermal and non-tumescent techniques such as cyanoacrylate glue and other mechanochemical methods.^{105,106} Initial results are encouraging, but additional studies are needed to further evaluate the benefits of these new techniques versus thermal ablation and surgery.

8. POTENTIAL BENEFITS OF MMP INHIBITORS IN VVS

Currently available treatment options for VVs focus mainly on the symptoms rather than the causes of CVD. The identification of the role of MMPs in the pathogenesis of CVD has prompted the search for inhibitors of MMP expression or activity. MMP inhibitors can be used to prevent MMPs from binding with their substrates and degrading the ECM, and thereby prevent the development or recurrence of VVs. MMP inhibitors include endogenous inhibitors such as TIMPs and α 2-Macroglobulin, as well as synthetic Zn^{2+} -dependent and Zn^{2+} -independent inhibitors.

8.1 TIMPs and MMP/TIMP Ratio

TIMPs are endogenous, naturally occurring MMP inhibitors that bind MMPs in a 1:1 stoichiometry.^{107,108} TIMPs include four homologous, TIMP-1, -2, -3 and -4. TIMP-1 and -3 are glycoproteins, but neither TIMP-2 nor TIMP-4 protein contain carbohydrates. TIMPs do not show high specificity toward a specific MMP, and inhibit different MMPs with different efficacies. For example, TIMP-1 is a poor inhibitor of MT1-MMP, MT3-MMP, MT5-MMP and MMP-19, while TIMP-2 and -3 can inhibit MT1-MMP and MT2-MMP.¹⁰⁹ Also, while TIMP-1 and -2 can bind MMP-10 (stromelysin-2), their binding is 10-fold weaker than that to MMP-3 (stromelysin-1).¹¹⁰ Importantly, TIMP-1 has a threonine-2 (Thr2) side chain that enters the MMP S1' pocket in a manner similar to that of a substrate P1' substituent, largely determining the affinity to MMP-3. Substitutions at Thr2 could affect the stability of the TIMP-MMP complex and the TIMP specificity for different MMPs. For example, a substitution of alanine for Thr2 is associated with a 17-fold decrease in binding of TIMP-1 to MMP-1 relative to MMP-3.¹¹¹

TIMPs have been localized in different regions within the veins. Studies have tested whether histological changes in VVs wall may correlate with alterations in the expression of MMPs and TIMPs. VVs were compared with great saphenous vein segments from arterial bypass, and with arm and neck veins from fistula and carotid operations. There was a higher expression of TIMP-2 and increased connective tissue accumulation in the tunica media of VVs compared with control arm and neck veins. TIMP-2 and -3 expression was higher in hypertrophic than atrophic segments, and in the thicker proximal segments compared to the distal segments of VVs. It was suggested that a higher TIMP expression would suppress protease activity, reduce ECM turnover, and favor deposition of connective tissue and thicker

vein wall.¹¹² Other studies showed TIMP-1, -2, and -3 in the intima and TIMP-1 and -2 in the media of control veins, as compared to TIMP-1 and -3 in the intima and TIMP-1, -2, and -3 in the media of VVs.⁴

An imbalance of MMP/TIMP ratio may contribute to the development of VVs. A change in either TIMP or MMP levels could alter the MMP/TIMP ratio and cause a net change in specific MMP activity. In one study, MMP-7 and -9, and TIMP-1, -2 and -3 levels were only slightly modified, while MMP-1, -2 and -3 levels were increased, and these changes were accompanied by an increase in the elastic network and accumulation of collagen type I, fibrillin-1 and laminin in both the veins and the skin of patients with VVs compared with control subjects undergoing coronary bypass surgery. These findings suggest that an imbalance MMP/TIMP ratio could lead to disruption of ECM production/degradation balance, and the observed remodeling in both the veins and the skin of patients with VVs suggests systemic alterations of the connective tissue.⁴ Other studies showed a decrease in MMP-2/TIMP-1 ratio in avulsed VVs and suggested that the decrease in MMP-2 proteolytic activity could be the cause of the extensive accumulation of ECM observed in hypertrophic regions of VVs.¹¹³ Also, marked increases in plasma levels of MMP-2 and -9, TIMP-1 and -2, and the MMP-2/TIMP-2 ratio were observed in patients with leg venous ulcers compared with normal controls. In subjects with healed venous ulcers, there was a decrease in MMP-9 and TIMP-1 levels and in the MMP-2/TIMP-2 ratio compared to the baseline values.¹¹⁴ These observations highlight the importance of further examining the levels of TIMPs in comparison with MMPs in different regions of VVs at different stages of CVD.

