CHAPTER 8

Anti-Angiogenic Therapy and Cardiovascular Diseases: Current Strategies and Future Perspectives

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Abstract: The process involving new blood vessel sprouting from already existing ones is regulated by a physiological complex mechanism, known as angiogenesis. It plays a key role in wound healing but is also present in pathophysiological conditions such as cancer and cardiovascular disease, which have the highest rates of morbidity and mortality worldwide. It is stimulated mechanically or chemically, with the latter involving several signaling pathways and proteins widely known as growth factors. Anti-angiogenesis has always been an appealing target for cardiovascular related diseases, such as atherosclerosis, with its role still eluding our grasp. In this chapter we focus on the latest trends in anti-angiogenic therapy and drug discovery as well as highlight the distinct pathways underlying it. Therapies can range from use of peptides, proteins as well as well-defined chemically synthesized molecules. Latest trends involve gene therapy related approaches, with delivery of anti-angiogenic factors to target areas. Furthermore, toxicity issues arising from the use of anti-angiogenic drugs are discussed and highlighted as many of the drugs employed can cause serious side effects, while others may not achieve maximum therapeutic effect. Anti-angiogenic therapy is a very dynamic field and will continue to evolve and improve in the future. A very interesting addition to the anti-angiogenesis drug arsenal can be achieved with the aim of nanotechnology, a novel but promising scientific field. It is certain that in the future new, more potent drugs will be discovered, posing greater therapeutic potential and lower side effects, providing a much needed boost in this continuously evolving scientific field.

Keywords: Angiogenesis, anti-angiogenic drugs, atherosclerosis, cardiovascular disease, endothelial cells, fibroblast growth factor, gene therapy, hypoxia-inducible factor, interleukins, liposomes, macrophages, matrix metalloproteinases, microRNAs, nanomedicine, nicotinamide adenine dinucleotide phosphate-oxidase,

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polyphenols, reactive oxygen species, statins, transforming growth factor-beta, vascular endothelial growth factor, vascular smooth muscle cells.

INTRODUCTION

Cardiovascular disease (CVD) is the pandemic of the 21st century, affecting not only developed countries but developing ones as well. Although there are various causes underlying CVD progression, atherosclerosis is the prevalent one. It affects all vascular beds and can manifest in the form of cardiac, cerebral, visceral, or peripheral vascular diseases. Atherosclerosis can be defined as a chronic low grade inflammation affecting the vascular wall, which over time and through multiple processes results to the development of atherosclerotic plaques (Fig. 1) [1].

While atherosclerosis was initially considered a lipid storage disease, later insights revealed that development and progression of atherosclerosis is a multi-variable process with many factors involved, namely hormones, cytokines, adhesion molecules, bacterial products and inflammatory cells [2]. In response to the endothelial injury triggered from the above inflammatory cell infiltration occurs. In addition, two major events of the atherogenic process are the deposition of low density lipoprotein (LDL) and sub-endothelial matrix remodeling. Reactive oxygen species (ROS) as well as several other biochemical mediators and enzymes participate in the oxidation of LDL and the formation of ox-LDL, with the latter being recognized by multiple receptors such as lectin-like oxLDL receptor-1 (LOX-1), scavenger receptor SR-B1, Toll-like receptors (TLRs) and CD205 [3]. Ox-LDL induced the over-expression of several inflammatory mediators, such as monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) as well as adhesion molecules like vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), which in turn favour macrophage and other inflammatory cell recruitment into the sub-endothelial area [4]. Subsequently ox-LDL is accumulated by macrophages, resulting in the formation of foam cells [5]. However, there are reports that ox-LDL may exert anti-inflammatory effects in
macrophages through, activation of the peroxisome proliferator-activated receptor gamma (PPARγ) pathway [6-8]. All the above conclude to the fact that ox-LDL is a pluripotent mediator, affecting several pathways [9]. Furthermore, apart from the classic factors, there has been a documented link between the immune system and atherosclerosis, with both innate and adaptive immunity playing key roles in atherosclerosis progression [10]. T helper 1 (Th1) cells have been implicated in the pathophysiology of atherosclerosis as they produce high levels of interferon-γ (IFN-γ) encountered in atherosclerotic lesions [11]. Studies with mice lacking the Th1 cell transcription factor T-bet, IFN-γ, or the IFN-γ receptor have reported resistance to high fat diet-induced atherosclerosis [12, 13]. Interlukin-12 (IL-12) is produced mainly by macrophages, dendritic cells (DCs) and B cells [14]. It can stimulate INF-γ production from T and natural killer (NK) cells in Th1 responses [14]. In mice treated with IL-12 stimulated INF-γ production and increased lesion size, mice lacking ApoE and p40, a subunit of IL-12 demonstrated smaller atherosclerotic lesions [15]. However, given the fact that p40 is also a subunit of another interleukin, IL-23, reduction of atherosclerotic size might occur due to combined deficiency of both IL-12 and IL-23 [15]. There are also recent studies in mice and humans claiming that IL-17-producing CD4+ T (Th17) cells are encountered in atherosclerotic lesions but their role is still debatable and needs to be clarified [16-18]. All these findings pinpoint the critical role of innate and adaptive immunity in atherosclerosis. On the other hand interleukin-10 (IL-10), protects early stage atherosclerosis development possibly by inhibiting INF-γ production, thus exhibiting strong anti-inflammatory action [19].

In addition, in early stage atherosclerosis smooth muscle cell migration from the media to the tunica is observed. Over the course of time, plaques are formed from mature lesions, which in turn can undergo calcification further reducing vessel elasticity [20]. Pro-inflammatory cytokines and proteinases eventually render the plaque unstable, making it prone to rupture. In the event of a plaque rupture, the pro-coagulant exposed lipid core results in the occlusion of the vessel lumen [20]. Depending on the area occluded, these events clinically manifest in the form of acute vascular syndromes, like acute myocardial infarction (AMI) or stroke [21]. The key mechanisms involved in atherosclerosis are displayed in Fig. 2.
Figure 1: A diagrammatic overview of the development and progression of atherosclerosis. Classic risk factors for the development of atherosclerosis, such as obesity, smoking, hypertension etc. can cause oxidative stress, oxidation of LDL to ox-LDL and subsequently promote inflammation. Ox-LDL accumulation in the sub-endothelial space along with secretion of cytokines and adhesion molecules, activate the endothelium, promoting macrophage infiltration. Macrophages accumulate ox-LDL and transform into foam cells further increasing the inflammatory burden. It later steps cytokines release causes VSMC migration and proliferation into the arterial wall, leading to neointimal formation. As a result vessels narrow, reducing blood flow and the atherosclerotic plaques formed may rupture, resulting to acute coronary syndromes.
Figure 2: Overview of the key mechanisms involved in atherosclerosis. Low density lipoprotein enters the sub-endothelial space where it is oxidized to ox-LDL in the presence of ROS and other biochemical stimulants. This event leads to the release of adhesion molecules and a subsequent increase in endothelial cell permeability. This allows for leukocyte and T-cell transmigration into the sub-endothelial space where they differentiate to macrophages, uptake ox-LDL and transform to foam cells. Furthermore, ox-LDL antigens are recognized by CD4+ T cells which differentiate to Th1 cells, favoring pro-inflammatory cytokine production. Finally, under the effect of cytokines and growth factors SMC proliferation and migration to the sub-endothelial space occurs, in response to vascular injury, thus contributing to fibrous cap formation.

