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Pleiotropic properties of statins via angiogenesis modulation in cardiovascular disease

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Inhibition of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase by statins is affected by inhibiting the active site of the enzyme in a competitive manner. Statins reduce plasma cholesterol by inhibiting its *de novo* synthesis. In addition, statins impart 'pleiotropic' activities that do not directly relate to their ability to decrease cholesterol. The proangiogenic and antiangiogenic characteristics of statins are among these pleiotropic effects. These angiogenic-modifying properties could offer new therapeutic applications. Statins stimulate or suppress angiogenesis in a biphasic manner. Whereas low doses of statin stimulate angiogenesis, high doses reduce protein prenylation and limit cell development and angiogenesis. In this review, we discuss how statins impact angiogenesis, with a particular focus on angiogenesis in stroke and cardiovascular disease (CVD).

Keywords: Statins; Angiogenesis; Vascularization; Cardiovascular; Ischemic heart disease

Introduction

Statins competitively inhibit HMG-CoA reductase and are widely used and highly effective therapeutic agents. They hinder the conversion of HMG-CoA to mevalonate, the upstream, rate-limiting step for the biosynthesis of cholesterol, by directly blocking the active site of the enzyme.[1] In humans, plasma cholesterol is derived either from food consumption or by cellular *de novo* production. Statins lower serum cholesterol by inhibiting *de novo* cholesterol production and altering the expression of the low-density lipoprotein (LDL) receptor.[2] Upstream of cholesterol, mevalonate is converted to isoprenoid lipids, such as isopentenyl pyrophosphate (IPP), farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP). These intermediate compounds are important in the post-translational modification of several oncoproteins, particularly small monomeric GTPases, such as the Rho and Ras families, which have been linked to cancer initiation and progression in diverse ways.[3] Furthermore, multiple clinical investigations have revealed inhibiting the mevalonate pathway as a potential cancer treatment and method of chemoprevention.[4]

The discovery of statins as a fungal metabolite during the 1970s sparked their development as 'cholesterol-lowering' medications, with the earliest of these compounds being a natural substance called mevastatin. Lovastatin, another naturally occurring statin, was found to have twice the potency for inhibition of HMG-CoA reductase compared with mevastatin. The first statin approved by the US Food and Drug Administration (FDA) for use in the treatment of hypercholesterolemia and CVD was lovastatin. Based on the naturally occurring statin structure, simvastatin and pravastatin

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(two synthetic statins) and atorvastatin, fluvastatin, rosuvastatin, cerivastatin and pitavastatin were developed.[5] Although newer lipid-lowering therapies have been introduced in recent decades,[6–8] statins are still a mainstay in dyslipidemia management.

The pharmacokinetics (PK) of the seven FDA-approved statins are comparable although not equal. There are two types of statin (lipophilic and hydrophilic) and their physicochemical features, such as lipo- and hydrophilicity, are used to distinguish among them.[9] To gain access to HMG-CoA reductase, their classical target, lipophilic statins diffuse passively through cell membranes. By contrast, hydrophilic statins frequently require transmembrane peptide transporters to pass through cell membranes and, therefore, are less widely distributed extrahepatically compared with lipophilic ones. The organic anion transporting polypeptide-1B (OATP1) transporter, expressed mainly in liver, is required for hydrophilic statins. Other subtypes of OATP are expressed in other cells and tissues and could be significant for statin trafficking.[10] This physicochemical feature illustrates why hydrophilic statins concentrate in the liver initially.[11]

Statins exert their pleotropic effects in several areas of cardiovascular pathobiology in addition to affecting lipid balance balance.[12–16] Reduced availability of FPP and GGPP reduces cellular signaling from small G proteins, with downstream cell-mediated effects.[17] For example, reduced Rho activation increases the production of endothelial nitric oxide synthase (eNOS), which maintains the function of the endothelium and slows the onset of CVD.[18] Statin safety and oral availability allow for potential novel therapeutic applications because of their angiogenic-regulating actions.[9]

