The Effect of Pressure-Induced Mechanical Stretch on Vascular Wall Differential Gene Expression

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Key Words

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Abstract

High blood pressure is responsible for the modulation of blood vessel morphology and function. Arterial hypertension is considered to play a significant role in atherosclerotic ischaemic heart disease, stroke and hypertensive nephropathy, whereas high venous pressure causes varicose vein formation and chronic venous insufficiency and contributes to vein bypass graft failure. Hypertension exerts differing injurious forces on the vessel wall, namely shear stress and circumferential stretch. Morphological and molecular changes in blood vessels ascribed to elevated pressure consist of endothelial damage, neointima formation, activation of inflammatory cascades, hypertrophy, migration and phenotypic changes in vascular smooth muscle cells, as well as extracellular matrix imbalances. Differential expression of genes encoding relevant factors including vascular endothelial growth factor, endothelin-1, interleukin-6, vascular cell adhesion molecule, intercellular adhesion molecule, matrix metalloproteinase-2 and -9 and plasminogen activator inhibitor-1 has been explored using ex vivo cellular or organ stretch models and in vivo experimental animal models. Identification of pertinent genes may unravel new thera-

peutic strategies to counter the effects of pressure-induced stretch on the vessel wall and hence minimise its notable complications.

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Introduction

Arterial hypertension is a progressive cardiovascular syndrome associated with functional and structural cardiovascular abnormalities that damage various organs including the heart, kidneys, brain and other vasculatures and lead to premature morbidity and death [1]. Similarly, venous valvular incompetence and elevation in the pressure of blood in the lower-limb superficial venous system is considered contributory to vein dilatation and tortuosity (varicose veins) [2]. Importantly, such increased venous pressure is also responsible for chronic venous insufficiency (CVI), which manifests as lipodermatosclerosis, oedema and ulceration [2].

There are three main cell types found in the main layers of the blood vessel wall, namely endothelial cells (ECs) lining the tunica intima, vascular smooth muscle cells (VSMCs) in the tunica media and fibroblasts within the adventitia. These cells have the ability to respond to various micro- and macro-environmental stimuli including

Fig. 1. Diagram showing haemodynamic forces in a blood vessel. Distension pressure results in a radial force applied to the vessel wall, which in turn leads to elongation of the wall and alignment of the vascular cells in a direction that is perpendicular to the applied force [38, 48].

mechanical stretch. The elastic ability of large vessels allows them to accommodate significant volumes of blood under physiological conditions. However, upon exposure to high pressure, the structural and functional properties of vessels are modified to accommodate this by a process termed 'vascular remodelling'. Remodelling encompasses alterations in the migration, proliferation and apoptosis of ECs and VSMCs and synthesis and degradation of extracellular matrix (ECM) [3]. More elastic vessels, such as the aorta, attenuate the effects of hypertension initially by way of remodelling, but prolonged hypertension in these large elastic arteries and particularly in less elastic vessels (such as smaller arteries and veins) tends to alter the vessel wall shape and composition, ultimately resulting in various clinical complications. The aim of this review is to summarise current knowledge relating to changes in gene expression as a result of high intravascular pressure and the morphological changes in artery and vein walls induced by the altered expression of these genes.

Methods

A literature review of the Pubmed database was conducted using the following search string: ('gene' OR 'genes' OR 'gene expression') AND ('stretch' OR 'distension' OR 'pressure' OR 'tension') AND ('vessel' OR 'arterial' OR 'artery' OR 'venous' OR 'veins'). The search was expanded using the 'related articles' function and article reference lists. Only English-language articles were included.

Blood Pressure and Mechanical Forces

Forces exerted by blood on the vessel wall include mechanical stretch and shear stress (fig. 1). Increased intraluminal pressure of the blood distends the vessel wall, exerting a force perpendicular to its surface. The vessel opposes this distension by applying a circumferential and longitudinal force. All the constituents of the vessel wall take part in counter-balancing the effect of blood pressure [4]. According to Laplace's law, this force per unit length of vessel wall (T) is directly proportional to the blood pressure (P) and wall radius (r) and inversely proportional to its thickness (h): $T = Pr/h$.

Shear stress is applied parallel to the vessel surface and is generated due to friction of the blood against the vessel wall. The effect of shear stress is borne directly by the endothelial lining of the blood vessel [4]. Shear stress (τ) is directly proportional to the blood viscosity (μ) and the rate of flow (Q): $\tau = 4\mu Q/\pi r^3$, where r = vessel radius.

The mechanical stretch (circumferential stress) in arteries is mainly cyclical due to pulsatile blood flow, increasing in the systolic phase of the cardiac cycle and gradually reducing during diastole. All elements of the vessel wall including all three main cell types and ECM are affected by the mechanical stretch. As a consequence, up- or downregulation of genes and their transcriptomic or proteomic products will form the basis of this review.

Morphological Effects of Mechanical Stretch on Blood Vessels

Forces exerted by blood pressure on the arterial wall affect the vessel wall at both the cellular and molecular level. Evidence has shown that the tension generated by intraluminal pressure affects the thickness and composition of the vessel wall [5, 6]. Mechanical stretch exerted by increased intraluminal pressure induces vascular smooth muscle hypertrophy and hyperplasia and changes in contractile and matrix proteins [7, 8]. Localised haemodynamic stresses in arteries are considered to play a role in attracting inflammatory cells into the arterial wall [9]. These inflammatory cells produce cytokines, chemokines, oxygen free radicals and matrix metalloproteinases (MMPs), the latter leading to degradation of wall structural components including elastin and collagen. Similarly, vascular ECs and VSMCs produce MMPs [9]. A weak arterial wall, in the context of ongoing exposure to such wall stresses, is susceptible to aneurysmal dilatation.