ANGIOGENESIS

Vascular endothelial cells which along with pericytes and the basal membrane have a pivotal role in orchestrating the angiogenic process. Under normal circumstances endothelial cells (ECs) maintain their function by autocrine signaling of VEGF, NOTCH, angiopoietin-1 and fibroblast growth factor (FGF) [22]. When the physiological conditions are disrupted by low oxygen, inflammation, tumor development or wound healing, endothelial cells starter.
expressing hypoxia-inducible factors such as HIF-2α and prolyl hydroxylase domain 2 (PHD2) [22] Angiogenesis occurs in several subsequent steps starting with the degradation of the basal membrane followed by reduced adhesion of endothelial cells. Afterwards, vascular sprouting occurs via the migration of endothelial leading (tip) and trailing (stalking) cells. Subsequently new lumen is formed by endothelial cells while pericytes are attracted to the newly formed area. Finally, vascular stabilization occurs by tightening of the basal membrane and of cell junctions [22]. On a protein level there are several molecules that regulate angiogenic responses by acting on several distinct signaling cascades. The most important ones are vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF2), transforming growth factor-beta (TGF-β), Notch ligands Jagged 1 (JAG1) and Delta like ligand 4 (DLL4) and angiopoietins (Ang-1 and Ang-2). VEGF consists of a family of seven members all sharing a common core region of cysteine knot motif. VEGF is a very specific mitogen of endothelial cells. Following binding to tyrosine receptors, a variety of responses can occur, leading to “endothelial cell proliferation, migration and formation” of new vessels [23]. Furthermore, VEGF interacts with vascular endothelial growth factor receptor-2 (VEGFR2) activating endothelial nitric oxide synthase (eNOS), SRC, RAS-ERK and PI3K-AKT pathways inducing vascular permeability in addition to endothelial proliferation migration and survival [24, 25]. Ang-1 and Ang-2 are the best characterized angiopoietins. They bind to Tie-2 tyrosine kinase receptor, expressed in vascular endothelial cells and in some macrophage subtypes involved in angiogenesis [26]. Angiopoietins are key molecules in the angiogenic process as they control angiogenic switch. Ang-1 is pivotal for endothelial cell proliferation, migration and survival, while Ang-2 disrupts endothelial and perivascular cell connections, thus leading to cell death and vascular regression [26]. Interestingly under the presence of VEGF, Ang-2 exerts pro-angiogenic effects [26]. As with VEGF, FGFs are a family of structurally similar polypeptides, with nine distinct members. FGF-1 and FGF-2, also termed as acidic and basic FGFs respectively have been well-characterized as key modulators of angiogenesis [27].

Delta-Notch signaling is also a critical part of angiogenesis as it regulates sprout formation. In this cell-cell signaling system, VEGF-A induced DLL4 production
in tip cells induces Notch receptor activation in stalk cells [28]. Apart from direct involving in angiogenesis VEGF, FGF, Notch and TGF-β signaling cascades crosstalk with other pathways such as the canonical WNT and Hedgehog pathways that regulate embryonic and stem cell responses [29, 30]. TGF- exerts diverse cell actions by binding and activating type I and II serine and threonine kinase receptors. TGF-β signaling is without dispute a critical element of angiogenesis and vascular remodeling [31]. TGF-β1 is the key molecule in the formation of the primary vascular structure and the subsequent creation of a more complex network [31].

ASSOCIATION OF ANGIOGENESIS WITH ATHEROSCLEROSIS

Since delivery of nutrients from the lumen to nearby cells is limited, many larger human arteries form a microvasculature at their outmost (adventitial) layers termed as vasa vasorum. Artery branching at common intervals that run longitudinally parallel to the vessel consists of first-order vasa vasorum, while artery arching from the first-order vasa vasorum around the perimeters coronary lumen is termed as second order vasa vasorum [32]. Association of intimal neovascularization and atherosclerosis was first mentioned at the end of the 19th century by Koester, while in the following years several similar observations were made. Almost a century later, it was hypothesized that atherosclerotic plaque progression beyond a critical point, was due to the proliferation of coronary vasculature, by supplementation of oxygen and nutrients [33]. Later on, they proposed that the neovascular network of coronary atherosclerotic plaques can be more rupture-prone, causing plaque destabilization thus promoting acute myocardial infarction development [34]. Since then, there has been ample evidence linking plaque neovascularization with the progression atherosclerosis both in vivo and in vitro [35]. Furthermore, in human atherosclerotic lesions where angiogenesis occurs, several proinflammatory cytokines are expresses further establishing the link between these two processes [35]. However, in order to fully elucidate the role of angiogenesis in atherosclerosis, further elucidation of the mechanisms and pathways is required. In the field of angiogenesis there is still much progress to be made.
ANGIOGENESIS AND NEOINTIMAL GROWTH

Following arterial stenting, angioplasty and venous bypass graft, aberrant neovascularization has been observed [36-39]. This can be justified by the hypoxic environment at the sites of intimal hyperplasia, which not only as aforementioned switches to an angiogenic profile but also leads to the up-regulation of several growth factors (mainly VEGF, FGF) and cytokines which in turn regulate vascular smooth muscle cell (VSMC) migration and proliferation [40]. The role of VEGF in several models of intima formation has yielded contradictory results [35]. This can be explained by the use of different animal models and the fact that VEGF effects are concentration dependent [41]. Lower concentrations of VEGF are atheroprotective accompanied with very low angiogenic response. On the other hand, at higher concentrations, the protective effect is nullified, along with an increase of angiogenesis [35, 41]. In even greater concentrations VEGF can exhibit proatherogenic action as demonstrated by intimal thickening, having a detrimental effect in the atherosclerotic progress [35]. There is also evidence that endothelial progenitor cells are a source of VSMCs encountered in atherosclerotic plaques [42, 43]. However, their role in angiogenesis and tissue revascularization still remains to be clarified.

HYPOXIA AND INTRAPLAQUE NEOVASCULARIZATION

Recent evidence emerging from cancer models suggesting that hypoxia is a critical factor for tumor growth, has provided insight on whether it can affect atherosclerotic plaque neovascularization [44]. Theoretically, when vessel thickness exceeds a threshold, as a result of injury or lipid accumulation, oxygen and nutrient supply to the tissues will decrease as the distance between the lumen or the vasa vasorum grows. When this distance surpasses 100\(\mu\)m [45], it forms a hypoxic environment, providing the stimulus for hypoxia-inducible transcription factors (HIF). More specifically HIF-1\(\alpha\) induces the expression of VEGF and other pro-angiogenic factor expression, commencing the angiogenic process [46]. Supporting this hypothesis, in atherosclerotic lesions, HIF-1\(\alpha\), FGF and VEGF levels were found elevated [47]. Given the fact that microvessels deliver oxygen and nutrients to both plaques and inflammatory cells, atherosclerosis continues perpetually [48]. However, it should be noted that angiogenesis only promotes
plaque formation but does not initiate it. Interestingly, angiogenic responses can be induced by a hypoxia-independent mechanism that of oxidative stress [48]. Overexpression of p22phox, a key subunit of β-nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in a transgenic mouse model demonstrated increased arterial lesion formation [49], suggesting that ROS might have a significant effect in triggering angiogenic responses.

