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REVIEW

Vasostatins: new molecular targets for atherosclerosis, post-ischaemic angiogenesis, and arteriogenesis

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Abstract

The chromogranin–secretogranin secretory proteins—granins—are acidic proteins localized in granules of endocrine cells and neurons. The chromogranin family includes chromogranins A (CgA) and B, as well as secretogranin II (once called chromogranin C). Members of this family undergo catalytic proteolysis to produce active peptides. The CgA-derived peptides vasostatin-1 and vasostatin-2, in particular, appear to protect against atherosclerosis, suppressing the expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, as well as exerting vasodilatory effects by enhancing nitric oxide bioavailability. Vasostatin-1 also suppresses vasoconstriction and abnormal angiogenesis. Vasostatin-1 and vasostatin-2 may be novel therapeutic targets for atherosclerosis and coronary heart disease, also protecting the myocardium against ischaemic damage.

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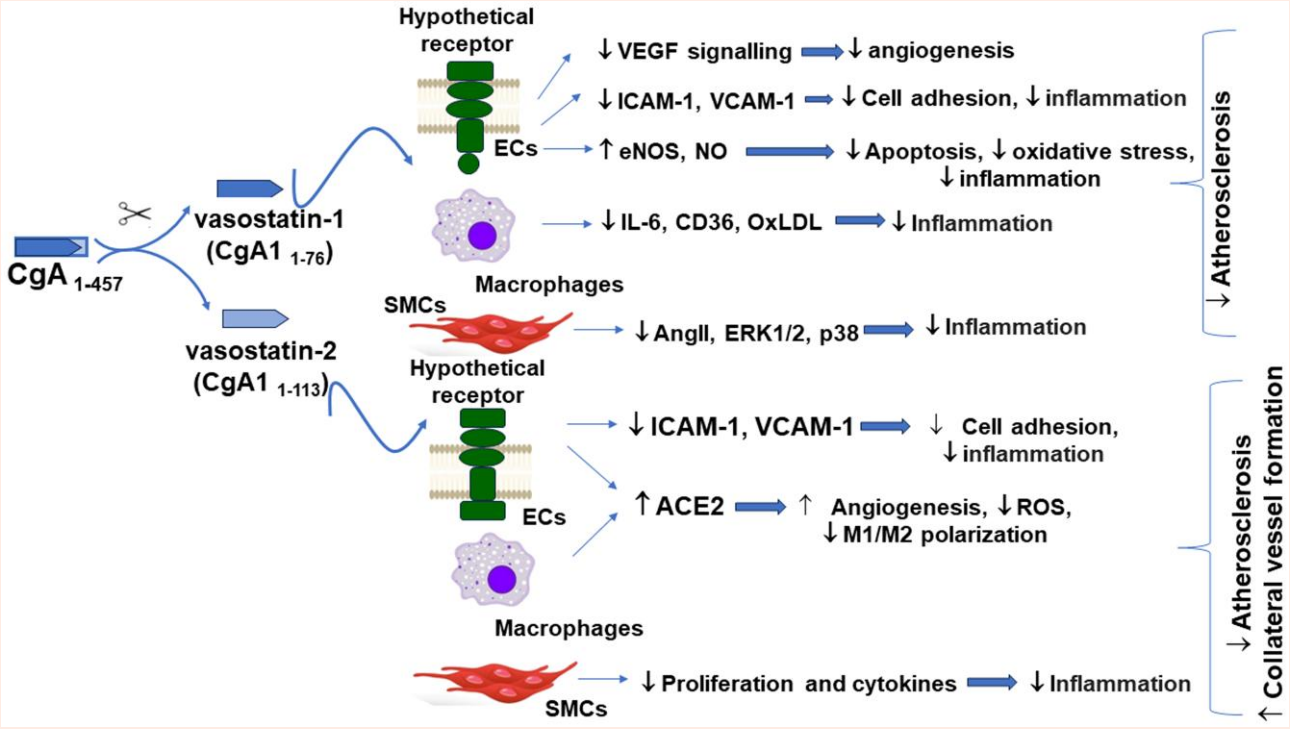
Graphical Abstract



Vasostatin-1 reduces atherosclerosis and plaque rupture via the reduction of abnormal angiogenesis and plaque hypervascularization. Vasostatin-2 reduces atherosclerosis and promotes angiogenesis and collateral vessel formation via ACE2. ACE2, angiotensin-converting enzyme 2; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; VEGF, vascular endothelial growth factor; OxLDL, oxidized LDL; AngII, angiotensin II; ROS, reactive oxygen species.