High arterial blood pressure certainly plays a part in the pathogenesis of atherosclerosis [10]. In response to endothelial injury, a complex interaction between these activated ECs, VSMCs, platelets and leukocytes results in an inflammatory cascade [11]. This interaction also changes the vasomotor status of the blood vessels, alters the coagulation cascade and fibrinolytic system and stimulates VSMC proliferation and migration. These processes contribute to the development of an atherosclerotic lesion in the vessel intima [12]. In the regions of arterial branching, there is greater susceptibility to atherogenesis owing to the greater effect of the haemodynamic forces of shear stress [13] and mechanical stretch [14].

The venous system functions as a blood capacitance reservoir and also a channel to return the blood to the heart. As veins are exposed to lower pressure, it is logical to expect less mechanical stretch and shear stress in veins in comparison to arteries. However, this hypothesis does not consider the thinner, less muscular and more compliant nature of the vein wall. The peripheral venous system in the lower limbs is divided into superficial and deep components. Superficial veins are located outside the musculo-fascial compartments and are connected to the deep venous system by perforating veins. From the clinical perspective, the peripheral venous system is important, as failure of this system is associated with CVI. The peripheral venous system is supported by valves and muscle pumps to help pump blood centrally towards the heart and prevent retrograde flow into the legs (termed venous reflux). Failure of the valves or muscle pump leads

to increased pooling of blood in the legs and high venous pressure exerting a static stretch on the vein wall. The resting standing venous pressure in a normal leg is about 80–100 mm Hg. During calf muscle contraction, the venous pressure normally drops to 20–30 mm Hg, followed by venous refilling. However, patients with venous reflux demonstrate a less than 50% venous pressure drop during calf contraction and a much shorter refilling time [2]. The overall increased venous pressure as a result of blood stasis may result in vein wall dilatation and the characteristic CVI dermal changes with hyperpigmentation, venous eczema, subcutaneous tissue fibrosis and ultimately ulceration [2, 15].

Under physiological conditions, veins do not normally develop atherosclerotic disease. However, the autologous superficial great saphenous vein is the most commonly used conduit in coronary artery bypass surgery and is also regarded as an appropriate conduit in the context of chronic and critical limb ischaemia [16–19]. Veins used as conduits for arterial reconstruction in heart and limb bypass operations are also known as ‘arterialised veins’, as they are subjected to relatively high arterial pressure. Arterialised veins are more likely to develop atherosclerotic disease, which may lead to vein graft failure [20]. The risk of vein graft failure is estimated to be around 50% over 10 years in coronary arterial disease and approximately 30% over 1 year for peripheral arterial disease [21–23].

Functional Aspects of in vitro Stretch Experiments

The vessel wall opposes the intraluminal stretch of blood pressure, and this resistance is manifested by changes in the vessel wall in both circumferential and longitudinal directions (fig. 1). Therefore, mechanical strain is a measure of deformation and change in the length of the cells. The percentage increase in the length of the deformed cell caused by stretch is calculated as the ratio of the circumference when stretched to the initial circumference. Human aorta undergoes an 8–10% oscillation in external diameter under physiological conditions due to expansion of its elastic fibres [24]. However, the peripheral arteries undergo an approximately 5% oscillation in diameter due to the elastin being well supported by collagen and smooth muscle cells (SMCs) in the tunica media of their walls [25]. However, when the blood pressure is high, including in hypertension, the change in the aortic diameter has been modelled in vitro as 20% [26–28]. Therefore, 20% stretch is often applied in cell culture studies to in-

investigate the effects of hypertension-induced stretch. For example, commercially available Flexercell FX-2000 devices are used in studies to apply an in vitro cyclical stretch on cultured vascular ECs and VSMCs on an elastic membrane. Mechanical strain to cultured cells under study is applied through a vacuum unit linked to a computer programme. Cultured cells resting on the flexible membranes are deformed by a sinusoidal negative pressure of 15 kPa (113 mm Hg) controlled by a computer at a frequency of 1 Hz (60 cycles/min) [29]. This stretching model produces a cyclical strain on cells ranging from minimal strain at the centre of the membrane to a peak value of 20% at the periphery [30].

The effects of stretch are studied in whole blood vessels in ex vivo experiments using vein or arterial organ culture models. For example, segments of blood vessels suspended in a culture medium are stretched with 0.5- or 2-gram weights (2-gram weight = 83 mm Hg) using stainless steel hooks [31]. Isolated aortic segments attached to bespoke cold perfusion circuits [32] and carotid artery segments cannulated to rods in a self-contained flow system have also been used to study the effects of stretch on the vessel wall [33]. Shunts and fistulae formed between arteries and veins have also been developed in experimental animal models to look at the effects of high arterial pressure on the vein wall [34, 35].

Cellular Mechanosensing and Intracellular Pathways

The cellular mechanosensitive mechanisms detecting and transmitting the effects of intraluminal stretch include cell adhesion sites, integrins, cellular junctions, tyrosine kinase receptors, ion channels and components of the lipid bilayer (fig. 2) [4, 26, 36–38]. Activation of such mechanosensors initiates multiple complex intracellular pathways which drive patterns of gene expression. These signalling pathways are numerous and often overlapping. Protein kinase C, Rho family GTPases such as Rho and Rac and activation of phosphatidylinositol-3-kinase and its downstream target Akt are involved in stretch signal transduction [27]. In addition, stretch also activates mitogen-activated phosphate kinases (MAPKs) [39, 40]. The MAPK cascade consists of three pathways including extracellular signal-regulated kinases 1 and 2 and stress-activated protein kinases (such as c-Jun N-terminal kinase and p38) [36, 39, 41–43]. Downstream of the MAPK cascade includes ternary complex formation with the serum response element on gene promoters and activation of transcription factors such as c-fos, c-Jun and activator

protein-1, which bind to DNA transcription promoter sites [4]. Cyclical strain also affects many transcription factors such as activator protein-1, cAMP, serum response element and nuclear factor- κ B (NF κ B). These transcription factors are known to be involved in the cyclical-strain-mediated regulation of gene expression [44, 45]. As a result of up- and downregulation of specific genes, key cellular processes such as cell proliferation, apoptosis, cell migration and the synthesis, degradation and reorganisation of the ECM may be modulated. This review underlines the effects of haemodynamic stretch on regulation of specific genes associated with particular morphological changes in the vessel wall.