INFLAMMATORY CELLS AND NEOVASCULARIZATION

In atherosclerotic plaques and especially in vulnerable ones, there is a higher concentration of macrophages [50]. These along with VSMCs secrete several cytokines and proinflammatory molecules, greatly contributing to the progression and development of the disease. Furthermore, they secrete a plethora of angiogenic factors [51]. Rupture prone areas consisting of higher numbers of inflammatory cells capable of secreting matrix metalloproteinases (MMPs) can contribute to plaque instability [52]. Comparison of normal coronary arteries with atheromatous ones in humans, revealed that interleukin-18 (IL-18), produced by macrophages is almost exclusively produced in the atheromatous human coronary artery [53]. IL-18 has been described to have similar actions to that of VEGF and FGF-2 [54]. In plaques, expression ICAM-1, E-selectin and VCAM-1 promotes even more inflammatory cell accumulation, thereby accelerating atherosclerosis [55]. A summary of the association between angiogenesis and cardiovascular disease is displayed in Fig. 3.

ANTIOXIDANTS IN ANGIOGENESIS AND ATHEROSCLEROSIS

All organisms are constantly exposed to free radicals and oxidants, which are either produced as a result of physiological processes or derived from exogenous sources [56]. Free radicals exert both beneficial and hazardous effects, creating a delicate oxidative balance [57]. Once this balance is disrupted, oxidative stress occurs, which has been associated with a multitude of disorders including atherosclerosis and cardiovascular disease [58]. In order to maintain this balance, organisms employ the use of antioxidants [57]. Antioxidants are created in situ (endogenous) and can be further classified as enzymatic or non-enzymatic or can
be obtained through diet (exogenous) [59]. Enzymatic antioxidants include: catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRx). Examples of endogenous non-enzymatic antioxidants are glutathione, coenzyme Q10, melatonin, transferrin and bilirubin, while exogenous antioxidants are vitamin C and E, flavonoids, polyphenols and carotenoids [59, 60]. Since atherosclerosis progression is mediated through ROS-induced oxidation of LDL, studies have demonstrated that antioxidants can inhibit atherosclerosis by preventing LDL oxidation and subsequent formation of ox-LDL [61, 62]. However, there are some studies claiming that prevention of atherosclerosis by dietary antioxidant vitamin supplementation still needs further
proving by conduction of more clinical studies [63]. Concerning angiogenesis, there are studies claiming that antioxidants have a favourable effect in angiogenesis prevention, by down-regulating inducible nitric oxide synthase (iNOS) [64] or by altering cell proliferation and migration profile [65]. However, just as in the case with atherosclerosis, there is still a debate on the favorable action of antioxidants as there are controversial reports and the exact mechanism of action has not yet been elucidated [66].

THERAPEUTIC APPROACHES FOR TREATING ANGIOGENESIS

Although the link between rupture prone atherosclerotic plaques and angiogenesis has been established, there is still more need to understand the mechanisms underlying these processes. Such effort will surely lead to the creation of new anti-angiogenic drugs designed to inhibit angiogenesis thus lowering the progression of atherosclerotic plaque destabilization (Table 1). As aforementioned, in atherosclerotic plaques, several growth factors have been found to be expressed like VEGF, acidic fibroblast growth factor (aFGF) and basic FGF [67, 68]. The major source of nutrients to the vessel wall is delivered via the vasa vasorum. It has been observed that in atherosclerotic plaques a much denser network of vasa vasorum is present, possibly promoting the destabilization of atherosclerotic plaques [69]. Stopping the expansion of vasa vasorum is critical to reducing atheroma progression [70]. There is also evidence of microvessels formation in restenotic lesion as well as in the neointimal [71, 72].

Table 1: Overview of anti-angiogenic therapeutic tools employed for treating cardiovascular diseases

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Target/Therapeutic Factor</th>
<th>Mode of Action</th>
<th>Refs.</th>
</tr>
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<tbody>
<tr>
<td>Antibody treatment</td>
<td>VEGFR-1 (Flt-1)</td>
<td>Reduction of early and intermediate lesion size at the aortic root Suppression of macrophage infiltration in the adventitia</td>
<td>[39]</td>
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<td></td>
<td>VEGF</td>
<td>Inhibition of neovascularisation without prevention of endothelization</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>FGF2</td>
<td>Inhibition of SMC proliferation</td>
<td>[41]</td>
</tr>
<tr>
<td>Synthetic drugs</td>
<td>Paclitaxel</td>
<td>Inhibition of neointimal formation Inhibition of in stent restenosis</td>
<td>[36, 37]</td>
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<tr>
<td></td>
<td>SU5402</td>
<td>Reduction of atherosclerosis by FGFR inhibition</td>
<td>[45]</td>
</tr>
<tr>
<td>Anti-angiogenic factors</td>
<td>Compound</td>
<td>Activity</td>
<td>References</td>
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<tr>
<td>SSR128129E</td>
<td>Novel multi-FGFR inhibitor, demonstrated significant Reduction of neointimal formation</td>
<td>[46, 51]</td>
<td></td>
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<tr>
<td>TNP-470</td>
<td>Inhibition of intimal hyperplasia Reduction in plaque growth</td>
<td>[52]</td>
<td></td>
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<tr>
<td>PI-88</td>
<td>Reduction of intimal thickening and VSMC proliferation</td>
<td>[39]</td>
<td></td>
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<tr>
<td>Angiostatin</td>
<td>Inhibition of neointimal formation</td>
<td>[36]</td>
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<tr>
<td>sFlt-1</td>
<td>Reduction of adventitial thickening</td>
<td>[47]</td>
<td></td>
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<tr>
<td>Endostatin</td>
<td>Reduction in plaque growth</td>
<td>[52]</td>
<td></td>
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<tr>
<td>Interleukin-10</td>
<td>Negative regulation of VEGF expression</td>
<td>[54]</td>
<td></td>
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<tr>
<td>PEDF</td>
<td>Blocking of NADPH oxidase mediated ROS generation in SMCs</td>
<td>[80]</td>
<td></td>
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<tr>
<td>sFGFR1</td>
<td>Inhibition of SMC proliferation</td>
<td>[42]</td>
<td></td>
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<tr>
<td>Fluvastatin</td>
<td>Down-regulation of angiogenic molecules (VEGF, HIF-1α, phospho-STAT3) Suppression of ICAM-1 expression Prevention of superoxide-induced lipid peroxidation</td>
<td>[60]</td>
<td></td>
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<tr>
<td>Cerivastatin</td>
<td>Inhibition of MMP-1,-3,-9 expression in SMCs and macrophage foam cells</td>
<td>[62]</td>
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<tr>
<td>Simvastatin</td>
<td>Down-regulation of PDGF and VEGF expression</td>
<td>[65]</td>
<td></td>
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<tr>
<td>miR-17-92 cluster</td>
<td>Inhibition of angiogenic activity in ECs Prevention of neovascularization</td>
<td>[105,109-111]</td>
<td></td>
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<tr>
<td>miR-23-27-24 cluster</td>
<td>Regulation of angiogenesis and postnatal retinal vascular development Repression of angiogenesis sprouting</td>
<td>[95, 113,114]</td>
<td></td>
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<tr>
<td>miR-208</td>
<td>Regulation of cardiac stress response</td>
<td>[103, 118]</td>
<td></td>
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<td>a,β3-targeted paramagnetic nanoparticles loaded with fumagillin</td>
<td>Inhibition of aortic atherosclerotic progression Possibility of MRI imaging for therapy assessment</td>
<td>[125,126]</td>
<td></td>
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<tr>
<td>Liposomes loaded with PLP</td>
<td>Anti-inflammatory action, imaging with 18F-FDG-PET/CT</td>
<td>[128]</td>
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Stent implantation represents a major breakthrough in the treatment of cardiovascular disease. Despite that there are still major issues that need to be addressed such as in-stent restenosis (ISR) and stent thrombosis (IST), with the former accounting for 15-30% of bare metal stent usage in percutaneous coronary interventions [73, 74]. The development of IST is accredited to the fact that during the balloon deployment of the stent, vessel injury occurs, resulting in neointimal hyperplasia and tissue proliferation [75]. Other factors influencing ISR formation are patient-related (diabetic or patients with small vessel diameter) while the stent coating physicochemical characteristics (elastic recoil, shape and surface properties) are also of great importance [76, 77]. Following stent implantation, endothelialization occurs [78]. This is mostly driven through the deposition of platelets and fibrin followed by migration and penetration of leukocytes into the tissue, with several cytokines regulating the process [78]. If the re-endothelization process is rapidly achieved rapidly and completely after intervention the formation of the neointimal can be significantly reduced [75]. One strategy is to inhibit key components of the most important signaling pathways such as VEGF/Ang-1 with the use of antibodies.