Keywords Chromogranins • Vasostatins • Angiogenesis • Arteriogenesis • Atherosclerosis

1. Introduction

Atherosclerosis is an inflammatory disease that initially arises from endothelial dysfunction following arterial damage due to acute or chronic exposure to cardiovascular risk factors.[1] Endothelial dysfunction leads to a cascade of events that can be summarized in four different phases: (i) lipid infiltration of the intima; (ii) homing of leucocytes and smooth muscle cells into the intima; (iii) foam cell formation; and (iv) lysis of extracellular matrix (ECM). This process may evolve into arterial narrowing and, eventually, in plaque rupture, with subsequent myocardial ischaemia.[2]

Abnormal angiogenesis due to plaque hypervascularization is involved in plaque rupture. Neocapillaries appear as fragile vessel structures without pericytes, with a consequent high potential for rupture, haemorrhage, and acute increase in plaque volume.[3,4] Angiogenesis is also involved in the formation of new capillaries after artery occlusion.[5–8] This response to these insults is the endogenous attempt to repair or limit the ischaemic/necrotic tissue following injury, and, therefore, angiogenesis can be viewed as a physiological reparative process. The impaired ability to repair myocardial tissue in response to ischaemia by new vessel formation is one aspect of endothelial dysfunction due to exposure to various risk factors, especially diabetes.[4] Angiogenesis is regulated by growth factors and cytokines that act in a subtle balance between proangiogenic and antiangiogenic signalling pathways.[9]

Chromogranins and their derived peptides are among emerging molecules implicated in endothelial dysfunction, atherosclerosis, angiogenesis, and tissue repair. In particular, the chromogranin A (CgA)–derived peptides vasostatin-1 and vasostatin-2 appear to exert protection against atherosclerosis by limiting endothelial activation and inducing vasodilation by enhancing nitric oxide (NO) bioavailability.[10] Vasostatin-1 also suppresses vasoconstriction and abnormal angiogenesis of the microvasculature (i.e. abnormal capillary formation in the diabetic retina), while promoting reparative angiogenesis and arteriogenesis in the ischaemic hindlimb or myocardium.[11] Vasostatin-1 and vasostatin-2 may thus be novel therapeutic targets for atherosclerosis and tissue ischaemia.

Here, we discuss the biochemical and functional aspects of the chromogranin system, specifically focusing on the role of vasostatins on atherosclerosis and post-ischaemic angiogenesis/arteriogenesis.[12]

1.1 The chromogranin family: origin and effects

The chromogranin–secretogranin secretory proteins are collectively referred to as granins. Granins are acidic proteins stored in granules of endocrine cells and neurons. The discovery that cardiomyocytes and endothelial cells (ECs) produce at least one of the three chromogranins belonging to the family, especially CgA, along with brain natriuretic peptide (BNP), has promoted interest in the cardiovascular effects of CgA and its fragments.

Chromogranins undergo a catalytic proteolysis to produce active peptides. There are three chromogranins: CgA, CgB (also called secretogranin I), and CgC (also called secretogranin II)[13] (*Figure 1*). Names of these mediators were derived from the initial discovery of 'granins' as proteins involved in the formation of secretory granules, initially localized in chromaffin cells ('chromo-'). The discovery of CgA dates back to 1965, when it was first isolated from chromaffin cells of the adrenal medulla. In 1991, CgB and CgC were identified in a rat pheochromocytoma cell line and in