Effect of Stretch on Genes Affecting Growth and Apoptosis

ECs serve various important functions including maintaining vascular haemostasis and secretion of a number of vasoactive and anti-thrombogenic mediators. VSMCs provide strength to the vascular structure. VSMCs also regulate ECM turnover and vascular tone. Under physiological conditions, there is a balance between cell proliferation and apoptosis to maintain function and prevent hyperplasia. Any stress, including mechanical stretch, may lead to cellular dysfunction and dysregulation.

Cyclical stretch affects the proliferation and apoptosis of ECs and VSMCs (for the effect on genes regulating proliferation/apoptosis and VSMC phenotype, see table 1). Studies involving both venous and arterial cultured ECs have shown an increased cell proliferation upon exposure to stretch [46, 47]. Cultured ECs on flexible membranes subjected to cyclical strain have shown cellular elongation and perpendicular alignment to the direction of stretch (fig. 1) [48]. Application of a 20% pathological cyclical strain to human umbilical vein ECs (HUVECs) has been shown to induce a 2.5-fold increase in activity of the c-Myc promoter after 2 h. Important upregulated downstream genes of c-Myc are vascular endothelial growth factor (VEGF), proliferating cell nuclear antigen and heat shock protein-60. These genes are thought to be involved in cell proliferation and matrix re-accumulation in stenotic diseases including neointima formation in vein bypass grafts [28]. Furthermore, stretch increases the sensitivity of ECs to growth factors [46].

The effects of cyclical stretch on the life cycle of VSMCs (proliferation and apoptosis) are not clear [27]. Evidence from studies applying stretch to cultured ve-

Fig. 2. Diagram summarising the effects of stretch on vascular ECs and VSMCs. AP-1 = Activator protein-1; CAT = catalase; Egr-1 = early growth response gene-1; ERK-1/2 = extracellular signal regulated kinases 1 and 2; FAK = focal adhesion kinase; GADD-153 = growth arrest and DNA damage-inducible gene 153; JNK = c-Jun N-terminal kinase; HSP-60 = heat shock protein-60; MCA = monocyte chemotactic antigen; NO = nitric oxide; PCNA

= proliferating cell nuclear antigen; REB = response element-binding protein; SGK = serum-glucocorticoid-induced protein kinase (a serine/threonine protein kinase); SM22-α = smooth muscle cells specific protein; TGF-β = transforming growth factor-β; THA-2 = thromboxane synthase-A2; TNF-α = tumour necrosis factor-α.

nous SMCs and ECs and a vein organ cultured ex vivo model has demonstrated increased apoptosis in VSMCs [49–52] (for the details of differential genes affecting growth, see table 1). This effect may be load dependent, as seen in a study in which 25% stretch caused DNA fragmentation in VSMCs whilst 7% stretch protected VSMCs from apoptosis [41]. Interestingly, similar degrees of stretch have been shown to result in both apoptosis and proliferation in VSMCs [53, 54].

On the other hand, evidence from studies of arterial cell cultures subjected to stretch has shown increased expression of hypoxia-inducible factor (HIF)-1α, VEGF and

transforming growth factor-β1, all favouring VSMC proliferation [29, 55]. Following static mechanical stretch of rat skeletal muscles for 4 and 7 days, cultured capillary ECs isolated by laser capture micro-dissection were found to display increased transcription of HIF-1α and -2α and their DNA-binding activity [56]. HIFs are key nuclear transcriptional factors that regulate genes mediating oxygen homeostasis [57, 58], including those involved in ECM metabolism (MMPs and tissue inhibitors of metalloproteinases), vascular tone, cell survival and apoptosis, glucose transportation, angiogenesis, erythropoiesis and oxygen delivery [57, 59].

Table 1. Mechanical stretch induces expression of various genes in ECs and VSMCs affecting cell growth/apoptosis and phenotype