In a study using New Zealand rabbits under a three week atherogenic diet, use of phosphorycholine coated stents with an anti-VEGF antibody inhibited neovascularisation without preventing endothelization [79]. Similarly, another group reported the use of a biodegradable polymeric stent coating releasing hirudin and iloprost, successfully inhibiting neointimal formation after coronary stenting in both sheep and pig models [80]. Another group reported that paclitaxel and angiostatin both offered protection against neointimal formation after administration of recombinant human VEGF in rabbits [81]. Furthermore, use of paclitaxel-coated balloon catheters proved beneficial in patients with in-stent restenosis [82].

**PLACENTAL GROWTH FACTOR**

Placental growth factor (PIGF) is a homolog of VEGF and has been implemented in pathological angiogenesis by acting through its receptor Flt-1 as well as in the development of atherosclerotic plaques and macrophage accumulation in mice [83]. Treatment with flt-1 antibodies in Apo E−/− and PIGF−/− deficient mice not
only reduced atherosclerotic lesion size and number but macrophage accumulation as well, compared to their Apo E<sup>-/-</sup> counterparts [83]. Interestingly, the number of plaque microvessels and the growth of advanced atherosclerotic lesion remained unaffected [84].

**FIBROBLAST GROWTH FACTOR INHIBITION**

Fibroblast growth factors are involved in several biological processes, by participating in a plethora of endocrine signaling pathways [85]. There are so far 22 FGFs identified in vertebrates, having a highly conserved gene structure as well as amino acid sequence. FGFs play key roles in both embryonic development and in adult organism functions. Regarding the former, FGFs play “diverse roles in cell proliferation, migration and differentiation”, while concerning the latter they regulate functions associated with tissue repair in response to injury [85]. Aberrant FGF expression has also been associated with cancer development and progression [86]. Concerning atherosclerosis, the role of FGFs still remains unclear. Given the fact that FGF is a potent stimulant for smooth muscle cell (SMC) and endothelial cells which both contribute to the stability of atherosclerotic plaques, there were many doubts as to whether inhibition of FGFs or its receptor could be beneficial for treating atherosclerosis. Two older studies demonstrated that SMC proliferation was inhibited after treatment with anti-FGF2 or administration of soluble FGFR1 after balloon injury or aortic transplants, pinpointing the role of FGFs in restenosis in different models of disease [87, 88]. However, despite the fact that FGF1 and 2 as well as their receptors have been identified as components of atherosclerotic plaques [89], the role of FGFRs in early atherosclerotic lesion formation still remains largely unknown. Recently, a study highlighted the effects of FGFR2 in acceleration of atherosclerosis in ApoE<sup>-/-</sup> mice over-expressing FGF-R2, through promotion of p21<sup>Cip1</sup>-mediated endothelial cell dysfunction as well as platelet-derived growth factor (PDGF) induced VSMC proliferation [90], further complicating the role of FGFRs in atherosclerosis progression. An older study using the compound SU5402, an FGFR inhibitor, demonstrated successful attenuation of atherosclerotic progression in ApoE<sup>-/-</sup> mice. It should be mentioned that this inhibition was not FGF specific and could be mediated through VEGFR signaling as well [91]. In a recent study, treatment with SSR128129E, a novel multi-FGF inhibitor was beneficial not only in mice
undergone vein graft but in ApoE\(^{-/-}\) deficient mice as well. The former showed significant reduction of neointimal formation, while the latter reduced lesion size in the aortic sinus [92].

**VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR**

As aforementioned, VEGF is a key molecule of the angiogenic process. It interacts via several receptors but mainly through VEGFR-1 also known as Flt-1. It has been proposed that Flt-1 is involved in pathological angiogenesis so it may be a lucrative target for treating atherosclerotic plaque formation as well as other angiogenesis related diseases [93]. Admission of a monoclonal anti-Flt-1 antibody in a model of ApoE\(^{-/-}\) mice for a period of five weeks demonstrated significant reduction of early and intermediate lesion size at the aortic root. Furthermore the Flt-1 antibody suppressed macrophage infiltration in the adventitia, reducing inflammation [83]. An interesting study highlighted the use of sFlt-1 to block VEGF and FGF with a (with a dominant negative form of FGF receptor 1 [FGF-R1DN]) attenuated adventitial thickening. Interestingly, the study concluded that adventitial thickening was not initiated by angiogenesis but only stimulated [94]. In a very interesting study, adenosine (Ado) was found to modulate the balance between soluble and membrane and Flt-1 receptor, switching from an anti-angiogenic to a pro-angiogenic profile respectively in human primary macrophages [95].

**VASCULAR ENDOTHELIAL GROWTH FACTOR -C,-D**

Although there is ample scientific information about VEGF and PLGF and their interactions with their inhibitors, little is known about other the other isoforms VEGF-C,-D and their receptor Flt-4 and their relation with angiogenesis. A recent study demonstrated that macrophages and monocytes express both these growth factors as well as their receptor in advanced atherosclerotic plaques both *in vivo* and *in vitro* [96]. This new link between angiogenesis and atherosclerosis can be exploited for developing new anti-angiogenic therapies.