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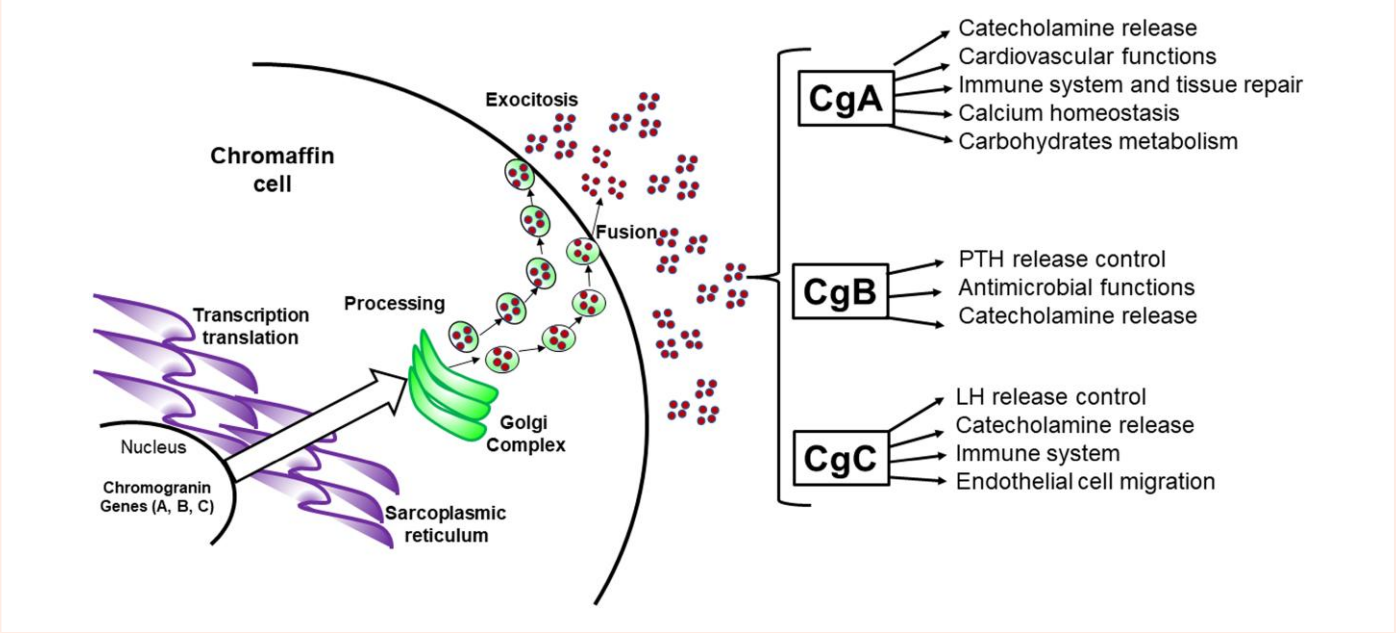

Figure 1 The granin family. Biological and biochemical activities of mature chromogranins A, B, and C. Cg, chromogranin; PTH, parathyroid hormone; LH, luteinizing hormone.

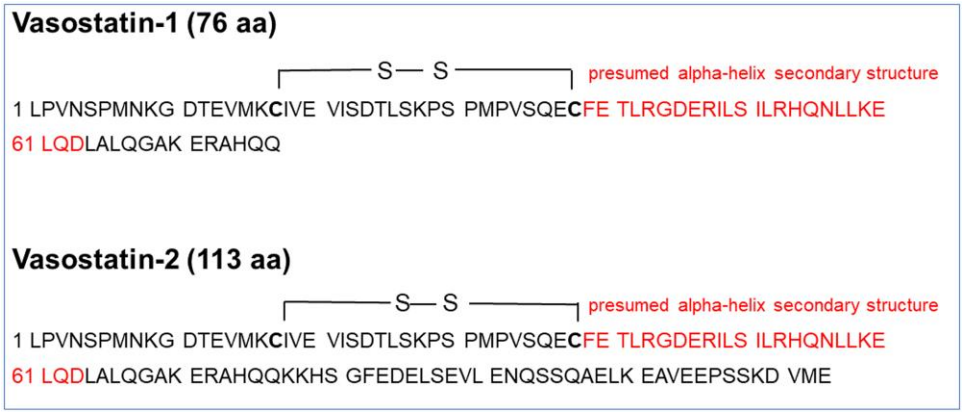

Figure 2 Structural features of vasostatins. Primary structure of vasostatin-1 and vasostatin-2, CgA 1-76 and CgA 1-113, respectively (from pro-CgA, https://www.ncbi.nlm.nih.gov/protein/215274270). Both contain a disulfide bridge (–S–S–) between cysteine residues 17 and 38 (C in bold), which generates a loop; the sequence in red can bear an alpha-helix structure, as present in CgA, which favours cell membrane adhesion and its subsequent activity. The RGD and KGD triplets promote the interaction with the VEGFR.

the anterior pituitary and prostate cancer cells, respectively. Granins also include less characterized peptides such as secretogranins III (SgIII), IV (SgIV), V (SgV, also called 'neuroendocrine secretory protein 7B2'), VI (SgVI or NESP55), and VII [SgVII, also called 'VGF (non-acronymic) nerve growth factor inducible protein']. Granins are ubiquitous acid secretory proteins, encoded by distinct genes localized in chromosomes 14q32.12 (CgA), 20pter–p12 (CgB), and 2q35–2q36 (CgC). They are characterized by a total negative charge and are localized in neurons, immune cells, and neuroendocrine organs (the anterior pituitary lobe, thyroid C cells, parathyroid glands, the adrenal medulla, the islets of Langerhans, and the enterochromaffin cells of the gastrointestinal tract). Human CgA, CgB, and CgC are proteins of 457, 677, and 617 amino acids with a molecular mass of 51, 78, and 68 kDa, respectively. CgA co-localizes with catecholamines in granules in the adrenal medulla, or with the parathyroid hormone in the parathyroid glands. CgA is also present in pancreatic islets, keratinocytes, cardiomyocytes, ECs, and macrophages. CgA is released in the tissue microenvironment and then into the circulation. Both CgA and CgB are involved in the blood pressure control. They also influence the immune system, as well as glucose and insulin metabolism.[14] CgC induces the release of dopamine, luteinizing hormone (LH), and catecholamines and contributes to EC migration.