Study	Cell type and origin	Stretch method employed	Genes regulated	Effect on the vessel wall
Hurley et al. 2010 [28]	HUVECs	cultured cells 20% cyclical stretch	↑ c-Myc (2.6-fold) at 1–2 h ↑ c-Myc-regulated genes: VEGF (3.5-fold at 4 h) HSP-60 (2.6-fold at 4 h) PCNA (1.75-fold at 3 h)	cell proliferation ECM remodelling
Frye et al. 2005 [47]	HUVECs	cultured cells 10% cyclical and non-cyclical stretch	↑ CAT (catalase) with cyclical but not non-cyclical stretch	cell proliferation
Stula et al. 2000 [50]	HUVECs	cultured cells 10% continuous stretch	↑ early growth response (Egr-1) at 10 min, reached peak at 30 min	chemotactic and mitogenic effects on VSMCs
Zheng et al. 2004 [46]	RCMEs HUVECs	cultured cells 15% cyclical stretch for 24 h	↑ protein expression of receptor tyrosine kinases: Flk 1 (both cell types) Tie 1 (only in RCMEs) Tie 2 (both cell types)	increase in sensitivity of ECs to growth factors
Cattaruzza et al. 2000 [67]	PAECs rat aortic VSMCs	cultured cells 20% cyclical stretch for 6 h	↑ ET-1 (8-fold) in PAECs ↑ ET-B receptor in rat VSMCs	ET-1 release from ECs causes vasoconstriction but in response to stretch causes ET-B-induced apoptosis of VSMCs
Milkiewicz et al. 2007 [56]	skeletal muscle capillary endothelial cells (rat)	10% static stretch to skeletal muscles	↑ HIF-1 α (3-fold on day 4 and 4-fold on day 7) ↑ HIF-2 α (2.5-fold on days 4 and 7)	increase in expression of HIF target gene VEGF ↑ permeability ↑ cell proliferation ↑ angiogenesis
Cheng et al. 2010 [101]	venous SMCs (mice)	cultured cells 15% cyclical stretch for 2 h	↑ SGK (19-fold) in venous SMCs	increased proliferation
Feng et al. 1999 [61]	human aortic SMCs	cultured cells 4% cyclical stretch for 12 or 24 h	↑ >2.5-fold: COX-1 tenascin PAI-1 ↓ >2.5-fold: MMP-1 thrombomodulin	reduced VSMC proliferation ECM remodelling
Cheng et al. 2008 [49]	VSMCs (rat)	cultured cells 20% cyclical stretch	↑ GADD-153 (4.5-fold over 18 h)	apoptosis
Chang et al. 2003 [29]	thoracic aorta VSMCs (animal)	cultured cells 20% cyclical stretch	↑ HIF-1 α (2.1-fold at 4 h)	increase in expression of HIF target gene VEGF ↑ permeability ↑ cell proliferation ↑ angiogenesis
Richard et al. 2007 [60]	thoracic aorta VSMCs (rabbit)	cultured cells 20% cyclical stretch	↑ PCNA	increase in nuclear protein import and nuclear protein complex (5-fold at 48 h) cell proliferation (cell hyperplasia and hypertrophy)
Mata-Greenwood et al. 2005 [55]	pulmonary artery SMCs (animal)	cultured cells 20% cyclical stretch	↑ VEGF (2.4-fold at 4 h) ↑ TGF- β 1 (3-fold at 2 h)	cell proliferation

Table 1 (continued)

Study	Cell type and origin	Stretch method employed	Genes regulated	Effect on the vessel wall
Albinsson et al. 2004 [62]	portal vein (rat)	portal vein organ culture stretched with 0.3–0.6 g of weight (equivalent to physiological conditions) for 72 h	↑ actin polymerisation ↑ smooth muscle-specific proteins: α-actin calponin SM22-α tropomyosin desmin	maintains the contractile phenotype of SMCs
Cornelissen et al. 2004 [51]	saphenous vein (human)	saphenous vein organ culture stretched to 200–250 mm Hg of pressure for 2 min	↑ phosphorylated p38 associated with increased apoptosis in saphenous vein SMCs	apoptosis
Goldman et al. 2003 [52]	IVC (rat)	IVC and diaphragm excised and placed in a pulsatile circulatory system exposure to both venous and arterial pressure (ex vivo culture model of vein graft)	↓ SMC α-actin filaments (down to 19% at 48 h on exposure to arterial pressure) ↑ caspase 3 (on exposure to arterial pressure)	apoptosis
De Waard et al. 2006 [32]	saphenous vein ex vivo perfusion model (human) carotid artery organ culture (mice) venous SMCs (human) SMCs from internal mammary artery (human)	saphenous veins placed in extracorporeal circulation during bypass surgery and exposed to autologous (non-pulsatile) arterial pressure carotid artery organ culture exposed to pulsatile stretch of 60–140 mm Hg for 48 h cultured VSMCs exposed to 10% cyclical stretch	↑ DNA synthesis (3.5-fold) ↑ PAI-1 (8-fold) ↑ TR3 (14-fold after 1–2 h of cyclical stretch) in VSMCs of veins but not arteries	change of phenotype of venous smooth muscles from contractile into synthetic phase on exposure to arterial pressure (arterial smooth muscles more resistant to this change)

ET-B = Endothelin-B; Flk 1 = fetal liver kinase 1; GADD-153 = growth arrest and DNA damage-inducible gene 153; HSP-60 = heat shock protein-60; PAECs = porcine artery endothelial cells; PCNA = proliferating cell nuclear antigen; RCMEs = rat coronary microvascular endothelial cells; SGK = serum-glucocorticoid-induced protein kinase (a serine/threonine protein kinase); SM22-α = smooth muscle cells protein22-α; Tie 1 = tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains 1; Tie2 = tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains 2; TGF = transforming growth factor; TR3 = human orphan receptor TR3.

Pulsatile stretch of 20% causes rabbit aorta VSMCs to enter and complete the cell cycle and induces cell hyperplasia and hypertrophy [60]. In human aortic SMCs exposed to cyclical strain, DNA microhybridisation analyses identified 5,000 putative functional genes, but only 3 were shown to be upregulated, including cyclooxygenase-1 (COX-1), tenascin and plasminogen activator inhibitor-1 (PAI-1), whilst downregulated genes included thrombomodulin and MMP-1. Tenascin and PAI-1 modulate ECM, and COX regulates VSMC proliferation [61].

Effect on VSMC Phenotype

Mechanical stretch above a certain physiological threshold is required to maintain VSMCs' differentiated phenotype (contractile) [36]. For instance, exposure of a

murine portal vein organ culture to physiological stretch increased actin polymerisation and proteins associated with a contractile cytoskeletal phenotype [62]. However, when the pressure is increased, the VSMC phenotype shifts from contractile to synthetic. This effect is seen when the vein is subjected to conditions akin to a vein bypass graft. An increase in PAI-1 and DNA synthesis in venous SMCs is noticed. The phenotype of arterial SMCs remains stable at a relatively higher pressure as compared to veins, owing to arteries being exposed to higher pressures physiologically [32].

