Targeting glycolysis in endothelial cells to prevent intraplaque neovascularization and atherogenesis in mice

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Chapter 2

Pharmacological strategies to inhibit intraplaque angiogenesis in atherosclerosis

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Abstract

Atherosclerosis is a complex multifactorial disease that affects large and medium-sized arteries. Rupture of atherosclerotic plaques and subsequent acute cardiovascular complications remain a leading cause of death and morbidity in the Western world. There is a considerable difference in safety profile between a stable and a vulnerable, rupture-prone lesion. The need for plaque-stabilizing therapies is high, and for a long time the lack of a suitable animal model mimicking advanced human atherosclerotic plaques made it very difficult to make progress in this area. Evidence from human plaques indicates that intraplaque (IP) angiogenesis promotes atherosclerosis and plaque destabilization. Although neovascularization has been widely investigated in cancer, studies on the pharmacological inhibition of this phenomenon in atherosclerosis are scarce, mainly due to the lack of an appropriate animal model. By using $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ mice, a novel model of vulnerable plaques, we were able to investigate the effect of pharmacological inhibition of various mechanisms of IP angiogenesis on plaque destabilization and atherogenesis. In the present review, we discuss the following potential pharmacological strategies to inhibit IP angiogenesis: (1) inhibition of vascular endothelial growth factor signalling, (2) inhibition of glycolytic flux, and (3) inhibition of fatty acid oxidation. On the long run, IP neovascularization might be applicable as a therapeutic target to induce plaque stabilization on top of lipid-lowering treatment.

Introduction

Atherosclerosis is a chronic inflammatory disorder of the arterial wall leading to coronary artery disease, stroke and peripheral arterial disease. [1-3] Not only the size but rather the stability of atherosclerotic plaques is determinant for acute clinical implications. When a plaque develops an unstable phenotype, it is prone to rupture, which can lead to myocardial infarction, stroke and sudden death. Unstable plaques have a relatively large lipid core, high macrophage content and a thin fibrous cap. Due to cholesterol-lowering drugs the lifespan and wellbeing of patients has been significantly improved. However, a large group of patients does not fully benefit from current lipid-lowering strategies. [3] Indeed, despite major advances in cardio- and cerebrovascular research, plaque rupture remains the leading cause of acute events. Therefore, additional therapy that reduces atherosclerosis or prevents plaque rupture and its complications is needed.

Recently, several new therapies have emerged to treat high-risk patients (e.g. PCSK9 monoclonal antibodies and inclisiran to reduce the residual cholesterol risk, and canakinumab, a monoclonal antibody against interleukin-1β to reduce plaque inflammation).[4-7] However, accumulating evidence indicates that also intraplaque (IP) angiogenesis promotes atherosclerosis and plaque destabilization. Clinical data link IP angiogenesis with progressive and unstable vascular disease. Autopsy studies, for example, revealed a higher density of IP vasa vasorum microvessels in symptomatic (ruptured) plaques compared to asymptomatic ones. [8] IP angiogenesis is a complex process that depends on the equilibrium between several pro- and anti-angiogenic molecules. [9] The presence of hypoxia in advanced atherosclerotic plaques correlates with IP angiogenesis in human carotid arteries. [10-12] Besides hypoxia, inflammation is a strong inducer of angiogenesis as it promotes the synthesis of various angiogenic factors. During acute inflammation, several pro-angiogenic molecules can induce cell permeability, contributing to the infiltration of leukocytes in the inflammatory core and thereby provoking chronic inflammation. [13] Because the newly formed vessels growing into the plaque are immature, they are inherently leaky, permitting inflammatory cell infiltration and influx of blood constituents (including erythrocytes and blood platelets) into the plaque. [14] Moreover,

IP microvessels can promote the entry of leukocytes into the plaque by upregulation of adhesion molecules such as ICAM-1 and VCAM-1. [15] The increased release of matrix metalloproteinases from activated macrophages and proteases secreted from mast cells cause further damage to the microvessels and facilitate IP haemorrhage.[16] Following IP neovascularization, IP haemorrhage has been linked to plaque progression [17], making it an important hallmark of plaque instability. Based on these findings, it is well accepted that IP neovascularization plays a significant role in atherosclerotic plaque destabilization and rupture.

