Matrix metalloproteinases in the development of varicose disease

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Abstract

Background: The imbalance between extracellular matrix components is a consequence of MMPs activity. Increased expression of matrix metalloproteinases (MMPs), in particular type 2 and 9, has been identified in varicose veins. MMP-2 and -9 have acute venodilatory effect in addition to their known effects on the extracellular matrix. The data suggest that protracted increases in venous pressure and wall tension increase MMPs expression, which in turn reduce venous contraction and lead to progressive venous dilation. Increases in magnitude and duration of wall tension are associated with reduced contraction and overexpression of MMP-2 and -9. MMP-2 and -9 promote inferior vena cava relaxation. MMP-2 did not inhibit venous contraction during membrane depolarization by high KCl, suggesting that MMP-2 induced relaxation likely involves hyperpolarization and activation of a K^+ channel. MMPs cause hyperpolarization of the smooth muscle cells of the vein wall, leading to prolonged opening of Ca^{2+}-dependent K^+ channel (BK_{Ca}).
Conclusions: MMP-2 causes significant inhibition of Phenylephrine and Angiotensin II-induced IVC contraction likely through a post-receptor mechanism involving activation of plasmalemmal K^+ channels, membrane hyperpolarization, and inhibition of Ca^{2+}influx. MMP-2 induced inhibition of the Ca^{2+} entry mechanism of venous smooth muscle contraction may play a role in the venous dilation associated with varicose vein formation. Studying the mechanisms of action of MMPs is an important step in the development of new treatment methods of varicose veins, such as synthetic inhibitors of matrix metalloproteinases.
Key words: varicose, dilatation, MMPs, extracellular matrix, hyperpolarization.

Introduction

Throughout time phlebology has been the subject of much research and workings, and today the theme is more actual than ever due to numerous progresses in research, to the development of diagnosis and treatment technologies, and the people's medico-sanitary conscience evolution [1]. Varicose veins affect up to 25 percent of women and 15 percent of men. By the age of 50, nearly 40 percent of women and 20 percent of men have significant leg vein problems [2].

Studying this theme is too important because currently there are a number of treatments: sclerotherapy, laser therapy and surgical treatment, all accompanied by administering of phlebotonics and wearing bandages compression, but none of them resolves the problem, and recurrent varicose veins is an evidence of that. Despite medical and socio-economic consequences of varicose veins, the pathophysiological mechanisms involved are not fully understood. Although there were identified risk factors for varicose veins, such as female gender, pregnancy, obesity, aging, family history, the molecular mechanisms underlying pathogenicity and progressive varicose veins remain unclear. The tendency of most studies is hereditary component orientation and the genetic pathogenesis [1,3,4].

For example, the genetic mutations in iron metabolism genes may play a role in varicose veins (VVs). Prolonged venous reflux is associated with iron overload and dermal hemosiderin deposition that is directly correlated with clinical symptoms of chronic venous insufficiency (CVI) including skin changes and lipodermatosclerosis. Iron deposition may induce the formation of free radicals which can cause further tissue injury, and progression to advanced forms of CVI and leg ulcers. Also, Factor XIII is a cross-linking protein that plays a key role in ulcer healing. Mutations in hemochromatosis C282Y (HFE) gene and Factor XIII V34L gene variants have been identified in patients with chronic venous disease (CVD) and have been associated with increased risk of severe forms of CVI, skin changes and the size of venous ulcers. Some clinical conditions support a genetic component of VVs. Patients with Klippel-Trenaunay Syndrome have congenital venous anomalies in the form of atresia, agenesis of the deep venous system, valve insufficiency, venous aneurysms, and embryonic veins [4].

The primary cause of varicose vein formation is not clear; however, both vein valve dysfunction and hydrostatic venous pressure appear to play a critical role in the initiation and progression of the disease. Although valve-reflux may precede vein-dilatation,there is a significant body of evidence supporting the view that vein dilation can precede venous reflux, and that valvular dysfunction may be an epiphenomenon of vein wall dilation. Clinical studies have demonstrated that venous insufficiency can occur in varicosities without axial reflux of the superficial, deep or perforated veins, and that an imbalance in extracellular matrix proteins may cause connective tissue changes prior to valvular insufficiency [5].

