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Pharmacological prevention of intimal hyperplasia: A state-of-the-art review

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ABSTRACT

Intimal hyperplasia (IH) occurs in a considerable number of cases of blood vessel reconstruction by stenting or balloon angioplasty, venous bypass grafting, and arteriovenous dialysis accesses. It is triggered by endothelial injury during the vascular intervention and leads to vessel restenosis with life-threatening consequences for patients.
To date, the drugs used for IH prevention in clinics—paclitaxel and rapalog drugs—have been focusing primarily on the vascular smooth muscle cell (VSMC) proliferation pathway of IH development. Limitations, such as endothelial toxicity and inappropriate drug administration timing, have spurred the search for new and efficient pharmacological approaches to control IH.
In this state-of-the-art review, we present the pathways of IH development, focusing on the key events and actors involved in IH. Subsequently, we discuss different drugs and drug combinations interfering with these pathways based on their effect on peripheral circulation IH models in animal studies, or on clinical reports. The reports were obtained through an extensive search of peer-reviewed publications in Pubmed, Embase, and Google Scholar, with search equations composed based on five concepts around IH and their various combinations.
To improve vascular intervention outcomes, rethinking of conventional therapeutic approaches to IH prevention is needed. Exploring local application of drugs and drug combinations acting on different pathophysiological pathways of IH development has the potential to provide effective and safe restenosis prevention.

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Abbreviations: API, active pharmaceutical ingredient; bFGF, basic fibroblast growth factor; EC, endothelial cell; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; GF, growth factor; IH, intimal hyperplasia; IL, interleukin; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; NO, nitric oxide; p70S6K, p70 ribosomal protein S6 kinase; PCSK9, proprotein convertase subtilisin-like kexin type 9; PDGF, platelet-derived growth factor; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; TGF-β, transdermal growth factor beta; TNFα, tumor necrosis factor alpha; TXA2, thromboxane A2; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell; YAP, yes-associated protein.

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1. Introduction

Peripheral vascular disease is a blood circulation disorder caused by the narrowing of vessels outside coronary and cerebral circulation. It is the most prevalent cardiovascular condition accounting for over 40% of all cardiovascular diseases worldwide (Fowkes et al., 2013). Patients with lifestyle-limiting symptoms of peripheral artery disease are candidates for vascular interventions such as stent implantation, balloon angioplasty, and bypass grafting. In stented peripheral arteries, restenosis occurs in up to 51% of the patients one year after the vascular intervention (Zen et al., 2017). In hemodialysis patients, stenosis can occur in arteriovenous fistulas, arteriovenous grafts, and other vascular accesses (Manns et al., 2005). The clinical consequences of restenosis include vascular access failure and acute limb ischemia in peripheral arterial disease.

The leading cause of restenosis is intimal hyperplasia (IH). To prevent IH, drug-eluting stents and drug-coated balloons eluting paclitaxel or sirolimus are the most widely used devices nowadays. These devices have improved the endovascular intervention outcomes (Simonton et al., 2007); however, they are also associated with certain risks (Katsanos et al., 2020; Katsanos, Kitrou, & Karnabatidis., 2018), and do not spare the endothelium, which is crucial for vessel healing.

In this state-of-the-art review, we present the pathophysiology of IH and therapeutic options for its prevention, classified according to the mechanism of action on different pathways of its development. A particular focus is set on peripheral artery applications, although several literature contributions relate to coronary applications.

This review includes alternative approaches to target the multifactorial process leading to IH by using combinations of drugs acting on different pathways of IH development. It emphasizes that a combination therapy strategy has the potential to enhance the efficacy and reduce the local toxicity of IH prevention.

The literature search was conducted using the PubMed, Embase, and Google Scholar databases to identify relevant peer-reviewed scientific reports published in English. The search terms included, but were not limited to, “intimal hyperplasia,” “restenosis,” “local drug delivery,” and “drug combinations.” The details of the search strategy are presented in Appendix 1.