Neovascularization has been widely investigated in cancer, but studies on the pharmacological inhibition of this phenomenon in atherosclerosis are scarce, mainly due to the lack of a suitable animal model. Scientific knowledge on the significance of IP neovascularization in atherosclerosis was mainly acquired through human specimens. In normal human arteries, vasa vasorum are found in the adventitia and outer media since diffusion of oxygen and other nutrients from the lumen is sufficient to nourish the intimal layer and the inner media [18]. However, during atherogenesis the progressive increase in plaque size is associated with development of hypoxic regions, increased oxidative stress and inflammation, which promote the formation of IP microvessels (angiogenesis) that reach the intima and infiltrate the atherosclerotic plaque.[19] Depletion of ATP in macrophages is also an important contributor to IP angiogenesis due to the extremely high rates of energy necessary for cholesterol uptake. [20, 21] Microvessels grow from the adventitial vasa vasorum through the media into the intimal lesion. Plaques can be particularly rich in microvessels at the shoulder region and base. IP microvessels in human carotid arteries have a diameter of 2-200μm and a surface of 20-20000 μm^2 [22]. Furthermore, the thin wall of plaque microvessels lacks proper structure, in terms of elastic laminae and smooth muscle cell (SMC) support, making them leaky, fragile and prone to rupture. [23, 24] Recent evidence supports the idea that blocking IP angiogenesis may represent a new approach to decrease plaque instability and thus cardiovascular risk. For example, it has been shown that inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. [25]

Although IP neovascularization is a typical feature of advanced human atherosclerotic plaques, it is rarely observed in animal models, including Apolipoprotein deficient ($ApoE^{-/-}$) mice. [26] Therefore, a causal and straightforward relation between plaque rupture and IP neovascularization has never been confirmed due to the lack of a relevant animal model of atherosclerosis with human-like characteristics such as IP neovascularization. In the past two decades, animal models of atherosclerosis merely generated a stable plaque phenotype, while plaque rupture almost never occurred. [27] The latter implied a substantial limitation in atherosclerosis-related research. Vein grafts in ApoE*3Leiden mice were among the first lesions in animals with features resembling those of human plaques, such as intimal dissection, intramural thrombosis and IP neovascularization. [28] Because this model is based on vein graft surgery, it requires a complex intervention to induce microvessel formation via neovascularization. We reported that $ApoE^{-/-}$ mice containing a heterozygous mutation ($C1039G^{+/-}$) in the fibrillin-1 (Fbn1) gene show very pronounced atherosclerosis and a highly unstable plaque phenotype on a Western-type diet [29], leading to plaque rupture and human-like complications, such as myocardial infarction, stroke and sudden death without any surgical interventions. [30] Interestingly, $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice reveal substantial IP neovascularization in the brachiocephalic artery and common carotid arteries. [30] Moreover, similar as in humans, both mature and immature microvessels are present, the latter being highly leaky. Because Fbn1 is the major structural component of the extracellular microfibrils in the vessel wall, neovascularization in $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice probably occurs because elastin fragmentation allows microvessel sprouting from the adventitial vasa vasorum through the media into the intimal lesion. Moreover, the high degree of stenosis and the presence of activated macrophages likely results in IP hypoxia, and triggers the growth of new vessels from the adventitia. IP haemorrhages are frequently observed in the proximity of microvessels, suggesting that they arise from leaky and/or ruptured microvessels.