CgA undergoes proteolysis generating smaller fragments with regulatory effects on the endocrine, cardiovascular, immune systems, and on glucose or calcium metabolism.[14] Proteolysis of mature CgA occurs through

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Table 1 Role of vasostatin-1 in different experimental models of vascular disease

PMID	Mechanism of action	Effect
34968165	↓ HS-CRP, ICAM-1, VCAM-1, and TNF-α expression	↓ Inflammatory response, diameter of abdominal aorta, incidence of abdominal aortic aneurysm, mortality in ApoE$^{-/-}$ mice
34839793	↑ AMPK/mTOR signalling	↓ Abdominal aortic aneurysm in rats
34093983	Binding to VEGFR, inhibition of VEGF pathway	↓ Angiogenesis, dose-dependent relaxation of arterial smooth muscle in rats
30401690	In macrophages: ↓ M1 phenotype and LPS-induced IL-6 secretion via NF-κB downregulation, ↓ OxLDL-induced foam cell formation via CD36 downregulation and ABCA1 upregulation In human SMC: ↓ AngII-induced migration and collagen-3 and fibronectin expression via decreasing ERK1/2 and p38 phosphorylation ↑ Elastin expression and matrix MMP-2 and MMP-9 activities via increasing Akt and JNK phosphorylation	↓Inflammation, atherogenesis in ApoE$^{-/-}$ mice
21884005	↑ eNOS and NO serum concentration	↓ Apoptosis, intracellular oxidative stress and inflammation
21825034	↓ Hypoxia-driven changes in EC, VE-cadherin redistribution, intercellular gap formation, and HIF-1α nuclear translocation	↓ Tumour neovascularization in *in vitro* models
21799030	↓ NGR-TNF-induced drug permeability	↓ Drug extravasation in tumours in EC monolayers
20217454	Binding to the fibroblast membrane via alpha-helix (residues 47–66) and C-terminal region (residues 67–78)	↑ Fibroblast cell adhesion in *in vitro* models
20213742	↑ eNOS phosphorylation, binding to heparan sulfate proteoglycans and caveolae endocytosis	↑ Vasodilation In BAEC
18697842	↑ PI3K-dependent eNOS phosphorylation	↑ Coronary dilation in Langendorff perfused rat heart
17566084	↑ VEGF-induced ERK phosphorylation	↓ EC proliferation and migration in *in vitro* assays
16725215	↓ Protein Gi/p38MAPK signalling, TNF-α-induced vascular dysfunction	↓ Vascular leakage in BAEC
16445995	Electrostatic and hydrophobic interactions with membrane phospholipids	↑ *In vitro* membrane fluidity in presence of saturated phosphatidylserines
11891009	↑ Protein Gi pathway and K^+ channel activation	Vasodilator effects in isolated bovine coronary arteries
7810329	Calcium binding	Vasoinhibitory activity in aortic VSMC

HS-CRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular adhesion molecule-1; TNF-α, tumour necrosis factor-alpha; ABCA1, ATP-binding cassette transporter A1; ApoE$^{-/-}$, apolipoprotein E knock-out; AMPK/mTOR, adenosine monophosphate–activated protein kinase/mammalian target of rapamycin; VEGFR, vascular endothelial growth factor receptor; IL-6, interleukin 6; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; OxLDL, oxidized LDL; VSMC, vascular smooth muscle cell; AngII, angiotensin II; MMP, metalloproteinase; JNK, c-Jun NH2-terminal kinase; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; EC, endothelial cells; HIF, hypoxia-inducible factor; BAEC, bovine aortic endothelial cells; p38MAPK, P38 mitogen–activated protein kinase.

proteases such as prohormone convertase 1 (PC1), PC2, furin, cysteine protease cathepsin 1, plasmin, thrombin, and kallikrein.[12,13,15] Post-translational modifications and proteolytic processing of different CgA cleavage sites give life to nine biologically active peptides including vasostatin-1 (CgA1-76) and vasostatin-2 (CgA1-113) near the N-terminal domain; chromofungin (CgA47-66) and chromostatin (CgA124-143, which share segments of the sequence of pancreastatin); chromacin and pancreastatin (CgA250-301), from the central domain; and WE-14 (CgA324-337), catestylin (CgA344-358), catestatin (CgA352-372), serpin (CgA402-439), and GE25 from the C-terminal domain, which contains parastatin and serpinin sequences.[16]