Angiogenesis is the growth of new blood vessels from existing ones. It is a complicated and dynamic process that involves smooth muscle cells, extracellular matrix (ECM), endothelial cells (ECs), vascular pericytes, and fibroblasts.[19] Angiogenesis is essential in a range of physiological situations, such as wound healing and embryonic development, as well as numerous pathological disorders, such as ischemic heart disease, stroke, cancer, diabetic retinopathy, and obesity.[20] Statins are among the therapeutic agents of interest in the study of angiogenesis. Although there has been much research on the effects of statins on angiogenesis, findings have been inconsistent. Some studies found statins to be proangiogenic, whereas others found them to be antiangiogenic.[21,22] However, when the statin effects on angiogenesis were studied at various statin concentrations, it was determined that statins had a dose-dependent biphasic effect on angiogenesis, with a low-dose proangiogenic effect and a high-dose antiangiogenic and proapoptotic effect.[23] The dose-dependent impact of statins on angiogenesis, first proposed in 2002, is now commonly accepted.[24] In this review, we outline the pleiotropic effects of statins on angiogenesis in stroke and CVD.

Proangiogenic properties of statins

Low-dose statins activate angiogenesis in a variety of animal models. The mechanism appears to relate to PI3 kinase/Akt signaling pathway activation, leading to eNOS phosphorylation/NO generation.[24–27] Bone marrow-generated endothelial progenitor cells (EPCs) have been shown to be involved in neovascularization, establishing postnatal vasculogenesis.[28] Statins improve EPC recruitment to neovascularization locations by promoting adult bone marrow-derived EPCs to survive, migrate, and differentiate via a mechanism dependent upon Akt.[29,30] In individuals with stable coronary artery disease (CAD), statin therapy lowers EPC senescence, promotes their proliferation, and mobilizes circulating $CD34^+$ EPCs.[30] eNOS is necessary for EPC mobilization, which improves overall heart function and leads to neovascularization of the myocardium.[31] Statin treatment might help EPC differentiation into cardiomyogenic cells in patients with CAD.[32] In this regard, simvastatin has been shown to improve the differentiation of ECs from peripheral blood mononuclear cells (PBMCs) in individuals with hypercholesterolemia and to boost the monocytic release of the proangiogenic cytokine IL-8.[33]

In human umbilical vein ECs (HUVECs), pravastatin, a hydrophilic statin, causes fibroblast growth factor receptor (FGFR) phosphorylation.[34] PI3K/Akt and MAPK activity, induced by pravastatin, was reduced by SU5402, an inhibitor of FGFR. Akt and MAPK activity were also suppressed by anti-FGF-2-blocking antibodies. In addition, phosphorylation of Akt and MAPK, induced by heparin, was also inhibited when extracellular FGF-2 was depleted. Proliferation, tube formation, and migration of pravastatin-enhanced ECs were reduced by FGF-2 antibody treatment. These findings suggest that the proangiogenic effects exerted by pravastatin in ECs are extracellular FGF-2 dependent.[34] (see Table 1).

Antiangiogenic properties of statins

In contrast to the proangiogenic effects outlined above, much research has demonstrated that statins reduce both migration and proliferation *in vitro* of ECs and angiogenesis in experimental models.[35,36] The antiangiogenic effects of statins are linked to mechanisms independent of cholesterol. Statins reduced VEGF expression in retinal pigment epithelial cells (RPEs) by downregulating the aging receptor (rage) and by Rho/focal adhesion kinase/Akt signaling pathway suppression.[35] Further evidence showed that statin-dependent angiogenesis suppression is associated with an increase in cell cycle inhibitors, such as Wnt5a, p19, and p21, as well as angiogenesis-related gene downregulation, such as VTNC, *PAI1*, *Notch4*, and *HoxD3*.[37] This is consistent with previous findings indicating that statins decrease angiogenesis via decreasing the geranylgeranylation of RhoA, a small GTP-binding protein.[38] The aforementioned contradictory results are likely dosage dependent. Low-dose statins might activate endothelial Ras and enhance Akt and eNOS phosphorylation, resulting in proangiogenic effects, whereas high doses block Ras and RhoA without affecting eNOS upregulation.[27]