Similarly, stretch in a SMC culture model switches the SMC contractile phenotype to a synthetic one, as shown by a loss of VSMC myosin, an increase in ECM and protein synthesis and a proliferative state [63, 64].

Stretch-Regulated Genes Affecting the Inflammatory Response and Vasomotor Activity in Blood Vessels

Exposure of HUVECs and saphenous vein ECs to 10% cyclical stretch increases the expression of a vasoconstrictor endothelin-1 (ET-1) messenger RNA (mRNA) but has no effect on endothelial nitric oxide synthase transcription. In contrast, shear stress increases endothelial nitric oxide synthase expression and decreases ET-1 expression. This suggests that the ET-1 activity is controlled by the balance between the two forces. The combination of these two mechanical forces acts to neutralise the effect on ET-1 (for expression of stretch-induced genes in inflammation, see table 2) [65, 66]. In addition to causing vasoconstriction, stretch-induced ET-1 mRNA from arterial ECs has been reported to affect VSMCs by increasing apoptosis [67]. Various vasoactive substances induced by cyclical strain in ECs include nitric oxide, prostacyclin, tissue plasminogen activator, reactive oxygen species (ROS) and monocyte chemotactic protein-1 (MCP-1) [68–72].

Exposure of HUVECs to cyclical stretch causes nuclear migration of zyxin (a zinc finger protein), which acts to coordinate the expression of proinflammatory genes such as interleukin (IL)-8, vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM) [73]. Similarly, an upregulation of inflammatory mediators, including IL-8 and MCP, has been seen in HUVECs in response to cyclical stretch [74]. Continuous stretch also upregulates the proinflammatory gene IL-6 through NF κ B in HUVECs [75]. IL-6 is also upregulated in mouse artery VSMCs exposed to cyclical stretch for 2 h [76]. NF κ B also plays a role in COX-2 transcription in HUVECs exposed to cyclical stretch [77]. COX-2 and thromboxane synthase enzymes are involved in the stepwise breakdown of arachidonic acid into prostacyclin and thromboxane-A₂. Prostacyclin is a vasodilator and has anti-inflammatory and anti-platelet activity, and thromboxane-A₂ is a vasoconstrictor and promoter of platelet aggregation. Upregulation of these genes suggests that vessel stretch may initiate an inflammatory response in ECs resulting in the activation of adhesion molecules and cytokines leading to endothelial barrier disruption and increased vascular permeability.

Stretch increases the expression of C-reactive protein (CRP) mRNA and protein in saphenous vein and internal mammary artery rings exposed to stretch [78]. CRP is a nonspecific marker of inflammation and is considered an

independent risk factor for atherosclerosis and atherosclerosis-related diseases [79, 80]. Elevated CRP has been measured in the blood of patients with high systemic or local strain including essential hypertension [81], abdominal aortic aneurysms [82] and vein grafts in coronary bypass surgery [83]. CRP directly activates inflammatory cells, MMPs and various cytokines [80, 84]. Locally produced CRP in vessel walls may therefore be an important mediator in the stretch-induced inflammatory response.

Mechanical Stretch and MMPs

ECM interlinks the cellular components such as endothelium and VSMCs and consists of collagen, proteoglycans, elastin, glycoproteins and fibronectin. It is a dynamic structure and maintains integrity and homeostasis through its interactions with the cellular components [85]. MMPs are zinc-dependant endopeptidases and belong to the family of proteases called metzincins. Their function is to cleave most of the constituents of ECM [15]. Out of 23 identified in humans to date, 14 are found in vascular tissue [86]. MMPs are produced as inactive pro-enzymes (zymogens) and are activated by various exogenous and endogenous stimuli [87]. Their activity is further controlled by their endogenous tissue inhibitors (tissue inhibitors of metalloproteinases) [85, 88, 89].

Stretch regulates MMPs in animal and human cells (for stretch-induced genes affecting ECM, see table 3) and organ culture models and in turn affects the matrix metabolism in vascular tissue [90]. Upregulation of MMP-2 and -14 expression in venous ECs and MMP-2 in capillary ECs has been observed [91, 92]. Increases in MMP-2 and MMP-9 expression have also been reported in venous and arterial SMCs and fibroblasts subjected to mechanical stretch [93–95].

Increases in the magnitude and duration of vein wall tension in segments of rat inferior vena cava (IVC) were associated with reduced contraction and overexpression of MMP-2 and MMP-9 [96]. MMP-2 and -9 belong to the gelatinase group of MMPs, which act to digest denatured collagens. MMP-2 and MMP-9 were also found to induce significant relaxation of rat IVC, proposed to occur through VSMC hyperpolarisation and activation of large-conductance calcium-dependent potassium channels, suggesting that MMPs may play a role in the early stages of venous dilation secondary to high venous pressure [97]. Using a similar model of IVC rings, Lim et al.