The full-length CgA can exert, in several biological pathways, a variety of regulatory functions, which are largely shared by its derived peptides.[13] CgA indeed has negative lusitropic (referring to the ability of the myocardium to relax following excitation–contraction coupling) and inotropic effects on the myocardium, as well as vasodilatory effects on the coronary arteries, mediated by the NO pathway in mammalian hearts.[17,18] CgA and its derivatives mostly exert antiangiogenic effects and can also regulate endothelial homeostasis by enhancing the endothelial barrier function.[19]

1.2 Vasostatins—origin, structural features, receptors, and cardiovascular effects

The term 'vasostatin' comes from the vasodilatory effect on isolated human vessels. Indeed, vasostatin-1 and vasostatin-2 can exert vasorelaxation in arterial vessels. Vasostatin-1 is the most abundant N-terminal peptide originating from CgA and contains 76 amino acids (*Figure 2*).

Vasostatin-1 carries three domains, two in the negatively charged CgA1–40 moiety and the third in the positively charged CgA47–66 moiety. The latter plays a role in innate immunity, as it features antifungal activities by penetrating through fungal cell membranes.[20] In vasostatin-1, the disulfide bridge is required for the cardiotropic action, while the C-terminal moiety is essential for antifungal activity.[21,22] The N-terminal region of CgA interacts with the calcium–calmodulin regulatory complex, inhibiting calmodulin-dependent enzymes. Together with the destabilization of fungal wall and plasma membrane, this may represent the mechanism by which vasostatin-I exerts antifungal activity.[23] The C-terminal domain (residues 47–78) of vasostatin-1 features two functional regions: an amphipathic alpha-helix (residues 47–66), which is critical for adhesion to the fibroblast membrane and for exerting its activity, and the hydrophilic C-terminal region (residues 67–78), which regulates its proadhesive activity by indirectly interacting with integrins and inducing the reorganization of the actin cytoskeleton. Membrane phospholipids can interact with the alpha helix, and the 69–75 residue hydrophilic sequence of the C-terminal region is structurally similar to the 50 kDa membrane cytoskeleton adapter protein ezrin–radixin–moesin-binding phosphoprotein.[24] The interaction between vasostatin-1 and the vascular endothelial growth factor receptor (VEGFR) has been analysed with a bioengineered vasostatin-1-derived peptide (V1DP). V1DP, which traces natural vasostatin-1, contains a domain highly conserved among vertebrates in its N-terminal region, including a

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Table 2 Role of vasostatin-2 in atherosclerotic vascular disease

PMID	Mechanism of action	Effects
36861348	↑ ACE2 expression	↓ Angiogenesis and atherogenesis in diabetic mice
27831589	↓ Rac1 (expression), ↓ Pak1 activation in primary monocytes and THP-1 cells	↓ Atherosclerosis, monocyte/macrophage recruitment and inflammation in ApoE$^{-/-}$ mice
26721428	↓ Inflammatory cytokine release from human VSMC, ↓ soluble adhesion molecules (sICAM-1, sVCAM-1)	↓ EC proliferation, inflammatory response, and cell adhesion in VSMC
22645192	↓ TNF-α-, angiotensin II-, and OxLDL-induced expression of adhesion molecules	↓ Atherosclerosis and inflammation in human coronary artery disease

ACE, angiotensin-converting enzyme; THP-1, human acute monocytic leukaemia cell line; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular adhesion molecule-1; TNF-α, tumour necrosis factor-alpha; ApoE$^{-/-}$, apolipoprotein E knock-out; OxLDL, oxidized LDL; VSMC, vascular smooth muscle cell; Pak1, p21-activated kinase-1; EC, endothelial cells.

disulfide bridge formed by two (cys17 and cys38) cysteine residues and two tripeptide recognition sequences, the amino acid triplets arginine–glycine–aspartate (RGD) and lysine–glycine–aspartate (KGD). The disulfide bridge, which produces a loop in the hydrophobic sequence, is located between these triplets. With these features, this synthetic peptide can bind VEGFR, blocking its signalling cascade.[19]

Vasostatin-2 is a peptide consisting of 37 amino acids, longer than vasostatin-1 (*Figure 2*). The sequence '38-FETLRGDERI LSILRHQNLL KELQD-54', present in both peptides is organized in an alpha-helix secondary structure. Furthermore, the N-terminal fragment has a disulfide bridge at amino acids 17 and 38 that confers various biological activities to the protein.[25,26] Vasostatin-2 from bovine CgA is phosphorylated at Ser-81.[27] This site is close to the cleavage sequence […QKK78HSS(p)81…] that yields vasostatin-1, suggesting the involvement of this modification in the production of vasostatin-1 in bovine chromaffin cells.[27] Comparing the two isoforms of vasostatin-2, the human isoform contains a Ser-80, which is not modified[14] (*Figure 2*).