Because IP neovascularization seems to have a major causative effect on atherosclerosis and plaque destabilization in humans [8, 31-33], we investigated whether inhibition of IP neovascularization might be a useful therapy for atherosclerotic plaque stabilization. Inhibition of pathological angiogenesis has become an accepted therapeutic strategy in cancer and diabetes mellitus. [34] Only recently, studies

unveiled the importance of endothelial cell (EC) metabolism in controlling angiogenesis and maturation of microvessels, [35] also in the field of atherosclerosis, showing the novelty of this research . [26, 28, 36] In this review, we discuss the following potential pharmacological strategies to inhibit IP angiogenesis: (1) inhibition of vascular endothelial growth factor signalling, (2) inhibition of glycolytic flux, and (3) inhibition of fatty acid oxidation (Figure 1, Table 1).

Targeting IP angiogenesis through inhibition of vascular endothelial growth factor signalling

Anti-angiogenic therapy in cancer research has demonstrated that vascular endothelial growth factor (VEGF)-A is a potent initiator of neovascularization. The VEGF family, consists of five closely related members, namely VEGF-A, B, C, D and placental growth factor. VEGF-A is released by various cell types including ECs, SMCs, astrocytes and macrophages. VEGF-A drives vasculogenesis, angiogenesis and blood vessel maintenance in physiological conditions. Activation of VEGFR-2, a receptor for VEGF-A, triggers several downstream pathways that promote EC survival, permeability, migration and proliferation.[37] VEGF-A is abundantly present within advanced human coronary and carotid atherosclerotic plaques [38, 39] and elevated VEGF-A concentration likely contributes significantly to promote IP angiogenesis. VEGF-A has been clearly observed in lipid-rich coronary lesions and stenotic coronary plaques particularly in ECs and macrophages surrounding microvessels.[38] It induces EC permeability via phosphorylation of VE-cadherin, which gets internalized, resulting in a loss of EC junctions. Accordingly, VEGF-A upregulation in plaques leads to highly permeable and leaky microvessels, which fail to mature properly.

In view of the above-mentioned findings, VEGF-A can be used as a promising target to inhibit IP microvessel formation. In the last decade, there has been a substantial increase in compounds targeting VEGF or its downstream pathways to counteract angiogenic growth. Bevacizumab, a monoclonal antibody against VEGF-A, inhibits IP neovascularization with smaller atherosclerotic lesions as a result [40] (Figure 1). This finding nourished the presumption that targeting IP neovascularization might

result in more stable lesions and opened a new field of interest in the treatment of atherosclerosis. Besides antibodies against VEGF-A [40, 41], antibodies blocking VEGFR-2 (DC101) revealed smaller vein graft lesions and less IP haemorrhages in ApoE*3 Leiden mice. [42] Also the stability of the vein graft lesion was increased in DC101-treated mice, as shown by a reduction in macrophage content and an increase in collagen and SMCs.

In the clinical practise in oncology, tyrosine kinase inhibitors are an important subgroup of new anticancer compounds specifically targeting VEGFR-induced neovascularization. [43] Compounds targeting VEGFRs may provide an interesting approach to investigate the effect of inhibition of the VEGF signalling in the stabilization of atherosclerotic plaques. Axitinib is a potent and selective inhibitor of VEGFR tyrosine kinases 1, 2 and 3 (Figure 1), and is clinically used for the treatment of advanced renal cell carcinoma. [44] Given the very promising results in oncology, we evaluated the potential plaque stabilizing effects of axitinib. This compound was chosen above other anti-VEGF receptor tyrosine kinase inhibitors due to its potency and acceptable safety profile in oncology research. [26] In atherosclerotic $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ mice, axitinib (35 µg/g i.p. 4x/week for 6 weeks) significantly reduces IP neovascularization by 50 %, with subsequent less prevalence of IP haemorrhages. [45] The SMC content doubles, whereas the amount of macrophages decreases by 30 %. Because entry of monocytes and macrophages is related to leakage of microvessels, a reduction in the amount of macrophages may be a direct result of the decreased microvessel network in the plaque. In addition, overall cardiac function is improved in axitinib-treated animals (fractional shortening: 27±2 vs. 19±3 %). Moreover, the number of animals with myocardial infarction decreases by 40 %. Coronary plaque formation is present in almost all control animals whereas axitinib-treated animals show a 30 % reduction in the occurrence of coronary plaques. Taken together, inhibition of VEGF receptor signalling by axitinib attenuates IP angiogenesis and plaque destabilization in mice. [45] However, the mechanisms responsible for these observations are not fully understood. It is very likely that inhibition of VEGFR-signalling affects a combination of several processes. Improved plaque stability can be a direct consequence of the decrease in neovascularization, with less leakage of destabilizing cells into the plaque. On the other hand, VEGF can