8.2 Synthetic MMP Inhibitors

Deep sea water components such as Mg^{2+} , Cu^{2+} , and Mn^{2+} may inhibit MMP activity via a mechanism involving interference with Zn^{2+} binding at the MMP catalytic active site.¹¹⁵ Utilizing the Zn^{2+} binding property, several MMP inhibitors have been developed.^{45,116} MMP inhibitors often have a Zn^{2+} binding group, e.g. hydroxamic acid, carboxylic acid, sulfhydryl group.¹¹⁷ Zn^{2+} binding globulins (ZBGs) displace the Zn^{2+} -bound water molecule in an MMP and inactivate the enzyme. A ZBG is also an anchor that keeps the MMP inhibitor in the MMP active site and allows the backbone of the MMP inhibitor to enter the MMP substrate-binding pockets.¹¹⁸ Hydroxamic acids include succinyl, sulfonamide, and phosphinamide hydroxamates.^{117,119,120} Batimastat (BB-94), marimastat (BB-2516), and ilomastat (GM6001) are broad spectrum succinyl hydroxamates with a structure mimicking collagen, and inhibit MMPs by bidentate chelation of Zn^{2+} .^{117,121} Other ZBGs include carboxylic acids, sulfonylhydrazides, thiols, aminomethyl benzimidazole-containing ZBGs, phosphorous- and nitrogen-based ZBGs, and heterocyclic bidentate chelators.^{118,122,123} Tetracyclines and mechanism-based inhibitors also inhibit MMPs by chelating Zn^{2+} ion.¹¹⁷ SB-3CT (compound 40) is a mechanism-based MMP inhibitor that coordinates with MMP Zn^{2+} , thus allowing the conserved Glu202 in the MMP molecule to perform a nucleophilic attack and form a covalent bond with the compound.¹¹⁸ When compared to the traditional competitive Zn^{2+} chelating MMP inhibitors, the strong covalent bond in SB-3CT prevents dissociation of the MMP inhibitor and decreases the rate of catalytic turnover, and therefore reduces the amount of MMP inhibitor needed to saturate the MMP active site.¹²⁴

Some MMP inhibitors such as compound 37 do not have ZBGs and do not bind to the highly conserved Zn^{2+} binding group.¹²⁵ Instead, these MMP inhibitors undergo non-covalent interaction with the S1', S2', S3', and S4' pockets in the MMP molecule in a fashion similar to that of the substrate P1', P2', P3', and P4' substituents. The specificity and efficacy of the MMP inhibitor are determined by which pockets it blocks in the MMP molecule.¹¹⁷