Potent inhibitors of angiogenesis include the fumagillin family of natural products. These are employed in combating tumor angiogenesis and metastasis. A
synthetic analogue of fumagillin is TNP-470 which is employed in clinical trials as an anticancer drug. In an older study, SMCs that underwent treatment with TNP-470 demonstrated inhibition of DNA synthesis [97]. More recently, in a study examining the effects of TNP-470 in SMCs and found that intimal hyperplasia was inhibited in a dose dependant manner highlighting its application for preventing vascular intimal hyperplasia [98]. As well as this, in ApoE−/− mice, treatment with TNP-470 or endostatin, a natural occurring fragment from type XVIII collagen with anti-angiogenic properties, resulted in significant reduction in plaque growth even when the treatment started after 32 weeks. However, there was no significant effect at the very early stages of atherosclerotic plaque formation [99].

Use of a synthetic polysulfated oligosaccharide, Phosphomannopentaose sulfate (PI-88) attenuated intimal thickening and reduced VSMC proliferation after balloon injury in rats [84]. It has been proposed that PI-88 has a dual mechanism of action: Firstly, by binding to FGF-2 and thus blocking FGF-2 receptor dependant ERK activation and secondarily by inhibiting heparinise activity, an enzyme that degrades heparin sulphate in both the extracellular matrix (ECM) and cell surface [84].

INTERLEUKIN THERAPY

Following the inflammatory reaction, several cytokines are being secreted in an effort to regulate the inflammation. A broad range of cytokines and especially interleukins are critical mediators of the atherogenic process, greatly contributing to plaque build-up [100]. There are some interleukins, such as IL-10, a potent deactivator of macrophages, thus exhibiting a significant anti-inflammatory action [101]. In addition, IL-10 down-regulates the expression of VEGF, TNF-α and MMP-9, preventing tumor growth-related angiogenesis [102]. In a mouse model of ischemia-induced angiogenesis IL-10 proved to have anti-angiogenic effects by negatively regulating VEGF expression [103].

STATINS FOR TREATING PATHOLOGICAL ANGIGENESIS

3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors better known as statins are a class of drugs are well known due to their pleiotropic lipid
lowering effects. However, it was later proved that the benefit of statins in vascular disease was “independent of their lipid lowering effects” [104]. Statins act by inhibiting the enzyme HMG-CoA reductase which is present at the early stages of the mevalonate pathway which yields several products such as cholesterol, coenzyme Q10, heme-A, and several isoprenylated proteins [105]. There have been several studies which highlight the beneficial use of statins. Statins are very intriguing as they have multiple effects by regulating cytokines, adhesion molecules redox sensitive transcriptional pathways thus expanding their beneficial effects as described by meta-analysis studies [104, 106].

Interestingly there are studies proving that statin treatment can reduce VEGF levels in serum as well as atherosclerotic plaque volume [107, 108]. In a study using statins and more specifically fluvastatin, in a mouse model demonstrated that statin treatment can have beneficial effects by inhibiting the up-regulation of angiogenic molecules such as VEF, HIF-1α and phospho-STAT3. In addition it suppressed expression of adhesion molecule ICAM-1 and prevented superoxide production and lipid peroxidation, suggesting that the anti-angiogenic effects of statins are because of their anti-oxidant and anti-inflammatory properties [109]. Furthermore, the anti-angiogenic effect of statins has been demonstrated due to their inhibitory effect in cyclooxygenase-2 (COX-2) and MMP-9 “expression and activity in endothelial cells”, thereby contributing to plaque stabilization [110]. In a study with human saphenous veins and rabbit aortic SMCs and macrophages 50nM cerivastatin dosage inhibited MMP-1,-3,-9 expression in both SMCs and macrophage foam cells [111]. Statin therapy has also been associated with reduced plaque angiogenesis in carotid therapy [112]. Another group reported that daily administration of simvastatin (40mg) can attenuate angiogenesis by down-regulation of PDGF and VEGF levels [113]. There are reports however, of biphasic effects of statins and more specifically of cerivastatin and atorvastatin. For example, low doses of statins can stimulate angiogenesis but higher statin doses possessed anti-angiogenic effects associated with increased endothelial apoptosis and decrease in VEGF expression [114]. Of great interest is the fact that statins are also utilized in other fields (i.e. oncology, hematology) due to their “anti-angiogenic and anti-tumor properties” [115]. However, long-term effects of statin treatment still remain under investigation. An interesting study highlighted
that chronic statin treatment in patients with AMI was associated with reduced positive remodeling in the culprit lesions [116].

**REDOX CONTROL OF ANGIOGENESIS**

Reactive oxygen species include a great variety of oxygen containing chemically reactive molecules that upon reaction with key biological molecules (i.e. lipids and proteins) can drastically lead to an increase of oxidant production leading to oxidative stress [117]. Oxidative stress is termed as the overproduction of ROS and reactive nitrogen species (RNS) and has been identified as a fundamental mechanism for cell damage [118]. Most notable examples of ROS are superoxide anion, hydroxyl radical and hydrogen peroxide [119]. It is worthwhile mentioning that low concentrations of radicals regulate several cellular functions and most notably cell communication and angiogenesis [120, 121]. From the vast majority of ROS, superoxide radicals have been implicated in several pathological conditions including atherosclerosis, vascular remodeling, myocardial infarction and ischemic stroke [122]. There are several oxidant enzyme systems that have been identified as key players for the production of superoxide. These include xanthine oxidase (XO), uncoupled NOS and cytochrome P450 [123]. These enzymatic sources, cannot account for the bulk amount of superoxide production under physiological and pathophysiological conditions [124]. NADPH oxidase is an enzyme with similar structure of the enzymatic complex found in phagocytic leucocytes. NADPH consists of at least 5 subunits: p47phox and p67phox, found at the cytosol and the membrane bound p22phox gp91phox (better known as Nox2) and Rac as small g protein [101]. Translocation of the cytosolic components to the membrane-bound complex triggers a superoxide production burst from the extracellular part of the membrane [101]. This is achieved via the reduction of oxygen and the use of NADPH as the electron donor [125].

In endothelial cells, several pro-angiogenic stimuli such as cytokines and growth factors can activate NADPH-dependent ROS production [126]. This effect can also work the opposite way, as increased ROS concentration may subsequently affect redox sensitive molecules, pathways or transcriptional factors [121]. More specifically, Nox2 and Nox4 isoforms in endothelial cells are responsible for ROS production and knockout of these two isoforms greatly reduced endothelial cell
proliferation and survival [127]. A group reported that in human microvascular and lung microvascular endothelial cells, Nox4 is a key mediator of angiogenesis through activation of the ERK signaling pathway [128, 129]. VSMCs treated with thrombin and PDGFAB, increased ROS production, following “activation of a p22-containing NADPH oxidase” [130, 131]. Furthermore, there is growing evidence linking oxidative stress and neointimal formation after angioplasty [132, 133], further associating NADPH oxidase with pathological angiogenesis and CVD. Given the role of NADPH-oxidase in cardiovascular diseases and inflammation, its inhibition may prove a new therapeutic solution for several diseases, by affecting the distinct pathways underlying these disorders.