act as an initiator of a well-organized signalling cascade, driving multiple mechanisms.[46, 47] Thus, not only the decrease in IP microvessels may account for the plaque-stabilizing effects of axitinib, also other mechanisms can be involved in determining the outcome. On the long run, it might be interesting to use VEGFR2 inhibitors as add-on therapy to statins for their plaque-stabilizing effects. Nevertheless, we must be careful because therapeutic angiogenesis is currently evaluated as a possible therapy in cardiovascular ischemic diseases, such as myocardial infarction and peripheral arterial disease. Importantly, in our study axitinib did not induce adverse effects on the heart; on the contrary, the heart function was even improved.

Endostatin, the C-terminal globular domain of collagen, is a naturally occurring anti-angiogenic protein. First discovered in Judith Folkman's lab, this protein was isolated for its ability to inhibit the proliferation of capillary ECs. Almost two decades ago, recombinant endostatin expressed in yeast was introduced into clinical trials. However, its instability diminished the efficacy. A new recombinant human endostatin, endostar, with an N-terminal modification was more stable and was at least twice as potent as compared to the parent compound. Endostar reduces inoculated tumour growth in mice by substantially inhibiting angiogenesis. [48] Endostar inhibits angiogenesis by blocking VEGF-induced tyrosine phosphorylation of VEGFR-2, but others associate its effects with decreased expression of β-catenin in the atherosclerotic artery. Suppression of angiogenesis through inhibition of Wnt/β-catenin signalling plays a major role in regulating fundamental aspects of development such as cell fate specification, proliferation, survival and overall organogenesis. [48, 49] B-catenin is a key intracellular signal transducer, which besides its role in the Wnt pathway, also binds to cadherins (VE- and N-cadherin in ECs), thus stabilizing cell-to-cell adhesion and tissue integrity. Down-regulation of the Wnt/β-catenin signalling pathway may be involved in the inhibition of angiogenesis. [49] Therefore, inhibition of the Wnt/β-catenin signalling pathway might be a future approach to inhibit IP angiogenesis. [19]

Targeting IP angiogenesis through inhibition of glycolytic flux

While VEGF and its downstream pathways have been widely investigated to regulate and to inhibit neovascularization, recent studies in the field of oncology present evidence from a different point of view. [50-52] Indeed, modulation of cell metabolism (glycolysis) has already shown beneficial effects in cancer research, and this approach could be of value in atherosclerosis as well. Proliferating ECs reveal high glycolytic activity (>200-fold higher than glucose, fatty acid and glutamine oxidation), which results in the generation of >85% of the total cellular ATP content. [53] The conversion of fructose-6-phosphate (F-6-P) to fructose-2,6-bisphosphate (F-2,6-P_2) is one of the three rate-limiting checkpoints during glycolytic flux and is modulated by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFBs) (Figure 1). PFKFB3-driven glycolysis is important for the migration of ECs, and knockdown of PFKFB3 in ECs exhibits defects in angiogenesis both *in vitro* and *in vivo*. [54] Interestingly, inhibition of PFKFB3 by intraperitoneal injection of the small molecule 3PO [3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one] reduces vessel sprouting in EC spheroids, zebrafish embryos and the mouse retina by inhibiting EC proliferation and migration. [51] 3PO reduces F-2,6-P_2 levels by blocking PFKFB3 in its kinase domain, which in turn suppresses glycolysis (but without abrogating glycolytic side pathways such as the pentose phosphate pathway necessary for the production of NADPH). It is however important to note that the inhibition of PFKFB3 by 3PO is partial (35–40%) and transient [51], albeit sufficient to reduce neovascularization. Indeed, 3PO targets the hyper-metabolism that is induced when ECs switch from quiescence to proliferation and migration. This could have some safety implication, since inhibiting glycolysis nearly completely and permanently may also lead to ATP depletion and thus to cell toxicity. [55]