Small interfering RNA (siRNA) may inhibit the transcriptional product of an MMP.¹²⁶ Also, sulodexide (SDX) is a glycosaminoglycan that decreases MMP-9 secretion from white blood cells without displacement of the MMP prodomain, and may inhibit MMPs with cysteine residues such as MMP-2 and -9.^{22,66} Statins such as atorvastatin inhibit the expression of MMP-1, -2, and -9 in human retinal pigment epithelial cells,¹²⁷ and decrease the release of MMP-1, -2, -3, and -9 from rabbit macrophages and rabbit aortic and human saphenous vein VSMCs.¹²⁸ Also, pravastatin suppresses the increase in cardiac MMP-2 and -9 activity in a rat model of heart failure.¹²⁹ Despite the marked advances in the design of MMP inhibitors, doxycycline is the only FDA-approved MMP inhibitor.¹³⁰ In a recent study examining the effects of doxycycline in leg venous ulcer, patients were randomized into two groups, one group received the most appropriate basic treatment including compression therapy followed or not by vein surgery plus oral low dose of doxycycline 20 mg b.i.d. for 3 months, while the second group of patients received basic treatment only. Patients receiving basic treatment plus doxycycline showed a higher healing rate of venous ulcers compared with patients receiving basic treatment only. In patients receiving basic treatment only, the lower healing rate was associated with higher levels of MMP-9, NGAL and VEGF in plasma, wound fluid and biopsies. It was suggested that doxycycline administration through its immunomodulatory and anti-inflammatory actions, and inhibitory effects on MMP, could improve ECM function and speed venous leg ulcer and wound healing.¹³¹ A major limitation of MMP inhibitors is that they cause musculoskeletal side effects manifested as joint inflammation, pain, stiffness, and tendonitis.¹³² MMP inhibitors have the potential to be used clinically in CVD if their selectivity against specific MMPs is enhanced and their general side effects are minimized using targeted site-specific delivery.¹³³

9. CONCLUDING REMARKS

MMPs play a major role in venous tissue remodeling and could be important biomarkers for the progression of CVD and potential targets for the management of VVs. However, understanding the role of MMPs in the pathogenesis of VVs has been challenging. More than one MMP are likely involved in VVs. Also, the changes in MMPs in the venous system may not be uniform. Vein remodeling is a dynamic process, and an increase in one MMP in one region may be paralleled by a decrease of other MMPs in other regions. Also, MMPs have different proteolytic activities towards different substrates, and their activities may vary during the course of CVD. MMP activity is also regulated by endogenous TIMPs and the MMP/TIMP ratio could vary in atrophic versus hypertrophic regions of VVs. Therefore, it is important to measure different MMPs and TIMPs in various regions of VVs and at different stages of CVD. Another challenge is that currently available MMP inhibitors have poor selectivity and many biologic actions, and may cause side effects.¹¹⁷ As more selective MMP inhibitors are developed, their effectiveness in treatment of VVs should be tested.

Targeted approaches to modify MMP expression/activity locally in the vicinity of VVs may also minimize any systemic side-effects.

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ABBREVIATIONS

ADAM	a disintegrin and metalloproteinase
ADAMTS	a disintegrin and metalloproteinase with thrombospondin motif
AP-1	activator protein-1
BK_{Ca}	large conductance Ca ²⁺ -activated K ⁺ channel
CEAP	clinical-etiology-anatomy-pathophysiology
CVD	chronic venous disease
CVI	chronic venous insufficiency
ECM	extracellular matrix
EDHF	endothelium-derived hyperpolarizing factor
EMMPRIN	extracellular matrix metalloproteinase inducer
GPCR	G protein-coupled receptor
GSV	great saphenous vein
HIF	hypoxia-inducible factor
ICAM-1	intercellular adhesion molecule-1
IL	interleukin
IVC	inferior vena cava
MAPK	mitogen-activated protein kinase
MMP	matrix metalloproteinase
MT-MMP	membrane-type MMP
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NGAL	neutrophil gelatinase-associated lipocalin
NO	nitric oxide

PAR	protease activated receptor
PDGF	platelet-derived growth factor
PGE₂	prostglandin E2
PI₃K	phosphoinositide 3-kinase
RGD	Arg-Gly-Asp
ROS	reactive oxygen species
SDX	sulodexide
siRNA	small interfering RNA
TGF-β	transforming growth factor- β
TIMP	tissue inhibitor of metalloproteinases
TNF-α	tumor necrosis factor- α
TRP	transient receptor potential
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
VSM	vascular smooth muscle
VSMC	VSM cell
VVs	varicose veins
Zn²⁺	zinc
ZBG	Zn ²⁺ binding globulin

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Lower Extremity Veins

Fig. 1.