Aside from the biphasic dosage response shown in statin effects on angiogenesis, another question concerns acute versus chronic *in vivo* dosing. Statins improve vascular outcomes in several acute scenarios as well as after chronic therapy.[39–41] Similarly, a negative impact has been shown following statin discontinuation.[42] Although an exact assessment of effects on angiogenesis in patients is challenging, one can deduce that statins have favorable, although contradictory, effects in terms of cancer prevention (antiangiogenic) and reduction of CVD development in humans (proangiogenic).[43,44] A

TABLE 1

Studies exploring proangiogenic properties of statins in CVDs.[a]

Statin	Combination therapy	*In vivo*/ *in vitro* model	Dose	Effect	Disease	Refs
Atorvastatin	–	*In vitro*	0.1–1 μmol/l	Induces tube formation via decrease in abundance of caveolin; inhibits interaction with eNOS, induces tyrosine phosphorylation of hsp90 and direct interaction of hsp90 with Akt, inducing NO-dependent angiogenic processes	CMECs and HUVECs	26
		In vivo	1.5 mg/kg	Upregulates proangiogenic proteins eNOS, phosphorylated eNOS, phosphorylated AMPK, phosphorylated extracellular signal-regulated kinase, and VEGF	Ischemic myocardium in model of metabolic syndrome	67
			3 mg/kg, 8 mg/kg	Causes overexpression of angiopoietin-1 and VEGF and downregulation of MMP9 expression, leading to increased vascular density and enhanced vascular maturation	SDH	69
Fluvastatin	TSP-5	*In vitro*	Fluvastatin 0.5 μM and TSP-5 20 mg/ml	Reverses antiangiogenic effects of TSP1 and TSP2; enhances angiogenesis; increases expression of proangiogenic genes (e.g., *PECAM1*, *TEK*, and *TIE1*, encoding CD31 receptor and two angiopoietin receptors, respectively); suppresses HGF	Human aortic ECs	66
	SDF-1	*In vitro* and *in vivo*	100 nM, and 5 mg/kg	Increases EPC proliferation and migration, Akt phosphorylation, NO generation, and MMP-2 and MMP-9 expression; reduces EPC apoptosis	Ischemic hindlimb C57BL/6 mouse model	58
Rosuvastatin	–	*In vitro* and *In vivo*	0.1 mg/kg	Enhances phosphorylated-Akt/phosphorylated eNOS levels in EPCs; improves bone marrow-derived ECs integrated at ischemic locations; increases capillary density and quickens blood flow recovery	Mouse model of surgically induced hindlimb ischemia	68
Simvastatin	–	*In vivo*	20 mg/day	Induces secretion of proangiogenic cytokine VEGF and IL-8 from monocytes	Hypercholesterolemia	33

[a] Abbreviations: CMEC, cardiac microvascular endothelial cell; pecam1, platelet and endothelial cell adhesion molecule; SDF-1, stromal cell-derived factor-1; SDH, subdural hematoma; TIE1, tyrosine kinase with immunoglobulin like and EGF like domains 1; TSP-5, thrombospondin-5.

possible relationship between statins and TGF-β was recently discovered In cells from several types of cancer. Statins block the Ras/MEK/ERK and Ras/PI3K/Akt pathways via mevalonate, which lowers TGF-β expression (an angiogenic factor).[45]

Antiangiogenic activity of statins is likely the result of elevated levels of cell cycle inhibitors and decreased expression of genes related to angiogenesis.[46] Cyclooxygenase (COX)-2 is a proinflammatory inducible enzyme with significant angiogenesis-promoting properties.[47] Although the molecular processes of COX-2 participation in angiogenesis are complicated and not fully understood, a meaningful link of COX-2 activity with release of metalloproteinases (MMPs) is established in numerous cell models, although, notably, not in ECs.[48,49]

The antiangiogenic statin properties explored in CVDs are summarized in Table 2.