Recently, we showed that in atherosclerotic ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice pharmacological inhibition of PFKFB3 by 3PO (50 µg/g, i.p) reduced IP neovascularization and haemorrhages by 50% in a preventive regimen and by 38 % in a curative regimen. [56] This compound had no effect on SMC, collagen and macrophage content in the plaques. Plasma VEGF-A levels decreased significantly (curative: 838±449 vs. 2871±653 pg/ml) and cardiac function improved after 10

weeks of treatment (fractional shortening 31±4 vs. 23±3 %). Thus, inhibition of PFKFB3 by 3PO significantly represses IP angiogenesis and haemorrhages in mice, demonstrating its potential in preventing plaque rupture.[56] However, up till now it is unclear whether the effects of 3PO are attributable solely to direct effects on PFKFB3 kinase. Previously, 3PO was investigated for its binding properties via computational modelling, whereas its activity was characterized via kinase activity assays. [57] However, recent evidence indicates that the specificity of 3PO for PFKFB3 is insufficient to attribute all its effects to PFKFB3 inhibition, meaning that there might be alternative mechanisms by which 3PO acts. [58] Recently, other PFKFB3 inhibitors such as PFK15 (a 3PO derivative that displays approximately 100-fold more activity against PFKFB3 than 3PO itself [58]), and indazole analogues that are structurally not related to 3PO have been developed. These drugs demonstrate high selectivity over related PFKFB3 isoforms and potent modulation of the target (IC_{50} PFKFB3: 3 nM)[59], though were not yet tested *in vivo*. Crystallographic studies highlight binding of these drugs at the ATP site of the enzyme. [59] In particular, a compound with a dimethylisoxazole substitution at the R position is a potent and selective inhibitor of PFKFB3. [59]Future studies are needed to evaluate whether these compounds are interesting as potential inhibitors of IP angiogenesis.

Targeting IP angiogenesis through inhibition of fatty acid oxidation

Recent evidence indicates that silencing or knocking out carnitine palmitoyltransferase 1a (CPT1a), a rate-determining enzyme of fatty acid oxidation (FAO), impairs vessel sprouting (but not migration) by reducing EC proliferation [60] (Figure 1). FAO is a multistep metabolic pathway during which fatty acids are broken down in order to produce energy in the cells. After the fatty acid enters into the cytosol it is transferred to the mitochondria where it undergoes β-oxidation. This process involves activation to acyl-CoA by conjugation with coenzyme A in the cytosol, conversion by carnitine palmitoyltransferase 1a (CTP1a) to acyl carnitine for transport across the mitochondrial membrane and conversion back to acyl-CoA inside the mitochondrion in which fatty acid oxidation (β-oxidation) takes place. β-oxidation involves a repeated sequence of four enzyme activities that results in the release of an acetyl-CoA unit, a molecule of FADH2 and a molecule of NADH.

Subsequently, the acetyl-CoA enters the mitochondrial tricarboxylic acid cycle where it is oxidized to CO_2 and H_2O with the generation of aspartate, used for dNTPs synthesis and essential for DNA replication in proliferating ECs. [61]

During angiogenesis ECs differentiate into “tip” (navigating) and “stalk” (proliferating) cells. While during the process of migration, tip cells seemed to rely more on a PFKFB3-driven glycolytic metabolism to rapidly produce enough ATP, during the process of proliferation, “stalk” cells depend on FAO, essential for sprout elongation. In contrast to 3PO, CPT1a deficiency does not alter ATP levels as FAO contributes to less than 5 % of the total amount of cellular ATP. Instead, FAO is important for the *de novo* synthesis of deoxyribonucleotides. [60] Thus, rather than using FAO for the production of energy, ECs use fatty acids for DNA synthesis, necessary for proliferation during vessel sprouting.[34] Therefore, a future strategy might be inhibition of IP angiogenesis by pharmacological blockade of CPT1a in ECs using etomoxir (Figure 1), which is an irreversible inhibitor of CPT1a enzyme that shows favourable effects during treatment of heart failure [62] and also reduces pathological angiogenesis in an ocular disease model. [60] Etomoxir impairs vessel sprouting [63, 64] but has not yet been tested in an animal model of atherosclerosis. However, the above-mentioned findings suggest its potential to inhibit IP angiogenesis.