The lower extremity venous system, and changes in VVs. The lower extremity has an intricate system of superficial and deep veins connected by perforator veins. Excessive vein wall dilation and incompetent venous valves could lead to superficial dilated spider vein or engorged and tortuous varicose veins.

Fig. 2.

Vein valves and blood flow in normal veins and VVs. Competent venous valves allow blood flow in the antegrade direction toward the heart (A). Vein dysfunction could progress to large dilated VVs with incompetent valves (B). VVs mainly show atrophic regions where an increase in MMPs increases ECM degradation, but could also show hypertrophic regions in which MMP/TIMP imbalance would promote VSMC hypertrophy and ECM accumulation, leading to tortuosity, dilation, defective valves, and venous reflux (B).

Fig. 3. Pathophysiology and management of CVD. Certain genetic, environmental and behavioral risk factors cause an increase in venous hydrostatic pressure in the lower extremity saphenous and femoral veins leading to valve dysfunction and venous reflux. Increased venous hydrostatic pressure also increases vein wall tension leading to increases in MMPs. Increased venous hydrostatic pressure could also cause endothelial cell injury, increased permeability, leukocyte infiltration, and increased adhesion molecules, inflammatory cytokines and reactive oxygen species (ROS) leading to further increases in MMPs. Increased MMPs may cause VSM hyperpolarization and relaxation as well as ECM degradation leading to vein wall dilation, valve dysfunction and progressive increases in venous hydrostatic pressure (vicious cycle). Increased MMPs generally promote ECM degradation particularly in atrophic regions. Other theories (indicated by interrupted arrows) suggest a compensatory anti-inflammatory pathway involving prostaglandins and their receptors that leads to decreased MMPs and thereby ECM accumulation, particularly in hypertrophic regions of VVs. Persistent valve dysfunction, and progressive vein wall dilation and tortuosity lead to different stages of CVD and CVI. Current treatment of CVD and CVI (presented in shaded arrows) includes physical, pharmacological and surgical approaches.

Inhibitors of the activity or action of MMPs (also presented in shaded arrows) may provide potential tools for the management of CVD/CVI.

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Fig. 4.

Mechanisms linking increased venous hydrostatic pressure to increased MMP expression and VVs. Increased venous hydrostatic pressure causes vein wall stretch, which increases HIF mRNA expression and protein levels, and in turn MMP levels. Increased wall stretch may also increase other MMP inducers such as EMMPRIN, chymase, hormones and NGAL. Increased MMPs may activate protease activated receptors in endothelial cells leading to NO production and venous dilation. MMPs may also stimulate endothelial cells to produce EDHF which in turn opens BK_{Ca} channels in VSM, leading to hyperpolarization, decreased Ca²⁺ entry through Ca²⁺ channels, and VSM relaxation. Loss of contractile function in VSM could cause a phenotypic switch to synthetic VSMCs. MMPs may also increase the release of growth factors, leading to VSMC hypertrophy. MMPs also cause ECM degradation leading to VSMC migration, further decreases in vein contraction and increases in venous dilation, and VVs. MMP-induced ECM degradation may also cause valve degeneration leading to further increases in venous hydrostatic pressure. As indicated in shaded arrows, inhibitors of MMP synthesis (U-0126, HIF siRNA, 17-DMAG, Echinomycin, MMP siRNA), activity (MMP Inhibitor) or actions (Iberiotoxin) may provide new tools for management of VVs. BK_{Ca}, large conductance Ca²⁺-activated K⁺ channels; DMOG,

dimethyloxaloylglycine, is an experimental inhibitor of HIF-prolyl hydroxylase; FGF, fibroblast growth factor; HIF, hypoxia-inducible factor; Hsp90, heat shock protein 90; IGF, insulin-like growth factor; MAPK, mitogen-activated protein kinase; NGAL, neutrophil gelatinase-associated lipocalin; TGF- β , transforming growth factor β ; VEGF, vascular endothelial growth factor.

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