Recent studies suggest that the balance between vascular proliferation, extracellular matrix deposition and degradation can be disturbed leading to loss of mechanical strength of the wall, venous dilatation and elongation. Fundamental changes in varicose dilatation involving cross linking and structural changes in the vein wall, inclusive fragmentation and disorganization of elastin smooth muscle [5].

Advanced stages of varicose veins are characterized by degeneration of the extracellular matrix, the endothelium and of smooth muscle cells of the vein wall. Histologically is modified extracellular matrix that shows the fragmentation of elastic blades, loss of circular and longitudinal fiber of smooth muscle and deterioration of endothelium [6,7]. Venturi [8, 9] and his co-workers have shown a decrease in desmosine, isodesomsinei and elastin collagen ratio in varicose veins compared to normal veins. C. Michiels [9] has shown that hypoxia induced leukocyte activation and generation of free radicals, activation of proteases, and therefore, the degradation of the extracellular matrix. Additionally, they have demonstrated that hypoxia activates endothelial cells to secrete growth factors and stimulate smooth muscle cell (SMC) proliferation and synthesis of extracellular matrix. These studies suggest that the disturbance in the synthesis and degradation of structural elements appear in the segments of varicose veins. The degradation of extracellular matrix triggeres the leukocyte infiltration and activates the inflammation which damages the vein wall. Vascular wall remodeling depends on the activity of macrophages, SMC, endothelial cells and fibroblasts. The extracellular matrix is made of SMC and fibroblasts and is degraded by enzymes secreted by the macrophages, such as matrix metalloproteinases (MMPs). The change in collagen-elastin ratio, which is observed in varicose veins, indicates an imbalance in the connective tissue matrix. Also, there are observed significant variations in the ratio of collagen type I and type II and increasing the activity of MMP, in particular 2 to 9 [4, 7]. All this has determined the shift towards the new direction of study. The research is based on the treatemet methods discovery which is focused physiopathologically at cellular and molecular level. The article is based in particular on the work of West Roxbury vascular surgeon and researcher Joseph D. Raffetto [3,5,7], professor at the University of Massachusetts.

Recent studies are based on the research of MMPs in the development and progression of varicose veins. MMPs were described initially by Jerome Grossand and Charles Lapiere [10, 11], who observed enzymatic activity (collagen triple helixdegradation) during tadpole tail metamorphosis (by placing a tadpole tail in a collagen matrix plate). Therefore, the enzyme was named interstitial collagenase (MMP-1). Later, it was purified from human skin [10]. Since then, the MMP family has grown to include at least 28 members in vertebrates, 23 in humans, and 14 in blood vessels [4].

MMPs are also called matrixins, they are multidomain zinc metalloproteinases that degrade various components of extracellular matrix (ECM) and belong to the larger superfamily of proteases called metzincins, which also includes adamalysins, serralysins, and astacins. Sequence homology with the catalytic domain of MMP-1 (collagenase 1) is a common feature of all members of the MMP family. To classify

MMPs there have been employed several methods. MMPs are classified as the matrixin subfamily of zinc metalloprotease family. The most common methods of classification MMPs are based on organization domain and substrate preference into collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT)-MMPs and others [4]. From the evolutionary point of view MMPs have been classified depending on their primary sequence into 6 subgroups (A–F): subgroup A (MMP-19, -26, -28), B (MMP -11, -21, -23), C (MMP-17, -25), D (MMP-1, -3, -8, -10, -12, -13, -27), E (MMP-14, -15, -16, -24), and F (MMP-2, -7, -9, -20) [13]. Thus MMP 1 is interstitial collagenase and its substrate is fibrillar collagen, MMP 2 and 9 are the gelatinases which acts on collagen type IV, V, collagen degraded and elastin, and MMP3 is stromelysin and has the substrate proteoglycans, fibronectin, laminin, pro MMP-1 9. Other subgroups include: matrilysin, enamelysin and metalloelastase macrophagal [13].