Table 2. Mechanical stretch induces expression of genes in ECs and VSMCs affecting vasomotor activity and the inflammatory response

Study	Cell type and origin	Stretch method employed	Genes regulated	Effect on the vessel wall
Toda et al. 2008 [65]	HUVECs	cultured cells 10% cyclical stretch	↑ ET-1 (1.6-fold at 24 h)	vasoconstriction apoptotic
Wojtowicz et al. 2010 [73]	HUVECs	cultured cells 10% cyclical stretch	cytoskeletal protein zyxin translocation into nucleus and increased expression of IL-8, VCAM, ICAM	apoptotic chemotaxis intercellular adhesions molecules promoting adhesion of cells to neutrophils, macrophages
Kobayashi et al. 2003 [75]	HUVECs	cultured cells continuous stretch of 125 or 150%	↑ IL-6 (4-fold at 2 h) NFκB mediated	proinflammatory and anti-inflammatory cytokines
Zhao et al. 2009 [77]	HUVECs	cultured cells 10% cyclical stretch	↑ COX-2 (2.5-fold within 3 h) ↑ thromboxane-A2 synthase (NFκB mediated)	regulation of inflammation and platelet activity control of vasomotor tone
Wung et al. 1997 [72]	HUVECs	cultured cells 12% cyclical stretch	↑ MCP-1	chemotactic to monocytes and macrophages
Okada et al. 1998 [74]	HUVECs	cultured cells 15% cyclical stretch	↑ IL-8 (2.8-fold at 6 h) ↑ MCA/MCP-1 (2.2-fold at 3 h)	chemotactic factor for T lymphocytes and VSMCs mitogenic for VSMCs
Iba et al. 1991 [70]	saphenous vein endothelial cells (human)	cultured cells 7–24% cyclical stretch	↑ tissue plasminogen activator at >7% stretch	fibrinolytic activity
Tsukurov et al. 2000 [66]	saphenous vein endothelial cells (human)	cultured cells pulsatile pressure and cyclical strain	↑ ET-1	vasoconstriction and regulation of apoptosis
Casey et al. 2001 [68]	saphenous vein endothelial cells (human)	cells cultured on a silicone tube exposed to similar conditions as seen in arteries (120/80 mm Hg, 115 ml/min flow, 2% cyclical stretch and 4.8–9.4 dynes/cm ² shear stress)	↑ endothelial nitric oxide (absence of pulsatility, cyclical strain and flow resulted in significantly lower production of nitric oxide)	vasodilation
Zampetaki et al. 2005 [76]	aortic VSMCs (rat)	cultured cells 7, 15 and 20% cyclical stretch	↑ IL-6 (NFκB mediated) peaked at 3 h	proinflammatory and anti-inflammatory cytokines
Huang et al. 2009 [78]	saphenous vein and internal mammary artery rings (human)	saphenous vein and internal mammary artery rings organ culture stretched with 0, 1, 2, 3 and 5 g of wall tension for 20 min	↑ CRP (>9-fold in internal memory rings and >10-fold in saphenous vein rings with 3 g of weight)	non-specific marker of inflammation considered a risk factor for hypertension and cardiovascular diseases

MCA = Monocyte chemotactic antigen.

[98] found increased expression of HIF-1α and -2α in association with MMP-2 and MMP-9 gene expression, highlighting the significance of MMP and HIF gene expression in venous wall remodelling under stretch. MMP-2 expression has been noticed in aortic tissue rings exposed to 100 mm Hg of pressure [99]. Porcine carotid arteries subjected to increases in axial strain (elongation) in an ex vivo perfusion circuit also showed an elevation of MMP-2 and MMP-9 protein expression [33].

Evidence from Studies Using Experimental in vivo Vein Bypass Grafts or Arteriovenous Fistula Models

The effects of stretch induced by arterial pressure on the vein wall have been established by in vivo experimental models of veins connected to the arterial circulation [developing a bypass graft or arteriovenous (AV) fistula] [34, 35, 100]. Expression of genes promoting proliferation of VSMCs in the neointima of an in vivo vein bypass graft

Table 3. Mechanical stretch induces expression of genes regulating ECM in vascular cells

Study	Cell type and origin	Stretch method employed	Genes regulated	Effect on the vessel wall
Wang et al. 2003 [91]	HUVECs	cultured cells 20% cyclical stretch	↑ (3-fold at 12 h) expression of: MMP-2 MMP-14 (mediated by TNF- α)	ECM remodelling (MMPs degrade ECM)
Milkiewicz and Haas 2005 [92]	skeletal muscle capillary ECs (animal)	static stretching of rat skeletal muscles	↑ MMP-2 (2.5-fold) ↑ HIF-1 α (13.5-fold)	ECM remodelling HIF regulates VEGF and other HIF- related genes
Stanley et al. 2000 [64]	human saphenous vein SMCs	cultured cells 22% cyclical stretch	↑ collagen α 1 (102 $\pm$ 30%) ↑ fibronectin protein (50 $\pm$ 21%)	major component of type 1 collagen fibronectin (major component of ECM) plays a significant role in cell- cell interaction, adhesion, differentiation and growth
Asanuma et al. 2003 [95]	human saphenous vein SMCs	cultured cells 5% stationary and 5% cyclical stretch	↑ MMP-2 (50-fold at 24 h) ↑ MMP-9 (both with stationary as compared to cyclical or no stretch)	ECM remodelling (MMPs degrade ECM)
Grote et al. 2003 [93]	aortic VSMCs (mice)	cultured cells 15% cyclical stretch	↑ (>2-fold at 3 h) expression of: MMP-2 PAI-1 CD44 integrin- α	ECM remodelling inhibition of fibrinolysis CD44: cell-cell interaction, adhesions and migration integrin- α : cell attachment to ECM and signal transduction
Tamura et al. 2000 [90]	VSMCs (animal)	cultured cells 20% cyclical stretch	↑ type 1 collagen (4.2-fold at 12 h) ↑ fibronectin (3.5-fold at 12 h) ↑ ACE	ECM remodelling (fibronectin plays a significant role in cell-cell adhesion, differentiation and growth)
Lim et al. 2011 [31]	IVC rings (rat)	IVC rings exposed to 2 g (84.3 mm Hg) of wall tension in an ex vivo model for 18 h	↑ HIF-1 α ↑ HIF-2 α ↑ MMP-2 ↑ MMP-9	HIF regulates VEGF and other HIF- related genes ECM remodelling
Raffetto et al. 2008 [96]	IVC rings (rat)	IVC rings subjected to 2 g of tension (equivalent to 83.4 mm Hg) for 24 h	↑ MMP-2 ↑ MMP-9	ECM remodelling
Ruddy et al. 2010 [99]	thoracic aortic ring apparatus (murine)	aortic rings subjected to tension equivalent to 0, 70, 80 and 100 mm Hg pressure for 3 h	↑ MMP-2 (1.57-fold at 100 mm Hg)	ECM remodelling
Nichol et al. 2009 [33]	carotid arteries (porcine)	carotid arteries cannulated onto stainless steel rods in a chamber connected to a self-contained perfusion loop arteries exposed to reduced or arterial haemodynamics for 9 days and either maintained their in situ length or elongated to 50% of in situ length	↑ MMP-2 and -9 protein expression by increased axial strain (elongation) and reduced haemodynamics individually ↑ MMP-9 by combined reduced haemodynamics and increased axial strain	ECM remodelling
ACE = Angiotensin-converting enzyme; TNF- α = tumour necrosis factor- α .				