Although we envision the future use of the above-mentioned strategies on top of a statin treatment, statins themselves might also partially affect IP angiogenesis. Statins reduce cholesterol levels via inhibition of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. In addition, growing evidence indicates that statins trigger pro-angiogenic effects at low (nanomolar) concentrations and anti-angiogenic effects at higher (micromolar) concentrations. [65] They display these pleiotropic functions beyond lipid lowering. HMG-CoA reductase regulates the synthesis of mevalonic acid, a precursor of cholesterol, as well as geranyl geranylpyrophosphate (GGP). The latter intermediate seems to play an important role in the anti-angiogenic properties of statins as supplementation of GGP reverses the angiostatic effects.[65] Interestingly, atherosclerotic $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice treated with atorvastatin show much less IP neovascularization as well as a reduction in cardiovascular morbidity and mortality without obvious changes in plasma cholesterol. [66] Accordingly, we presume that

patients suffering from atherosclerosis can benefit from the anti-angiogenic properties of statins, even without elevated cholesterol levels.

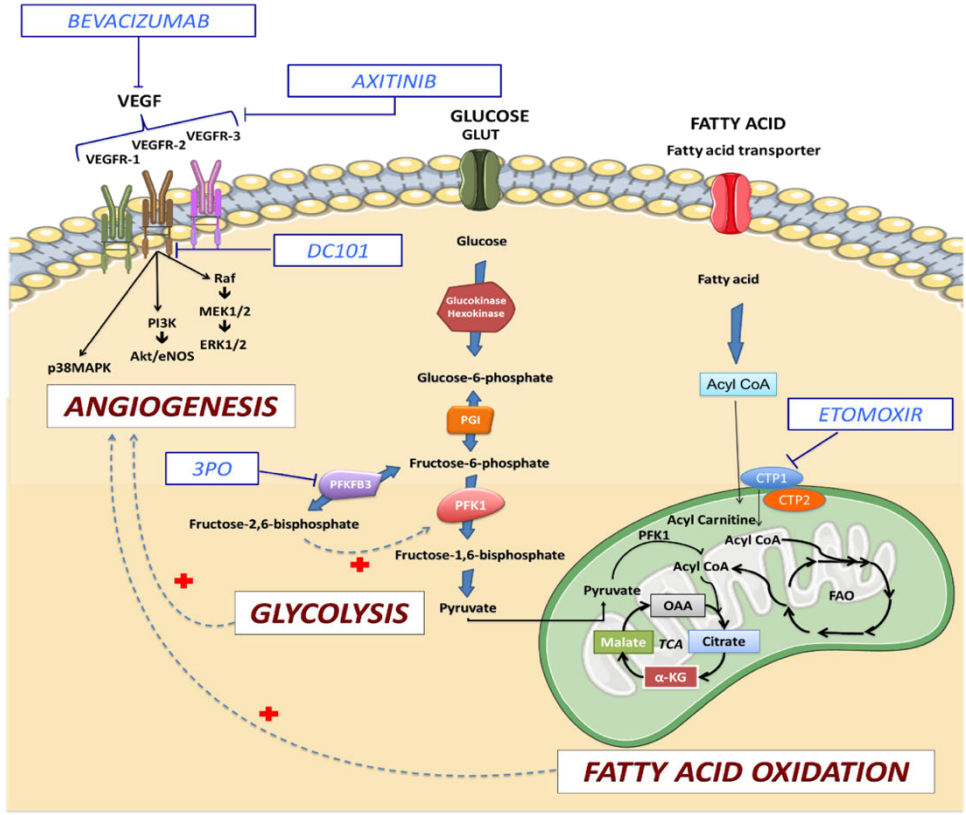

Figure 1. Overview of selected pathways in EC metabolism and their possible targets to inhibit IP angiogenesis