In a rat carotid artery balloon injury model, treatment with Pigment epithelium-derived factor (PEDF) drastically inhibited neointimal hyperplasia after vascular injury [134]. PEDF is a glycoprotein and belongs to the family of serine protease inhibitors [135]. It is a potent inhibitor of angiogenesis and blocks TNF-α and angiotensin II (Ang II) induced EC activation, due to its anti-oxidant properties [135]. The inhibition observed from PEGF treatment was derived by blocking of NADPH oxidase mediated ROS generation in SMCs [134]. Pharmacological inhibition of NADPH oxidase is also been applied for the treatment of retinopathy, which involves aberrant neovascularization of the retina, and suppression of tumor growth [136].

NATURAL POLYPHENOLS AS ANTI-ANGIOGENIC DRUGS

Polyphenols are a class of natural occurring chemicals found in plants, although some can be synthetic or semisynthetic [137]. Their main characteristic is the presence of multiple phenol rings in their structure. The most important characteristic of polyphenols is their strong anti-oxidant properties [137]. Depending on their structure they can act as chain breakers or free radical scavengers [138]. Polyphenols are believed to inhibit atherosclerotic plaque progression not only to their anti-oxidant abilities but also through inhibition of new blood vessel formation [139]. Apart from these effects, the beneficial actions of polyphenols may expand to prevention of LDL oxidation, inhibition of MCP-1 and tissue factor as well as activation of platelets [139]. Recently there is growing evidence of the anti-angiogenic effect of polyphenols in vitro and in vivo models.
In smooth muscle cells, red wine polyphenolic compounds (RWPCs) attenuated VEGF expression. It was also highlighted that the RWPCs reduced PDGF<sub>AB</sub> via the redox sensitive p38 MAPK pathway [140]. Polyphenols also inhibit “enzymatic sources of ROS production such as NADPH oxidase and xanthine oxidase in cells”, [141, 142] while in the meantime “enhance the activity of antioxidant enzymes such as catalase and glutathione peroxidase” [143]. These last two enzymes play a vital role in maintaining oxidative homeostasis by decomposing hydrogen peroxide to oxygen and water. In addition RWPCs offered sustained inhibition of PDGF<sub>AB</sub>, attenuating VEGF expression. Interestingly other antioxidant compounds like vitamin C, N-acetylcysteine and diphenylene iodonium demonstrated only partial reduction in PDGF<sub>AB</sub>-induced VEGF expression [140].

A major feature during the progression of the atherosclerotic process is the extensive remodeling of the arterial wall [144]. This procedure is tightly regulated by the MMPs, which can degrade components of the extracellular matrix. In vascular tissues MMP-1, -2 and MMP-9 have key roles “in the turnover of type IV collagen”, promoting angiogenesis [145] and atherosclerosis [146]. Since the role of MMPs in angiogenesis and atherosclerosis is well established [147], inhibition of MMPs can provide a potent therapeutic target for treating pathological angiogenesis. Polyphenols can inhibit thrombin and membrane bound MT1-MMP activity in VSMCs, resulting in significant reduction in MMP-2 levels [148]. Another hallmark event in angiogenesis, atherosclerosis and restenosis is the aberrant “proliferation and migration of ECs and VSMCs” [149]. Several polyphenols have been associated with inhibition of such cellular events. These effects have been associated with “decreased expression of CREB and ATF-1 transcription factors and subsequent down-regulation of cyclin A gene” [150]. Also the anti-angiogenic action exerted from Polyphenols is due to “specific inhibition of p38 MAPK and PI3-kinase/Akt pathways” [150]. Another polyphenol of interest is honokiol, which was found in pre-clinical models to have significant anti-angiogenic and anti-inflammatory effects with minimal toxicity [151].

Despite the fact that there are several in vitro studies for the anti-angiogenic properties of polyphenols, there are few in vivo models used to determine their
action. “Local application of RWPCs to chick embryo chorioallantoic membrane”, had a strong reduction of small blood vessel number and length, marking decreased angiogenesis after treatment for a period of 48 hours [152]. There are also older studies that pinpoint the anti-angiogenic action of several polyphenolic compounds and highlight their potential application in several angiogenesis related disorders [153]. Despite the distinct observed anti-angiogenic effect, there is still much to be elucidated about the mechanism of action of natural polyphenols.

**GENE THERAPY**

RNA interference (RNAi), represents a post-transcriptional gene regulation process that is conserved in many different organisms. Small non-coding RNAs (ncRNAs) play critical roles in several biological processes and dysregulation of ncRNAs is associated with several diseases including developmental timing, skeletal muscle proliferation, tumor progression, neurogenesis, brain morphogenesis, transposon silencing, viral defence, and many other cellular processes using the same RNA-processing complex to direct silencing [154-156].

Recent studies suggested that atherosclerosis is an angiogenic disease. The formation of microvessels, contributes to the development of plaques making them rupture-prone. Neovascularisation is proposed to greatly contribute to plaque progression and is frequently observed in human coronary arteries [48, 71]. Recent evidence has strongly pinpointed the role of microRNAs (miRNAs) in CVDs as well as other diseases. Mi-RNAs are small non-coding RNAs that “bind to a target mRNA, causing either degradation or translational repression thus regulating gene expression” [36]. Several studies indicate that these crucial regulators of gene expression have great potential as therapeutics, especially in the regulation of the angiogenic process and cardiogenesis. The identification of circulating miRNAs in patients with CVDs renders them as potential biomarkers for clinical diagnosis, leading to novel therapeutic approaches [157, 158].

Generation of miRNAs is mediated by two enzymes, Dicer and Drosha and is achieved in a two-step processing pathway. When cells encounter long double-stranded RNA molecules, Dicer, a ribonuclease III type enzyme, cleaves them
into small interfering RNAs (siRNAs) of 21–23 nucleotides [36, 155, 159]. “Dicer is constitutively expressed in endothelial cells, with its expression remaining unaffected by either response to stimuli, such as VEGF, or by cell proliferation status” [160]. “This small RNA attaches to an RNA interference silencing complex (RISC) and is directed to the messenger RNA (mRNA) of interest” [157, 161]. Several studies indicate that inhibition or hindrance of miRNA biogenesis pathway provides new therapeutic opportunities for treating diseases “characterized by aberrant angiogenesis (cancer or macular degeneration) or irregular angiogenesis (myocardial ischemia or peripheral vascular disease)” [162]. “The role of miRNAs in ECs was assessed by specific silencing of Dicer, by use of short interfering (si)RNA in human umbilical endothelial cells”, indicating diminished tube formation and cell migration with remarkable effect on several angiogenic regulators, such as TEK/Tie-2, KDR/VEGFR2, Tie-1, angiopoietin-like 4 (ANGPTL4), IL-8 and eNOS [163-166]. The silencing of Drosha, the other nuclear type-III ribonuclease which processes the pri-miRNAs has been dissected, producing less pronounced effects on angiogenesis than Dicer [163-167].