Vasostatin-1 and vasostatin-2 exert a large series of vascular effects summarized in *Tables 1* and *2*, respectively. In addition to vasodilation, vasostatins modulate the adhesion of fibroblasts and smooth muscle cells to ECM proteins and exert a cardio-inhibitory effect on isolated beating hearts from eels, frogs, and rats by negative chronotropic, inotropic, and lusitropic effects.[17] The inhibitory cardiovascular actions of vasostatins on heart rate, blood pressure, and myocardial contractility occur by increasing the vagal tone and reducing the sympathetic tone. Specifically, vasostatin-1 induces inhibitory cardiovascular reflexes through the Akt/NO/cyclic guanosine

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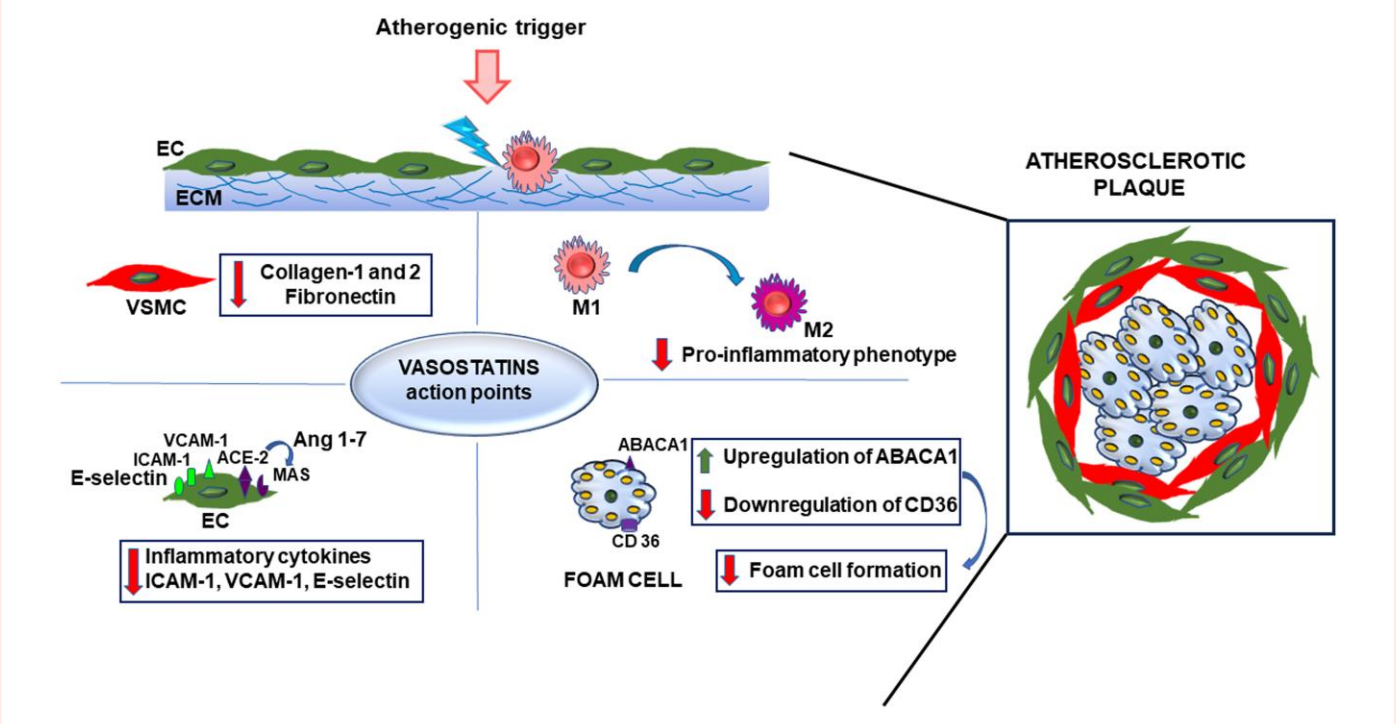

Figure 3 Main action points of vasostatins in atherosclerotic plaques. Vasostatins are important modulators of vascular permeability, inflammation, and elasticity by targeting ECs, VSMCs, macrophages M1 and M2, and ECM. Vasostatins intervene in the composition of the atherosclerotic plaque matrix through the reduction of fibronectin and type 1 and 2 collagen secretion by VSMC. Vasostatins reduce the expression of adhesion molecules and the secretion of proinflammatory cytokines by ECs, with a shift from M1 macrophage polarization to the M2 phenotype, leading to attenuated inflammation. Finally, vasostatins reduce foam cell formation through downregulation of C36 and upregulation of ABACA1 in human macrophages. ACE2, angiotensin-converting enzyme 2; MAS, angiotensin-(1–7) receptor; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; ABCA1, ATP-binding cassette transporter A1.