Schematic representation and simplified overview of selected metabolic pathways known to be involved in angiogenesis, and their respective possible targets. Vascular endothelial growth factor (VEGF) is a potent initiator of neo-angiogenesis which acts through its main receptors VEGFR1, VEGFR2 and VEGFR3. Bevacizumab inhibits VEGF-A. On the other hand, axitinib blocks VEGF receptor tyrosine kinase 1,2 and 3. DC101 is an antibody against VEGFR-2. All these targets interfere with angiogenesis.

PFKFB3 and CTP1a are key enzymes in glycolysis and FAO respectively, and are critical metabolic regulators of vessel sprouting. 3PO reduces fructose-2,6-bisphosphate levels – a potent allosteric activator of glycolysis – by blocking PFKFB3 in its kinase domain. This

inhibition is transient and partial, yet sufficient to reduce neovascularization. Pharmacological inhibition of CTP1a with small chemical compound etomoxir leads to reduced endothelial cell proliferation and defects in vessel sprouting in oncology studies. VEGF, Vascular Endothelial Growth Factor; VEGFR, Vascular Endothelial Growth Factor Receptor; eNOS, endothelial nitric oxide synthase; GLUT-1, glucose transporter 1; PGI, phosphoglucose isomerase; PFK1, 6-phosphofructokinase 1; PFKFB3, phosphofructokinase-2/fructose-2,6-bisphosphatase isoform 3; 3PO, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one; OOA, oxalacetate; α-KG, α-ketoglutaric Acid; FAO, fatty acid oxidation; CTP1a, carnitine palmitoyltransferase 1a.

Table 1. Compounds studied to inhibit IP angiogenesis

IP angiogenesis inhibitor	**Mechanism of action**	**Animal model of IP angiogenesis**	**Reference**
Bevacizumab	Monoclonal antibody to VEGF-A	Aortic balloon denudation in hypercholesterolaemic rabbits	[40]
Axitinib	VEGFR1,2,3-inhibitor	Hypercholesterolaemic $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice	[45]
DC101	VEGFR2 blocking antibody	Vein graft in hypercholesterolaemic ApoE3*Leiden mice	[42]
3PO	Glycolysis inhibitor	Hypercholesterolaemic $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice	[56]
Endostar	Blocking VEGF-induced tyrosine phosphorylation of VEGFR-2, decreased expression of β-catenin	Porcine model of atherosclerosis	[49, 67]

Conclusion

In conclusion, inhibition of IP angiogenesis may represent an attracting novel pharmacological target to stabilise vulnerable atherosclerotic plaques. While an association between angiogenesis and progression of human atherosclerosis have been reported multiple times, pharmacological approaches to target this process have been started to be explored only recently. Although previous studies highlighted that pro-angiogenic therapy enhanced atherosclerosis, while anti-angiogenic therapy reduced atherosclerotic complications, the majority of *in vivo* studies in animal models of atherosclerosis were based on assessing adventitial microvessels but not IP angiogenesis. Novel, more suitable animal models such as the ones described in this review, will permit a better evaluation of this novel therapeutic target in atherosclerosis. Recent studies with these models show the ability of anti-angiogenic drugs like bevacizumab, axitinib or DC101 to inhibit IP angiogenesis in atherosclerosis and to reduce IP haemorrhage. However, further investigations will be required to explore EC metabolism as a new target in atherosclerosis as already applied in tumour angiogenesis. On the long run, this approach can lead to novel therapeutic interventions to treat patients who do not fully benefit from current lipid-lowering therapies. Furthermore, the acquired knowledge will allow a significant advance in the fundamental understanding of IP neovascularization.

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