Matrix metalloproteinases are excreted by connective tissue and a variety of pro-inflammatory cells including fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes. These enzymes are expressed as zymogens, which are subsequently processed by other proteolytic enzymes (such as serine proteases, furin, plasmin, and others) to generate the active forms. Typically MMPs consist of a propeptide of about 80 amino acids, a catalytic metalloproteinase domain of about 170 amino acids, a linker peptide (hinge region) of variable lengths and a hemopexin domain of about 200 amino acids. The catalytic domain contains the Zn^{2+} binding motif HEXXHXXGXXH and a conserved methionine [11].

Most of the matrix metalloproteinases consist of four distinct domains, which are N-terminal pro-domain, catalytic domain, hinge region, and C-terminal hemopexin-like domain. This may be responsible for the macromolecular substrate recognition as well as for interaction with tissue inhibitors of metalloproteinases (TIMPs). The membrane-type MMPs contain an additional transmembrane domain that anchors them in the cell surface [14].

MMPs are synthesized as pre-proenzymes. The signal peptide is removed during translation and proMMPs are generated. Activation of these zymogens is therefore an important regulatory step of MMP activity.

Thirteen MMPs are secreted from the cell as proMMPs. The presence of a proteinase susceptible "bait" region in the propeptide allows tissue and plasma proteinases or opportunistic bacterial proteinases to activate proMMPs. Cleavage of the bait region removes only a part of the propeptide and complete removal of the propeptide is often conducted in trans by the action of the MMP intermediate or by other active MMPs [15].

MMPs degrade different components of ECM including collagen, casein and laminin. MMPs also modulate many bioactive molecules at the cell surface, and may regulate the cellular environment via interaction with G-protein coupled receptors. MMPs may play a role in cell proliferation, migration (adhesion/dispersion), differentiation and apoptosis, as well as physiological processes such as immune function, tissue healing, and angiogenesis. Matrixins participate in many normal biological processes (embryonic development, blastocyst implantation, organ morphogenesis, nerve growth, ovulation, cervical dilatation, postpartum uterine involution, endometrial cycling, hair follicle cycling, bone remodeling, wound healing, angiogenesis, apoptosis, etc.) and pathological processes (arthritis, cancer, cardiovascular disease, nephritis, neurological disease, breakdown of blood brain barrier, periodontal disease, skin ulceration, gastric ulcer, corneal ulceration, liver fibrosis, emphysema, fibrotic lung disease, etc.). Although the main function of matrixins is the removal of ECM during tissue resorption and progression of many diseases, it is notable that MMPs also alter biological functions of ECM macromolecules by specific proteolysis. For example, MMP-2 released by growth cones promotes neurite outgrowth by inactivating neurite-inhibitory chondroitin sulfate proteoglycans, thereby unmasking the neurite-promoting activity of laminin [11]. Among lung injuries, MMPs have been implicated in acute respiratory distress syndrome and chronic obstructive pulmonary disease, which encompasses both emphysema and chronic bronchitis. There has been considerable interest in the role that MMPs play on the progression of tumours to metastatic neoplasms. In neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, MMPs have been shown to play an important and complex role. MMPs have been shown to increase blood-brain barrier permeability that can cause oedema, haemorrhage and cell death and it is thought to play a role in white matter damage [16].

MMPs are regulated at multiple levels including transcription, secretion, and activation of the zymogen forms, extracellular inhibition and internalization by endocytosis. MMP activity is positively modulated by ions and reagents that induce MMP cleavage and activation. Zn^{2+}chelators suppress MMP activity by depriving MMPs from the Zn^{2+}critical for their activity. Cu^{2+}ions may decrease MMP-2 secretion. MMPs are also inhibited by both endogenous and exogenous inhibitors. Under normal physiological conditions, the proteolytic activity of the MMPs is controlled at any of the following three known stages: activation of the zymogens, transcription, and inhibition of the active forms by various TIMPs. In pathological conditions this equilibrium is shifted toward increased MMP activity leading to tissue degradation [14].