2. Physiology and development of intimal hyperplasia

IH is a multifactorial process governed by an array of cellular and extracellular phenomena. Vascular injury, unavoidable during vascular interventions, is the starting point of a cascade of events involving cells and tissues present at the injury site. This process develops over time and is divided into three stages: hyperacute, acute, and chronic, as presented in Fig. 1.

In the hyperacute stage, immediately after the vascular injury, platelets accumulate at the injury site, adhering to the subendothelial von Willebrand factor and collagen (Wilentz et al., 1987). In damaged endothelium, the production of NO, prostacyclin, and other endogenous vascular smooth muscle cell (VSMC) proliferation inhibitors is downregulated, whereas that of prothrombogenic factors is increased (Reidy, Fingerle, & Lindner, 1992).

The coagulation cascade is initiated in the acute stage, extending from several hours to several days after the injury. This results in further platelet activation and fibrin deposition, followed by the production of thrombin, a mitogen for VSMC (Maruyama et al., 1997). From the initial

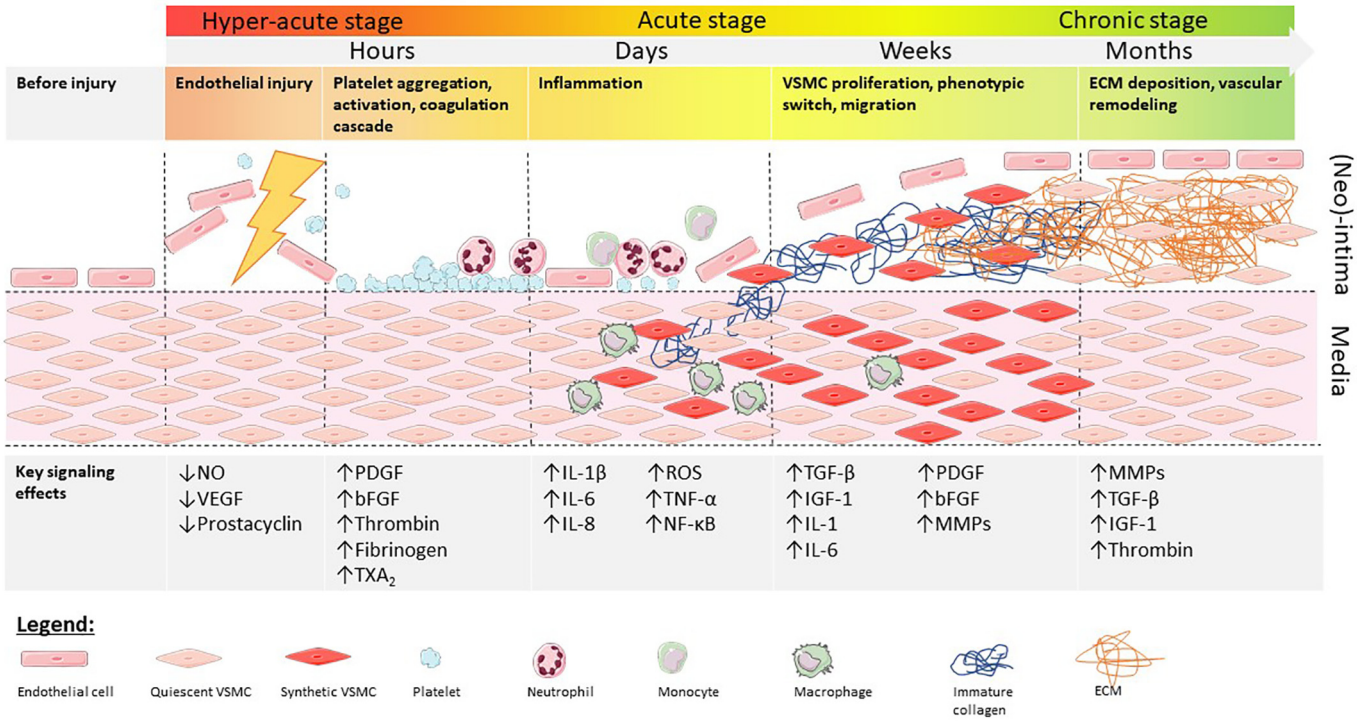

Fig. 1. Stages of intimal hyperplasia (IH) development with the key signaling events. IH occurs in 3 consecutive stages: hyper-acute, acute and chronic stage. These stages are driven by cellular events, taking place at the site of the impaired endothelium. The trigger of IH is the endothelial injury, upon which the platelets start aggregating and adhering to the injury site. The platelets release mitogenic growth factors, attracting macrophages and causing vascular smooth muscle cells (VSMC) to switch their phenotype to synthetic. Under the influence of growth factors and inflammation mediators, synthetic VSMC proliferate, secrete extracellular matrix (ECM) components and migrate into the subintimal space. In the chronic stage, ECM is resynthesized, definitively thickening the neointima. At this point, VSMC occupy the minority of the tissue volume in the neointima, while the majority of the neointimal volume is taken up by ECM.
(ECM indicates extra-cellular matrix; bFGF, basic fibroblast growth factor; IGF-1, insulin-like growth factor-1; IL, interleukin; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NO, nitric oxide; PDGF, platelet derived growth factor; ROS, reactive oxygen species; TGF-β, transdermal growth factor-β; TNF-α, tumor necrosis factor-α; TXA_2, thromboxane A_2; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell).