Biphasic properties of statins

Statins have a dose-dependent, biphasic angiogenic effect.[24,27] They promote angiogenesis at low dosages while inhibiting it at higher ones. The features of the biosynthetic pathways starting from mevalonic acid serve to explain the biphasic actions of statins on EC biology. Mevalonic acid, in a similar manner to cholesterol, serves as a precursor for different biological components, such as prenylated proteins, isopentenylated transfer RNAs, and ubiquinone. Intermediates, particularly GGPP and FPP, exhibit a greater affinity for nonsterol product-producing enzymes than for enzymes producing cholesterol. Thus, low-dose statins will primarily inhibit cholesterol production while also promoting the synthesis of GGPP/RhoA and FPP/Ras, which are essential for both cellular proliferation/migration.[50] By contrast, high-dose statins significantly reduce FPP and GGPP synthesis, leading to a reduction in cell proliferation and migration, although eNOS activation is further increased by RhoA suppression and activation of PI3K/Akt signaling. The control of eNOS activation, and of the migration and proliferation of ECs has been further validated by FPP or GGPP replenishment reversal effects.[46]

According to Colakoglu *et al.*, low-dose (0.5 mg/kg/day) atorvastatin therapy boosted KDR/Flk-1-dependent angiogenesis, resulting in increased regeneration. By contrast, high-dose atorvastatin (2.5 mg/kg/day) treatment reduced angiogenesis while having no effect on long-term regeneration outcomes. Moreover, statin, regardless of dosage, can enhance liver regeneration through extended expression of IL-6.[51]

Penumathsa *et al.* used a combination therapy that demonstrated the efficacy of statin and resveratrol therapies, either individually or combined, to enhance both cardiac microvascular construction and performance.[52] Statin and resveratrol-induced neovascularization in the infarcted myocardium appears to offer persistent cardioprotection toward ventricular remodeling. Additionally, because both resveratrol and statins show biphasic effects on angiogenesis, their impact might be dose dependent. In the work by Penumathsa *et al.*, the 1 mg/kg statin dose was not angiogenic, whereas that of resveratrol was. Increased Akt and eNOS phosphorylation was detected in all treated groups, causing a reduction in the degree of cardiomyocyte apoptosis. In addition, VEGF mRNA expression and β-catenin translocation were shown to be increased. As a result, it can

TABLE 2

Studies exploring anti-angiogenic properties of statins in cardiovascular diseases.[a]

Statin	*In vivo*/ *in vitro* model	Dose	Effect on angiogenesis	Disease	Refs
Atorvastatin	*In vitro*	1, 5, 10 mM	Downregulates VEGF expression, CD31, and Bcl-2 induces expression of caspase-3; effects are dose dependent	Glioma spheroids and HUVECs	72
	In vivo	0.6, 3 mg/kg	Downregulates VEGF, TNF-α, and TGF-β1	Murine sponge model of inflammatory angiogenesis	76
Lovastatin	*In vitro*	0.1–10 mmol/l	Inhibits endothelial cell migration and tube formation via upregulation of actin-binding protein transgelin 2	Endothelial cells (HUVECs)	71
Simvastatin	*In vitro* and *in vivo*	0.1 mg/kg	Increases tube formation, angiographic score, and capillary density; activates Akt and eNOS	Ischemia	70
	In vitro	0.30–48.25 μg/ml	Inhibits capillary tube formation	Glioma cell line and HUVECs	73
		15 or 5 mg/kg	Downregulates HIF-1α by increasing phosphorylation level of AMP kinase; reduces VEGF and FGF-2 expression in dose-dependent manner	Breast cancer cell line, HUVECS and BALB/c mice	74
		2.5 μM	Inactivates Ras/Raf/ERK	HUVECS	75
Simvastatin and atorvastatin	*In vitro*	0.1–10 mmol/l	Reduces COX-2 and MMP-9 expression and activity	Endothelial cells	49