Table 4. Changes in vein wall and altered gene expression on exposure to arterial pressure (in vivo AV fistula or vein bypass graft model)

Study	Model type	Stretch method employed	Genes regulated	Effect on the vessel wall
Takase et al. 2004 [103]	femoral artery and vein fistula formation (rat)	AV fistula development between femoral artery and vein for 21 days	↑ granulocyte, monocyte, T lymphocyte infiltration of valve ↑ P-selectin and ICAM-1 in ECs	increased inflammatory reaction extensive apoptosis
Pyle et al. 2010 [125]	saphenous veins retrieved from coronary artery bypass surgery VSMCs from human aortic tissue	arterialised saphenous veins from coronary artery bypass surgery VSMCs subjected to cyclical stretch of 15–20%	↑ MMP-2 (2.9-fold at 48 h) ↑ elastin (8.8-fold at 72 h) ↑ AIE genes in VSMCs exposed to cyclical stretch: sciellin (4.6-fold) periplakin (1.5-fold) SPRR3 genes (4.9-fold)	ECM remodelling
Chung et al. 2005 [35]	carotid artery bypass graft using internal jugular vein (porcine)	arterialised internal jugular vein for 2 weeks	↑ MMP-2 ↑ MMP-9 ↓ TIMP-1 ↓ TIMP-2	ECM remodelling neointima formation in arterialised vein
Misra et al. 2008 [34]	femoral artery and vein fistula formation (rat)	AV fistula development between femoral artery and vein for 28 days (exposing veins to arterial pressure)	↑ pro-MMP-2 ↑ active MMP-2 at days 14 and 28 ↑ pro-MMP-9 at day 28 (at the site of venous stenosis) ↑ intima to media ratio by 28% (α -actin)	ECM remodelling linked to vein graft narrowing and failure
Pascarella et al. 2008 [100]	femoral artery and vein fistula formation (rat)	AV fistula development between femoral artery and vein for 21 days (exposing veins to arterial pressure)	↑ MMP-2 ↑ MMP-9 ↑ granulocyte and macrophage infiltration into the venous wall and valve	ECM remodelling vein valve scarring
SPRR3 = Small proline-rich protein 3; TIMP = tissue inhibitor of metalloproteinase.				

model suggests the important role of stretch in the development of neointima in arterialised veins (table 4) [101]. The potential for venous hypertension to initiate vein wall and valvular damage leading to venous insufficiency is shown in a rat model by inducing acute venous hypertension through the creation of an AV fistula between the femoral artery and vein [34, 100, 102, 103]. The limbs with the AV fistula developed venous reflux and oedema, which worsened over time (3–6 weeks) compared to control limbs. Valvular and vein wall injury, fibrosis, increased infiltration of inflammatory cells including granulocytes, monocytes and macrophages and upregulation of P-selectin and ICAM-1 expression were found in saphenous vein walls in the presence of an AV fistula compared to those without [100, 102, 103]. Interestingly, rats that received oral treatment with micronised purified flavonoid fraction (a drug used in the treatment of CVI) demonstrated reduced leg oedema and venous reflux in response to the AV fistula [100].

Moreover, MMP-2 and MMP-9 were found to be over-expressed at the site of neointima in both the AV fistula

and vein bypass graft model after 2 weeks of exposure to arterial pressure (further increased after 4 weeks) [34, 35, 100]. Increased scarring of the venous valve was also noted [100]. These models clearly explain the important role of MMPs in the development of neointimal lesions in arterialised veins.

ROS and Stretch

ROS are produced by steady reduction of oxygen and include both unstable free radicals (such as superoxide and hydroxyl radicals) and stable non-free radicals (such as hydrogen peroxide). Increased production of ROS is implicated in the pathogenesis of vascular diseases such as hypertension, atherosclerosis, diabetic vascular complications and heart failure [104, 105]. ROS are produced by all vascular cell types including ECs, VSMCs and adventitial cells [55, 106, 107]. Pressure-induced mechanical stretch generates ROS from sources including xanthine oxidoreductase, nicotinamide adenine dinucleo-

tide phosphate oxidases and uncoupled nitric oxide synthases [69, 104, 108, 109]. These cyclic stretch-induced ROS further activate MAPK signalling, increasing NF κ B transcription factor production and, hence, playing a significant role in upregulating stretch-induced gene transcription (fig. 2) [110–112]. ROS have been linked with stretch-induced upregulation of VCAM-1, MMP-2, VEGF, IL-1, IL-6 and MCP-1 [55, 72, 93, 104].