The role of MMPs in VV has been largely attributed to their proteolytic effects on ECM, degradation of the valve leaflets and weakening of vein wall structure. The localization of MMPs in the VV wall adventitia and fibroblasts is consistent with a role in ECM degradation [11].

The last two decades have made a great progress in understanding the role of MMPs in the development and progression of varicose veins. Although MMP effects are generally attributed to the degradation of extracellular matrix, their effects on the mechanisms of contraction / relaxation vein are unclear. In order to loose this enigma Joseph D. Raffetto and his team conducted a series of research.

The purpose of the first study was to test the hypothesis that prolonged increases in vein wall tension cause overexpression of MMPs and decreased contractility promotes

venous dilation. The purpose of the second study was to test whether MMP-2 induced venous relaxation involves an endothelium-dependent mechanism, or an endothelium-independent inhibition of venous smooth muscle contraction. The task was to test this novel hypothesis, on evaluated effects of MMP-2 in inferior vena cava in the presence and absence of endothelium, in the presence and absence of inhibitors of endothelium-derived vasodilators, and during modulation of K^+channel activity by depolarizing solutions and by activators and blockers of K^+channels. Another study determined the mechanism which inhibits MMPs Ca+2 channels and generates venous dilation.

For this were used inferior vena cava obtained from rats of 12 weeks, weighing 250-300g, housed in the animal facility and fed standard rat chow and tap water in 12 hours / 12 h light / dark, then they were euthanized by CO2 inhalation. Inferior vena cava (IVC) were quickly excised, placed in Krebs solution, carefully dissected and cleaned of connective tissue under the microscope. IVC has been divided proportionally in four rings of 3 mm.

For the first experiment 4 vein segments were mounted on the wire hooks in different tissue baths at no specific order to minimize the effects of variability in tissue size on the observed contractile response. Unless indicated otherwise, vein segments were stretched under 0.5g of basal tension and allowed to equilibrate for 1 hr in a temperature controlled, water-jacketed tissue bath, filled with 50 mL Krebs solution continuously bubbled with 95% O_2, 5% CO_2 at 37°C. The changes in isometric contraction were recorded on a Grass polygraph. To determine the control contraction, IVC segments were stimulated twice with 96 mmol/L KCl solution. To test the IVC relaxation function, the tissues were stimulated with phenylephrine (Phe 10^{-5}mol/L) to achieve a steady-state contraction, and then treated with acetylcholine (Ach, 1^{-5}mol/L) to confirm the presence of intact endothelium. The veins were frozen to determine the expression and localization of MMPs using immunoblots and immunohistochemistry. These MMP-2 and -9 concentrations are physiologically consistent with the plasma and vein tissue levels in human which range between 1000 ng/g tissue (~1 mcg/ml) and 100 mcg/g tissue (~0.1 mcg/ml) [5].

In the second experiment there were measured the effects of MMP-2 on Phe- and KCl-induced contraction. To study the role of endothelium-derived vasodilators, the experiments were performed in the presence and absence of endothelium, L-nitro-arginine methyl ester (L-NAME), inhibitor of nitric oxide (NO) synthesis, indomethacin, inhibitor of prostacyclin (PGI_2) synthesis, cromakalim, activator of ATP-sensitive K^+channel (K_{ATP}); and iberiotoxin, blocker of large conductance Ca^{2+}-dependent K^+channel (BK_{Ca}) and smooth muscle hyperpolarization [3].