[a] Abbreviations, BiP, binding immunoglobulin protein; KLF2, Kruppel-like factor 2; MCP-1, monocyte chemoattractant protein-1; TGF-β1, transforming growth factor beta 1; TNF-α, tumor necrosis factor alpha.

be concluded that the acute and chronic protection provided by resveratrol and statin combination therapy might be attributable to proangiogenic, antiapoptotic, and antihyperlipidemic impacts, while long-term impacts might be the result of enhanced neovascularization resulting in less ventricular remodeling.[52]

Recently, Hu *et al.* discovered that the impact of pravastatin on the angiogenic activity of human cardiac microvascular ECs (HMVEC-Cs) under H_2O_2-induced oxidative stress conditions was biphasic, counteracting H_2O_2-induced angiogenic suppression at low (nanomolar) doses, with this effect diminishing at higher doses.[53] At low nanomolar doses, pravastatin had no effect on the angiogenic potential of HMVEC-Cs under normal physiological conditions and, therefore, was protective; however, at high (micromolar) levels, it dramatically inhibited HMVEC-C activity. Furthermore, pravastatin increased the angiogenic function of HMVEC-Cs at nanomolar concentrations by reducing oxidative stress and damage induced by H_2O_2, whereas, at micromolar doses, it suppressed the function of HMVEC-Cs by inhibiting prenylation. Furthermore, when pravastatin was at a high, yet therapeutically relevant, dose, its aforesaid protection of HMVEC-Cs disappeared. This reveals the biphasic impact of pravastatin on angiogenesis under pathological conditions.[53] The effects of statins on angiogenic-related molecular pathways are shown in Fig. 1.

Statins and angiogenesis in stroke and ischemic heart disease

Angiogenesis is a vital pathway in both physiology and pathology. This mechanism is required for wound healing, reproduction, fetal development, tumor progression, and metastasis.[54] The production and growth of new blood vasculature might also be a significant treatment for pathologically compromised or nonexistent blood flow to crucial tissues and organs. A healthy circulatory system is required for appropriate tissue and organ maintenance by supplying oxygen and nutrition, eliminating waste and facilitating immune cell movement. A disrupted blood flow can have serious repercussions, such as in ischemic heart disease (IHD), the result of inadequate blood flow to the myocardium because of myocardial ischemia or coronary insufficiency, which is a serious health issue and a major cause of mortality globally.[54] The primary underlying pathophysiological feature of IHD is atherosclerosis, which is characterized by a progressive restriction in blood flow that results in poor oxygen perfusion into the cardiac tissue, as well as dysfunction of ECs and reduction in angiogenic response with age.[55] Myocardial infarction (MI), myocardial ischemia, and ischemic cardiomyopathy are all caused by coronary atherosclerosis, which results in obstruction of the coronary arteries. Treatment for this condition includes statins, β-blockers, and antiplatelet drugs, therapies also used to promote disease stability and decrease acute events, such as MI. Surgery is, of course, another available treatment to enable prompt restoration of the blood supply.[56,57]

In addition, combining drugs used to treat CVD with other growth factors is a potential alternate strategy for improving cardiovascular health. In ischemic hindlimb animal models, stromal cell-derived factor 1 plus statins boosted angiogenesis by boosting progenitor cell mobility and integration into newly formed capillaries.[58]

The angiogenic pathway in ischemic tissue is a complicated process that involves a localized elevation in growth factors that stimulate angiogenesis formation, tissue regeneration immunomodulation, and cell activity restoration.[59] By stimulating the development of tubes by ECs, mesenchymal stem cells (MSCs) can assist vessels to proliferate and invade from nearby tissues. Smooth muscle cells and pericytes can be recruited by MSCs to aid the development of newly created blood arteries. MSCs can also develop into ECs, which help to improve vascularization.[59,60] The NO signaling pathway is a PI3K/Akt regulated pathway that is linked to angiogenesis. A set of angiogenic fac-