monophosphate (GMP)/protein kinase G pathway in hypertensive rat hearts.[18] Through this cardio-inhibitory action, vasostatins appear to intervene in the maintenance of cardiovascular homeostasis in conditions of sympathetic overstimulation, such as in stress conditions. Whether all effects of vasostatins are receptor mediated is not known, as specific receptors have not yet been identified. One theory is that vascular effects would depend on a hypothetical intracellular signalling system, activated by an as yet unidentified membrane receptor.[17] However, while it is not clear how vasostatin-2 elicits its effect on target cells, some experimental evidence suggests that it can interact with phosphatidylserine and other membrane-associated phospholipids.[28] In bovine aortic ECs, vasostatin-1 binds heparan sulfate proteoglycans and phosphoinositide 3-kinase and induces endothelial NO synthase (eNOS) phosphorylation,[29] contributing to antiatherogenic effects. Integrins and laminins may also act as important transducers of the vascular effects of vasostatin-1. At nanomolar concentrations, vasostatin-1 selectively interacts with the αvβ6 integrin, which is an endothelial and epithelial-specific cell surface receptor. By binding to laminin, vasostatin-1 interferes with EC attachment to the ECM and, by doing so, would derail the angiogenic process in the atheroma.[30]

1.3 Pathophysiology, diagnostic and therapeutic aspects of vasostatins, with a focus on atherosclerosis and post-ischaemic angiogenesis and arteriogenesis

Vasostatin-1 and vasostatin-2 protect against atherosclerosis, in particular vasostatin-1 suppresses vasoconstriction and angiogenesis (*Tables 1* and *2*). Vasostatin-1 is inversely associated with the progression of aortic atherosclerosis and has been proposed as a potential marker of atherosclerosis progression.[31] Low levels of circulating vasostatin-2 have been shown in diabetic patients with poor coronary collateral vessels and chronic total occlusions.[31] Low levels of vasostatins-2 have been shown in atherosclerotic plaque from endarterectomy samples and have been associated with increased coronary artery disease severity.[10] CgA, the precursor molecule of vasostatins, seems to play a role in the pathogenesis of diabetic vascular complications, mostly associated with the progression of atherosclerosis, although current knowledge on the role of CgA in diabetes is limited and conflicting. Compared with healthy controls, in patients with type 2 diabetes and poor glycaemic control (glycated haemoglobin ≥7.0%), CgA is increased in biological fluids, such as the saliva and serum.[10] Conversely, however, CgA levels in patients with type 2 diabetes were found comparable to those of healthy controls.[32] Less ambiguous are data in patients with type 1 diabetes, in whom CgA is consistently increased in >20% of cases, correlating with glycated haemoglobin levels.[32] CgA knock-out mice do not undergo type 1 diabetes and have a lower incidence of insulitis. These data indicate that CgA contributes to the pathogenesis of type 1 diabetes and also to disease progression. Whether vasostatins also play such an intimate role in the pathogenesis of diabetic vascular disease is not yet known. Nevertheless, decreased serum vasostatin-2 levels are associated with diagnosed coronary artery disease.[10] Vasostatin-1 may exert inhibitory effects on atherogenesis by suppressing oxidized LDL (OxLDL)–induced foam cell formation in macrophages, decreasing CD36 expression, and increasing ATP-binding cassette transporter A1 (ABCA1) expression in human macrophages (*Table 1* and *Figure 3*). Moreover, vasostatin-1 suppresses both vascular cell adhesion molecule-1 (VCAM-1) and E-selectin in ECs. Finally, vasostatin-1 exerts anti-inflammatory effects by suppressing macrophage migration.[31] Infusion of vasostatin-1 in apolipoprotein (Apo) E-deficient ($ApoE^{-/-}$) mice suppresses both the development of aortic atherosclerotic lesions and macrophage infiltration and leads to a reduction of plasma glucose levels, suggesting an inhibitory effects of vasostatin-1 on atherogenesis in diabetes.[31] Similarly, vasostatin-2 attenuates the atherosclerotic burden in $ApoE^{-/-}$ mice fed a high-fat diet,[33] an effect attributed to inhibition of monocyte/macrophage recruitment as well as to reduced production of tumour necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and VCAM-1 after treatment with vasostatin-2.[33] Vasostatin-1 inhibits EC migration and proliferation by blocking vascular endothelial growth factor (VEGF), therefore preventing the formation of new capillary vessels.[34] In addition, vasostatin-1 inhibits TNF-α-induced gap formation in ECs (*Table 1*). These studies highlight the importance of vasostatin-1 as key modulator of endothelial permeability,[35,36] which has a critical role in atherogenesis.[37] Indeed, vasostatin-1 also prevents ocular angiogenesis in the mouse model of diabetic retinopathy, suggesting that this peptide could reduce the angiogenesis and the hyperpermeability that characterize eye diseases in diabetic humans.[38] On vascular smooth muscle cells (VSMCs), vasostatin-1 and vasostatin-2 both induce elastin but suppress collagen-1 and collagen-2 expressions.[13] Both vasostatins appear to suppress human VSM, C migration,[31] but only vasostatin-2 suppresses human VSMC proliferation and secretion of inflammatory cytokines[39] (*Table 2*). Along with the other antiatherosclerotic effects, these results also suggest that vasostatins help suppress plaque progression and preserve vascular elasticity (*Figure 3*).