To evaluate the effects of MMP-2 on Ca^{2+} entry, IVC segments nontreated or pretreated with MMP-2 were incubated in Ca^{2+}-free Krebs solution for 5 min, stimulated with Phe (10^{-5}M), then increasing extracellular $CaCl_2$ concentrations ($[Ca^{2+}]_e$) were added and the contractile response was measured after 5 min in each $[Ca^{2+}]_e$. To further test the effect of MMP-2 on Ca^{2+} entry, the effect of MMP-2 on Phe contraction was tested in IVC segments treated with the Ca^{2+} channel blocker diltiazem (10^{-5}M) [7].

Later it was tested if the prolonged increases in vein wall tension affect venous contraction. Phe activates α-adrenergic receptors, and angiotensin II (AngII) activates angiotensin type 1 receptors. It was found that the prolonged increases in basal tension are associated with reduction in Phe-induced IVC contraction, which may not be specific to α-adrenergic mediated responses as AngII-induced contraction was similarly reduced under these conditions. The observation that the reduced IVC contraction in tissues subjected to prolonged basal tension was reversed in tissues treated with TIMP-1, suggested possible involvement of MMP-2 and -9. The immunoblot analysis in IVC suggested increased expression of MMP-2 and -9 during prolonged increases in basal tension. Also, immunohistochemical staining suggested localization of MMP-2 and -9 in the three layers of the vein wall. Additionally, prolonged increases in wall tension were associated with relative increases in MMP-2 and -9 in the vicinity of the smooth muscle layer, suggesting an effect on the contractile cells. Also, the observation that prolonged tension was associated with reduced contraction not only to Phe but also to AngII indicates that the reduction in contraction is not specific to a particular receptor. Furthermore, the Phe and AngII contraction were restored in veins treated with TIMP-1, suggesting that the reduction in contraction was not due to reduction in the Phe or AngII receptors, but rather it was due to increased MMP. It was also shown that MMP-2 causes significant inhibition of Phe- and AngII-induced IVC contraction likely through a post-receptor mechanism involving activation of plasmalemmal K+channels, membrane hyperpolarization, and inhibition of Ca2+influx. However, these studies do not exclude the possibility that the presence/activation of MMP-2 may lead to activation of other MMPs. MMP-2 and -9 have acute venodilatory effect in addition to their known effects on the extracellular matrix. MMP-2 and MMP-9 induced inhibition of Phe contraction in vascular segments is dose-dependent. Further, it was demonstrated that the venodilator effects of MMP-2 and -9 are time-dependent. MMP-2 could cleave big endothelin-1 yielding a novel vasoconstrictor and thereby enhance vascular contraction, an effect that will not be manifested in endothelium-denuded tissues. MMP-2 induced venous relaxation could also be due to the enhanced release of nitric oxide (NO), prostacyclin (PGI2) or endothelium-derived hyperpolarizing factor (EDHF). It was established that the NO synthesis (NOS) inhibitor L-NAME did not attenuate, and instead, enhanced MMP-2 induced IVC relaxation. MMP-2 did not increase NO production. Although the data suggest that MMP-2 induced venous relaxation may not involve increased NO production, they do not rule out possible interaction between the NO pathway and MMP. NO, being a major vasodilator may downregulate the effects of MMP-2 on other endothelium-derived vasodilators, and NOS inhibition may unmask these effects. As well, the studies have shown that NO inhibition may increase MMP-9 expression in rat vascular smooth muscle cells. MMP-2 may also directly or indirectly through increased endothelin-1 production, induce NO synthesis (NOS) uncoupling and lead to