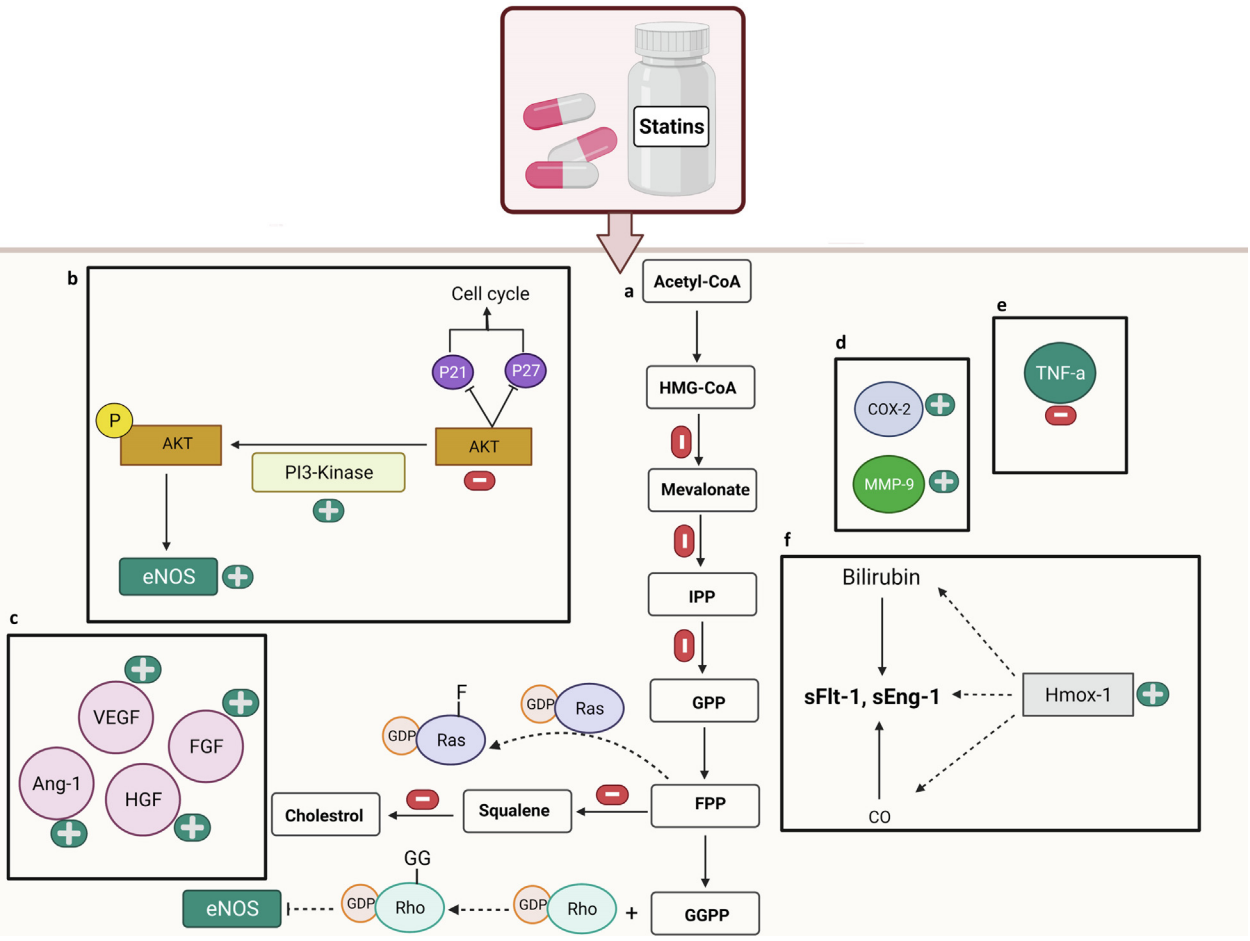

FIGURE 1

Effects of statins on angiogenic-related molecular pathways via **(a)** reducing the availability of farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), thus reducing Ras and Rho activation and increasing endothelial nitric oxide synthase (eNOS) production, maintaining the function of the endothelium, **(b)** stimulating angiogenesis by virtue of their ability to activate the Akt-nitric oxide pathway, **(c)** increasing proangiogenic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and angiopoietin (Ang)-1, **(d)** reducing cyclo-oxygenase (COX)-2 and matrix metalloproteinase (MMP)-9 levels, **(e)** reducing proinflammatory cytokine tumor necrosis factor (TNF)-α, and **(f)** increasing Hmox-1, thus increasing the proangiogenic factors sFlt-1 and sEng-1.

tors, including VEGF, are released through the PI3K pathway. The VEGF receptor 2 has a crucial role in angiogenesis induced by VEGF. VEGF promotes EC migration and the appearance of capillary-like structures in a PI3K/Akt-dependent way. The major mediator of smooth muscle tone, NO, is produced by the endothelium and promotes vasodilation through the action of eNOS. NO, along with VEGFs, FGFs, angiopoietin, and different integrins, can operate as a signal transduction molecule and an angiogenic factor in the activation, promotion, and stability of neovascularization. Lower NO bioavailability is associated with endothelial dysfunction, which can result from decreased production of NO synthase from ECs or oxidative stress-induced impaired activation/rapid inactivation of NO. Statins can boost eNOS expression by blocking Rho GTPase isoprenylation. Statins can also activate and phosphorylate eNOS rapidly via the PI3K/Akt pathway, thus stimulating angiogenesis and promoting ischemic tissue restoration.[61]

Statins have been demonstrated to improve microvascular function in *in vivo* stroke studies. Pretreatment with high-dose simvastatin and mevastatin increased cerebral blood flow and endothelial NOS, while decreasing infarct volume in mice.[62] Oral treatment with lower doses of atorvastatin (1 mg/kg and 3 mg/kg) within 24 h of the event improved VEGF expression, neovascularization, and proliferation of neural progenitor cells in rat embolic stroke models.[63] These data imply that early statin therapy, at dosages comparable to those used in clinical practice, could enhance tissue regeneration following ischemic damage by increasing angiogenesis.