In addition to their vasorelaxant properties occurring via increased NO production from ECs,[40] vasostatins exert cardioprotection through several mechanisms, among which induction of angiogenesis seems to be a main one. Vasostatin-1 protects from ischaemia/reperfusion-induced myocardial dysfunction in rats[41,42] through an NO-dependent pathway. Although vasostatin-2 was shown to inhibit the proliferation of ECs and to decrease the proliferation of VSMCs, a protective effect of vasostatin-2 has also been suggested, promoting angiogenesis and arteriogenesis in diabetic mice with hindlimb or myocardial ischaemia, in hypoxic conditions.[11] Vasostatin-2 administration was found to have a significant impact on macrophage polarization in such an ischaemic tissue, inducing a shift from an M1 to a proangiogenic M2 phenotype. This shift appears to be modulated by angiotensin-converting enzyme 2 (ACE2) activity (*Table 2*). Interestingly, angiotensin-(1–7) generated through ACE2 catalysis can induce angiogenesis after myocardial ischaemia.[43] In the atherosclerotic plaque, vasostatins play an antiatherosclerotic effect likely mediated by reduced infiltration of macrophages into lesions and reduced production of proinflammatory cytokines. Here, the activation of ACE2 and the angiotensin-(1–7) receptor (also called Mas, a G protein–coupled receptor) shifts M1 macrophage polarization to the M2 phenotype, leading to attenuated inflammation.[42] Notably, vasostatin-2 administration did not increase angiogenesis in atherosclerotic plaques of diabetic mice, possibly due to a different effect from that occurring in hypoxic conditions.[42]

A concern common to all angiogenic therapies aimed at the resolution of tissue ischaemia is that the proangiogenic agent may exacerbate plaque angiogenesis and thereby affect coronary artery disease progression or plaque stability. In clinical trials using exogenous VEGF or gene therapies aimed at upregulating endogenous VEGF production, the concern was that the permealizing effect mediated by VEGF could induce plaque ingrowth and rupture. Furthermore, VEGF could stimulate the progression of coronary narrowing by stimulating the growth of fibroblasts and medial VSMCs. Indeed, prolonged infusion of VEGF was found to be associated with progression of post-angioplasty restenosis in a dog study.[44] Unlike VEGF, vasostatin-2 does not stimulate VSMC proliferation[39] and does not increase angiogenesis in atherosclerotic plaques of diabetic mice,[42] thus dispelling the fear of adverse effects on atherosclerosis stability. In summary, vasostatin-2 appears to induce tissue repair and regeneration through increased angiogenesis and arteriogenesis *in vivo*.

2. Conclusions

Vasostatin-1 and vasostatin-2 both exert antiatherosclerotic and cardioprotective effects, albeit through slightly different signalling pathways (Graphical Abstract). Vasostatin-1 and vasostatin-2 suppress foam cell formation in macrophages and the expression of adhesion molecules in ECs.

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Both appear to suppress human VSMC migration, but only vasostatin-2 reduces human VSMC proliferation and secretion of inflammatory cytokines. Vasostatin-1 reduces plaque instability and protects from ischaemia/reperfusion-induced myocardial dysfunction through an NO-dependent pathway. Vasostatin-2 stimulates ischaemia-induced angiogenesis and protects against ischaemic myocardial damage. Because of these properties, a selective modulation of tissue levels of these peptides could represent an additional strategy capable of contributing to the slowing down of atherosclerotic vascular disease and its consequences on the myocardium. Additional preclinical and clinical intervention studies based on the modulation (in most cases increase) of tissue levels of these two peptides are necessary to confirm these hypotheses.

Author contributions

R.D.C. and R.M. contributed to the conception of the manuscript; R.M., S.B., and S.G. drafted the manuscript; R.D.C., L.L., and W.-F.S. critically reviewed and contributed to the final draft.

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