Alterations in Gene Expression with Stretch: Similarities with the Biological Features of Vascular Diseases

Varicose Veins

Several risk factors have been identified for primary varicose vein disease including age, sex, genetics and family history, pregnancy and occupation involving prolonged standing [113]. Primary wall changes including intimal hyperplasia, smooth muscle hypertrophy, alteration in elastin and collagen content and imbalances of MMPs and their tissue inhibitors have been reported in the context of varicose veins [114, 115].

Infiltration of inflammatory cells and increased expression of surface markers including VCAM-1 and ICAM-1 have been seen in the ECs of varicose veins [114, 116, 117]. Elevation of venous pressure activates inflammatory processes in the venous system including expression of E-selectin, ICAM-1 and VCAM-1 in the plasma obtained from leg veins of patients with and without varicose veins [118]. In another study, high venous pressure induced through venous occlusion with an inflated cuff elevated the expression of IL-1 β , IL-6 and tumour necrosis factor- α in patients with and without varicose veins, and more so in the former [119, 120]. Imbalances of vasoconstrictor and vasodilator levels have been reported, with a reduction in vasoconstrictors and an upregulation of vasodilators reported in the context of varicose veins [15, 114]. MMP-2 and -9 are also implicated in the development of varicose veins and CVI [87].

Chronic Venous Insufficiency

The mechanical stretch seen with blood stasis in the legs has been proposed as a mechanism responsible for primary vein wall changes; however, the evidence remains inconclusive. Several hypotheses, such as the white cell trapping theory, fibrin cuff theory and growth factor trapping theory, have been considered in the development of CVI [2]. Inflammation and an imbalance of

growth factors are considered to play a significant role in the pathogenesis of CVI, causing movement of capillary contents into the interstitial space, hyperpigmentation, oedema and ulceration. For example, upregulation of transforming growth factor- β , which has pro-proliferative and anti-inflammatory effects, is associated with healing of venous ulceration [121]. MMP-9 along with endothelial membrane intercellular adhesion molecule 1 and L-selectin are found to be elevated in blood samples taken from lower-extremity veins following 30 min of postural stasis, suggesting an interaction between proteolytic enzymes, ECs and leukocytes in situations resembling venous hypertension [122].

The majority of studies on cell culture models have looked at the effect of cyclical stretch. This approach limits the application of results to explaining the pathogenesis of venous diseases where the stretch pattern is more static than cyclical. Therefore, there remains a need for experimental evidence to correlate the role of static stretch in the pathogenesis of varicose veins and CVI. A better understanding of the impact of stretch due to blood stasis and its effect on the vessel wall may help open new therapeutic avenues for arterial and venous conditions.

Vein Graft Disease

Cellular changes such as loss of the EC layer, intimal hyperplasia, SMC proliferation and migration into the intima are reported in veins used as a conduit in arterial bypass leading to graft stenosis or occlusion [21]. Davies et al. [123] and Wilson et al. [124] have shown diffuse changes in intimal hyperplasia and muscular hypertrophy in saphenous and cephalic veins prior to their use in bypass surgery, suggesting that pre-existing disease may predispose vein grafts to stenosis due to inherent susceptibility of diseased veins to intimal hyperplasia.

Certain genes are isolated under physiological conditions in arteries but not veins. Arterial intima-enriched (AIE) genes are expressed by VSMCs and include sciellin, periplakin and small proline-rich protein-3 (SPRR3). AIE proteins are largely cytoskeletal and contribute to the protective properties of stratified epithelium, but their role in vascular tissue is unclear. Exposure to 24–72 h of stretch increases the expression of AIE genes in human arterial cells. Expression of AIE genes has been seen in arterialised saphenous veins (used as a conduit for coronary artery bypass surgery), suggesting that under conditions of luminal stretch, gene expression in veins approaches that seen in arteries [125].

By further understanding the differential gene expression and specific cellular pathways responsible for initiating and potentiating the pathological vein wall remodeling seen with vein arterialisation when exposed to pressure, development of putative therapeutic agents will be facilitated which may be employed in pre-conditioning veins prior to or during bypass graft surgery. The aim in this context is to improve long-term vein graft patency, reducing the death and limb loss related to vein bypass graft failure.

Aneurysmal and Atherosclerotic Arterial Diseases and Similarities with Venous Diseases

In aneurysmal arterial disease, inflammation is present with a cascade activating cytokines, chemokines, MMPs and ROS. In patients with abdominal aortic aneurysms, the expression of aortic wall MMP-9 was associated with aneurysm diameter [126]. Weakness of the medial layer and arterial dilation results in susceptibility to further enlargement with continued exposure to high pressure and, ultimately, rupture. Atherosclerotic arterial disease is also characterised by interaction between multiple vascular cell types with activation of a complex and self-perpetuating inflammatory cascade, again with protease activation [127].

Evidence has shown similarities among arterial and venous conditions. Exposure to altered pressure stretch

and/or shear stress in both arteries and veins results in activation of inflammation and remodelling of ECM. This results in the development of atherosclerotic lesions in arteries and arterialised veins. Veins have been shown to require less pressure than arteries to show alterations in their gross morphology. For instance, in a recent study, animal internal jugular vein and carotid arteries were subjected to increasing degrees of distending pressures, and the authors demonstrated that veins buckle at a much lower pressure than arteries [128]. This has clinical relevance in conditions where prolonged intraluminal pressure-induced stretch may lead to constant buckling, including varicose veins, venous valvular insufficiency, diabetic retinopathy and vein grafts [128].

Conclusion

Modulation of gene expression by stretch is seen in both vascular ECs and VSMCs. The effects of these transcriptional alterations and subsequent protein production lead to changes in cell growth and apoptosis, increased cytokine secretion, upregulation of adhesion molecules on ECs and phenotypic alteration in VSMCs. Similarly, the effect of stretch on vascular cells results in imbalances of MMPs and their inhibitors, in turn altering the composition of the ECM.

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