Plaque progression and instability have been linked to intraplaque angiogenesis. In this context, Koutouzis *et al.* compared the density of capillaries in carotid plaques removed from statin-treated and untreated individuals.[64] The intensity of intraplaque angiogenesis was measured using the CD34 antibody in immunohistochemistry. Statin medication was found to be linked

to decreased intraplaque angiogenesis in the carotid arteries. This might explain why this method of treatment is beneficial to patients with atherosclerosis.[64]

Concluding remarks

Statins are known to have cardioprotective effects through mechanisms other than their serum lipid-lowering effects, which appear to be driven by enhanced endothelial function and NO production. Statins have been shown to have both proangiogenic and antiangiogenic properties. Low statin doses tend to exert proangiogenic effects, mainly via activation of Akt, which causes eNOS phosphorylation and NO generation. By contrast, high statin doses significantly impede the synthesis of nonsterol products of mevalonate, resulting in decreased protein prenylation, cellular growth suppression, and toxicity.

Some of the elements that might influence the angiogenic properties of statins include: (i) dosage: according to the statin biphasic action theory, low doses are proangiogenic while high doses are antiangiogenic; (ii) specific disease: the impact of a statin might vary simply based on underlying illness; for example, the same dose of simvastatin that enhanced angiogenesis in response to hypoxic circumstances was shown to prevent angiogenesis driven by inflammation; and (iii) type of statin: although statins are thought to have comparable effects, individual statins have varied pharmacological and PK features, which might change their angiogenic ability.[65] The angiogenic-regulating actions of statins, as well as their other pleiotropic properties, could offer novel therapeutic applications. However, clinical trials are required to validate these additional potential uses.

Declaration of interests

None declared by authors.

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