increased superoxide production and decreased NO bioactivity. It has also detected that the MMP-2 induced venous relaxation was not affected by the cyclooxygenase inhibitor indomethacin, suggesting that it does not involve increased PGI2synthesis and activation of PGI2-cAMP relaxation pathway. The inability of NOS and cyclooxygenase inhibition to block the MMP-2 induced venous relaxation raises the possibility of MMP-2 mediated release of EDHF leading to enhanced K+ efflux via K+ channels and venous smooth muscle hyperpolarization and relaxation. The high extracellular KCl creates a K+ concentration gradient that does not favor K+ efflux through plasma membrane K+ channels. MMP-2 did not inhibit venous contraction during membrane depolarization by high KCl, suggesting that MMP-2 induced relaxation likely involves hyperpolarization and activation of a K+channel. K+ channels include the ATP-sensitive K+ channel (KATP), large conductance Ca2+-activated K+channel (BKCa), intermediate and small conductance Ca2+-activated K+ channel, voltage-gated K+ channels, and inward rectifier K+ channels. Activation of K+ channels likely causes smooth muscle hyperpolarization, and leads to decreased Ca2+influx through voltage-gated channels. MMP-2 can also activate directly the K+ channels. MMP may facilitate a conformational change in the BKCa channel from a closed state to an open state, during changes in voltage and intracellular Ca2+. As well the MMP-2 may activate K+ channels through specific protease-activated receptors (PARs). PARs are activated by serine proteases such as thrombin, trypsin, and tryptase. The trypsin-induced PARs-mediated relaxation was inhibited by KCl-precontraction, or pretreatment with apamin or charybdotoxin, blockers of small and intermediate Ca2+-activated K+channels, respectively. These facts suggest that PARs-mediated relaxation of the vasculature involves hyperpolarization of vascular smooth muscle and activation of Ca2+-activated K+ channels. In vascular smooth muscle, agonist-receptor interaction is coupled to increased release of inositol-1,3,5-trisphosphate (IP3) and activation of plasma membrane Ca2+ channels. IP3 induces Ca2+ release from the intracellular Ca2+ stores. Parallel activation of Ca2+ channels promotes Ca2+ influx from the extracellular space. In Ca2+-free Krebs solution, Phe and AngII produced transient contraction, suggesting that they activate IP3-induced Ca2+ release. Diltiazem inhibited Phe-induced contraction, suggesting the presence of functional voltage-gated Ca2+ channels in IVC. Moreover, diltiazem did not cause any further relaxation in MMP-2 treated IVC, indicating that the L-type channels are already inhibited by MMP-2. Importantly, MMP-2 caused further relaxation in diltiazem treated IVC, suggesting that during blockade of L-type Ca2+ channels, MMP-2 may inhibit other subclasses of voltage-gated channels (T- or N-type) or perhaps ligand-gated, store-operated Ca2+ channels or non-specific cation channels [3, 5, 7].

Conclusions

While inherent differences in the physiologic behavior and structure of rat veins and human veins make it difficult to extrapolate the response of rat IVC to human veins, the results indicate that the magnitude and duration of vein wall tension have significant impact on venous function and MMP expression. It was established that increases in basal tension cause a significant and irreversible reduction in IVC contraction, suggesting structural changes and irreversible vein damage. Increases in magnitude and duration of wall tension are associated with reduced contraction and overexpression of MMP-2 and -9. MMP-2 causes relaxation of Phe contraction in IVC segments by a mechanism involving hyperpolarization and activation of BKCa. Large amounts of MMP-2 have been detected in the plasma and venous tissues of patients with varicose veins. In addition, it is possible that some of the effects of MMP-2 can be due to activation of other endogenous MMPs. However, MMP-1, -3, -9, and -13 are also expressed in human varicose veins. The obtained results are the first step in the establishment of a new phlebology development direction. Understanding the molecular basis of VVs formation and MMPs-induced changes in endothelial cells (Ecs) and vascular smooth muscle (VSM) function and vein wall remodeling will provide valuable information on the mechanisms involved in CVD development and progression. Using human veins, obtained by minimal surgical manipulation can reveal not only the effects of MMPs on the veins, but can also divert treatment for use of MMPs inhibitor in the treatment of varicose veins. Previous attempts to study the effects of MMP inhibitors, such as doxycycline, batimostat and marimostat have been carried out, but they have significant side effects, especially on the musculoskeletal system. A deeper study of MMPs and their effects can lead to the creation of new synthetic inhibitors and their potential use. In the context of accelerated technological progress all they may become phlebology targets tomorrow.

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