Dysregulation of miRNAs has been widely studied in angiogenesis and several miRNAs included in this function process. “Knockdown of Dicer in ECs is rescued by adding individual miRNAs in the miR-17-92 cluster, a polycistronic miRNA gene categorized into four families (miR-17-, miR-18-, miR-19- and miR-92 family) and characterized as negative regulators of angiogenesis” [164, 168-170]. “Overexpression of miR-92a targets ITGa5 and inhibits angiogenesis in ECs, while administration of antagomir-92a blocks neovascularization in a mouse hindlimb ischemia model and minimizes tissue injury in myocardial infarction” [171]. It has been demonstrated that KLF-2 and its regulated-genes such as eNOS and thrombomodulin (TM), up-regulation by atheroprotective shear flow in primary Sjögren's syndrome and laminar shear stress were “repressed by over-expression of miR-92a in ECs” [171].

“The miR-23-27-24 cluster participates in angiogenesis and endothelial apoptosis in cardiac ischemia and retinal vascular development and miRNAs encoded by the miR-23-27-24 gene clusters are elevated in endothelial cells and highly vascularized tissues. Inhibition of miR-23 and miR-27 function by locked nucleic
acid-modified anti-miRNAs represses angiogenesis \textit{in vitro} and \textit{in vivo}” [154, 172]. MiR-23 and miR-27 silencing, represses angiogenesis, consequently up-regulating Sprouty2 and Sema6A proteins and subsequent attenuation of MAPK and VEGFR2 signaling by Raf activation [173]. MiR-27 is involved in early stage atherosclerosis, while miR-27b inhibited thrombospondin-1, a multifunctional protein which “binds to the reelin receptors, ApoER2 and VLDLR, thereby affecting neuronal migration in the rostral migratory stream” [174, 175].

“Human atherosclerotic plaques were compared to non-atherosclerotic left internal thoracic arteries (LITA) concerning their miRNA expression profile, their correlation between miR/mRNA expression profiles and processes in atherosclerosis” [154, 176]. The expression levels of miR-21, -34a, -146a, -146b-5p, and -210 in patients with CVDs were investigated and “predicted targets of these miRNAs” were found to be down-regulated [162]. Additional studies were carried out including “a few highly expressed miRNAs (miR-2, -15b, -16, -20, -21, 181a, -191, -221, -222, -320, let-7, let-7b, and let-7c), with receptors of angiogenic factors (Flt-1, Nrp-2, Fgf-R, c-Met, and c-kit) as putative mRNA targets” [163, 165].

“Furthermore, miR-208 belongs to a cardiac specific miRNA, encoded by an intron in the gene that encodes \(\alpha\)-myosin heavy chain and functions within a regulatory network, controlling cardiac stress responses” [162, 177]. Additionally, miR-126, a highly-characterized EC-specific miRNA, is enriched in tissues characterized by a high vascular component, like the heart and lung [162, 178, 179]. “MiR-126 is encoded by intron 7 of the EGF-like domain 7 gene also known as VE-statin, which encodes an EC-specific secreted peptide that acts as a chemoattractant and inhibitor of smooth muscle cell migration” [180, 181]. Finally, there are also indications that anti-angiogenic gene delivery even locally can exhibit beneficial action. In a study using a rabbit model, local gene delivery of sFlt-1 proved beneficial by suppressing plaque formation and angiogenesis within the atheromatic plaque [182].

Although pharmacological manipulation of miRNAs is still at its infancy, much more research is required before the above key players in angiogenesis can be taken into clinical practice, the overall body of evidence indicates that miRNAs might prove to be potent therapeutic tools in the future for controlling vascular
inflammation and regulate the progression of atherosclerosis by controlling the angiogenic switch.

**NANOMEDICINAL APPROACHES FOR TREATING PATHOLOGICAL ANGIOGENESIS IN CARDIOVASCULAR DISEASES**

Over the past few years scientific discoveries in the field of nanotechnology have been achieved with tremendous speed. Nanotechnology is the scientific field of synthesizing materials with distinct compositions, sizes and properties at the nanoscale level (nm) and utilizing them according to their properties. Nanoparticles are molecular assemblies that due to their unique composition and size exhibit extraordinary physicochemical, optical and mechanical properties [183]. Employment of these devices for medicinal applications gave rise to the field of nanomedicine. Although nanomedicine is still at an infant stage, there has been remarkable progress of nanomedicinal applications for almost every type of disease. Lately, efforts are being made to use such nanodevices for treating pathological angiogenesis.

In an effort to create a platform for sustained delivery of anti-angiogenic agents a group used a single injection in a rabbit model of a α_β_3-targeted paramagnetic nanoparticle formulation for site-specific delivery of fumagillin successfully inhibiting aortic atherosclerotic progression for a period of 3 weeks [184]. This treatment when combined with oral administration of atorvastatin prolonged its beneficial effects [185]. Furthermore, the paramagnetic nature of the nanoparticles used rendered MRI imaging possible for monitoring and evaluating the progress of the therapy [185]. Also in an effort to successfully monitor the progress of angiogenesis, dendritic biodegradable nanoprobes targeting specific α_β_3 integrin were utilized for detection of peripheral artery disease via positron emission tomography (PET) [186]. Novel nanomedicinal approaches can also aim to improve the efficiency of already tested anti-inflammatory and anti-angiogenic therapies. Such an example is the construction of a liposomal nano-formulation loaded with glucocorticoid, prednisolone phosphate (PLP). Glucocorticoids are a drug class with significant anti-inflammatory action in several models of atherosclerosis. However, extensive use of this drug class has been avoided due to poor pharmacokinetics and several side effects. In a rabbit model of experimental
atherosclerosis, these liposomal formulations containing a mixture of lipids, polyethylene glycol (PEG) and gadolinium with diethylenetriaminepentacetate and bis(stearylamide) (Gd-DTPA-BSA) were successfully delivered in atherosclerotic plaques, as verified by magnetic resonance imaging (MRI) and $^{18}$F-fluoro-deoxy-glucose positron emission tomography combined with computed tomography ($^{18}$F-FDG-PET/CT) by monitoring the uptake of $^{18}$F-FDG at the atherosclerotic aortas after injecting the rabbits [187].

ANTI-ANGIOGENESIS DRUG TOXICITY

Several years ago anti-angiogenenic drug development and therapy has been proposed in order to combat angiogenesis related diseases (i.e. vascular, cancer, rheumatoid) [188]. The excitement from the promising results yielded from in vivo and in vitro studies soon gave place to disappointment as clinical trials gave very poor results [189]. This was often due to the fact that the mechanisms underlying these processed are often not thoroughly understood. There are still many concerns about the safety of the anti-angiogenic drugs often employed. This is especially important in CVD, given the fact that it is a multivariable disease. Thus, successful treatment of one parameter many have several negative effect to other target cells or organs.

One of the most frequently observed, site-specific side effects of anti-angiogenic therapies is hypertension. There are several studies that describe this phenomenon as a result of anti-angiogenic drug treatment [190, 191]. The mechanism of hypertension development after these treatments still eludes our grasp. There are reports that hypertension is caused due to VEGF related inhibition of nitric oxide (NO) synthesis as well as the rarefaction of the capillary bed [192]. Furthermore, anti-angiogenic therapy has also been associated with increased cardiotoxicity, arterial and venous thrombosis and bleeding [193]. Furthermore, some anti-angiogenic therapies on phase II have exhibited a great increase in the rate of vascular toxicity (26.1%) along with lower but still significant rates of transient ischemic attack (4.3%) and cerebral vascular incidents (4.3%) [194].

Another issue when administering long term anti-angiogenic therapy are potential delayed toxicity issues. Since most of the therapies under development are at an
early stage, there is a possibility that toxic effects will not be detected in animal models or early phase clinical trials. There is still much that need to be done in order all potential side effects of anti-angiogenic therapy to be fully assessed before being administered for therapeutic purposes.

**EXCITING FUTURE PROSPECTS**

The first idea for developing anti-angiogenic drugs was conceived over 25 years ago, mainly for cancer therapies. Despite this initial hesitations and disappointments, anti-angiogenic therapy has evolved, and is now slowly starting to be applied for treating several other pathophysiological conditions associated with aberrant angiogenesis such as atherosclerosis, diabetic retinopathy, age-related macular degeneration and more. Given the fact that the common link between these diseases is angiogenesis, pharmacological advances to one field will surely be beneficial to the others as well. Furthermore, the progress achieved in this exciting scientific field is also verified by the increased number of drugs currently undergoing different stages in clinical trials. These drugs are assessed not only for their chemopreventing role but also aim in optimizing treatment (Table 2). Given the fact that our knowledge about the molecules involved in the angiogenic processes increases, new drug targets are identified, expanding our anti-angiogenesis drug arsenal. However, it is of the utmost importance that better *in vivo* and *in vitro* models are created in order to fully assess the potential action and side effects these drugs might pose. The advances in gene therapy and nanotechnology are surely considered to be pioneers in the efforts to create new potent anti-angiogenic therapies.

**Table 2:** List of anti-angiogenic drugs, their targets and stage of development

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target Molecule(s)</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>VEGF-A</td>
<td>FDA approved</td>
</tr>
<tr>
<td>Ranibizumab</td>
<td>VEGF-A</td>
<td></td>
</tr>
<tr>
<td>Pegaptanib</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Sorafenib</td>
<td>VEGFR-2/3, PDGFR-β, FLT3 and c-kit</td>
<td></td>
</tr>
<tr>
<td>Erlotinib</td>
<td>Epidermal growth factor (EGFR)</td>
<td></td>
</tr>
<tr>
<td>Sunitinib (SU11248)</td>
<td>VEGFR-2/3, PDGFR-β, FLT3 and c-kit</td>
<td></td>
</tr>
<tr>
<td>VEGF-trap</td>
<td>VEGF-A, PIGF</td>
<td>Phase III</td>
</tr>
</tbody>
</table>
Table 2: contd....

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effects</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combretastatin A-4</td>
<td>Vascular endothelial cells</td>
<td></td>
</tr>
<tr>
<td>Neovastat</td>
<td>VEGF, MMP-2,-9,-12</td>
<td></td>
</tr>
<tr>
<td>BMS 275291</td>
<td>MMP-1, -2, -8, -9, -13, -14</td>
<td></td>
</tr>
<tr>
<td>Pegaptanib</td>
<td>VEGF</td>
<td>Phase II/III</td>
</tr>
<tr>
<td>cyclic RGD peptide</td>
<td>$\alpha_{v}\beta_{3}$ integrin, Endothelial cell proliferation, migration</td>
<td></td>
</tr>
<tr>
<td>PTK787</td>
<td>VEGF</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

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Declared None.

CONFLICT OF INTEREST

The authors confirm that this chapter contents have no conflict of interest.

ABBREVIATIONS

$^{18}$F-FDG-PET/CT = $^{18}$F-fluoro-deoxy-glucose positron emission tomography combined with computed tomography

aFGF = Acidic fibroblast growth factor

AMI = Acute myocardial infarction

Ang II = Angiotensin II

Ang-1 = Angiopoietin-1

Ang-2 = Angiopoietin-2

ANGPTL4 = Angiopoietin-like 4

COX-2 = Cyclooxygenase-2

CVD = Cardiovascular disease
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCs</td>
<td>Dendritic cells</td>
</tr>
<tr>
<td>DLL4</td>
<td>Delta like ligand 4</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>ECs</td>
<td>Endothelial cells</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>FGF</td>
<td>Fibroblast growth factor</td>
</tr>
<tr>
<td>FGF-R1DN</td>
<td>Dominant-negative form of FGF receptor 1</td>
</tr>
<tr>
<td>FLT3</td>
<td>Fms-related tyrosine kinase 3</td>
</tr>
<tr>
<td>Gd-DTPA-BSA</td>
<td>Gadolinium with diethylenetriaminepentacetate and bis(stearylamide)</td>
</tr>
<tr>
<td>GPx</td>
<td>Glutathione peroxidase</td>
</tr>
<tr>
<td>GRx</td>
<td>Glutathione reductase</td>
</tr>
<tr>
<td>HIF</td>
<td>Hypoxia-inducible transcription factor</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>3-hydroxy-3-methyl-glutaryl-CoA</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule-1</td>
</tr>
<tr>
<td>IL-10</td>
<td>Interleukin-10</td>
</tr>
<tr>
<td>IL-12</td>
<td>Interlukin-12</td>
</tr>
<tr>
<td>IL-18</td>
<td>Interleukin-18</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1β</td>
</tr>
<tr>
<td>INF-γ</td>
<td>Interferon-γ</td>
</tr>
</tbody>
</table>
iNOS = Inducible nitric oxide synthase
ISR = In-stent restenosis
IST = In-stent thrombosis
JAG 1 = Jagged-1
LDL = Low density lipoprotein
LITA = Left internal thoracic arteries
LOX-1 = Lectin-like oxLDL receptor-1
MCP-1 = Monocyte chemoattractant protein-1
MHC class 2 = Major histocompatibility complex class 2
miRNAs = microRNAs
MMPs = Matrix metalloproteinases
MRI = Magnetic resonance imaging
mRNA = Messenger RNA
NADPH = β-nicotinamide adenine dinucleotide phosphate
ncRNA = Non-coding RNA
NK cells = Natural killer cells
ox-LDL = Oxidized-low density lipoprotein
PDGF = Platelet-derived growth factor
PDGFR-β = platelet-derived growth factor receptor β
PEDF = Pigment Epithelium-Derived Factor
PEG = Polyethylene glycol
PET = Positron emission tomography
PHD2 = Prolyl hydroxylase domain 2
PI-88 = Phosphomannopentaose sulfate
PIGF = Placental growth factor
PLP = Prednisolone phosphate
PPAR-γ = Proliferator-activated receptor gamma
RISC = RNA interference silencing complex
RNAi = RNA interference
RNS = Reactive nitrogen species
ROS = Reactive oxygen species
RWPCs = Red wine polyphenolic compounds
siRNAs = Small interfering RNAs
SMC = Smooth muscle cell
SOD = Superoxide dismutase
TGF-β = Transforming growth factor-beta
Th1 cells = T helper 1 cells
TLR = Toll-like receptor
TM = Thrombomodulin
TNF-α = Tumor necrosis factor alpha
VCAM-1 = Vascular cell adhesion molecule-1
VEGF = Vascular endothelial growth factor
VEGFR = Vascular endothelial growth factor receptor
VSMC = Vascular smooth muscle cell
XO = Xanthine oxidase

REFERENCES