hours to several days, platelets release growth factors (GFs) and attract immune cells. Under the influence of GFs, the VSMCs proliferate and change their phenotype from a quiescent contractile state to a synthetic and motile state. The extracellular matrix (ECM) breakdown mediated by platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and matrix metalloproteinases (MMPs) is responsible for VSMC migration into the subintimal space, where the dedifferentiated VSMCs become the predominant population (Myit et al., 2003).

In the chronic stage, extending from the first week to several months after the injury, the VSMC proliferation continues under the influence of GFs, while the ECM is resynthesized, definitively thickening the neointima (Wilentz et al., 1987). The definitive vascular remodeling leads to a partial-to-total lumen occlusion; if the lumen diameter is reduced by at least 50%, it is called restenosis. At this stage, VSMCs occupy only around 10% of the tissue volume in the neointima, while the remaining 90% is ECM (Schwartz, Holmes, & Topol, 1992).

3. Key pathways and growth factor-mediated effects in intimal hyperplasia

GFs are key players in the progression of IH. The GFs released at the site of the vascular injury induce VSMC proliferation, motility, survival, and phenotypic switch, ECM breakdown and re-synthesis as well as recruitment of immune cells and mural thrombus formation; they also have a role in endothelial cell (EC) regrowth after injury. The most notable mitogenic GFs are PDGF, insulin-like growth factor 1 (IGF-1), bFGF, transforming growth factor- β (TGF-β), and vascular endothelial growth factor (VEGF).

3.1. VEGF

VEGF plays a major role in supporting the healing and growth of vascular endothelium. It also promotes endothelial NO production by upregulating endothelial NO synthase (eNOS) (Hood, Meininger, Ziche, & Granger, 1998). These properties make VEGF an attractive approach to IH prevention, particularly relevant in the early stages of its progression.

3.2. PDGF-bb

PDGF is one of the most notable GFs involved in IH development. It has three isoforms: PDGF-AA, PDGF-AB, and PDGF-BB. The most relevant isoform in the context of IH is PDGF-BB as it activates the PDGF-β receptor (Dandré & Owens, 2004). The effects of PDGF on VSMCs can be attenuated by PDGF-BB antagonists or by blocking the PDGF-β receptor.

Huang et al. reported that PDGF-BB induces VSMC phenotypic modulation through the Hippo- Yes-associated protein (YAP) pathway (Huang, Zhou, & Zheng, 2019). The Hippo pathway is a tumor-suppressor pathway involved in cell survival, proliferation, and apoptosis. Its dysregulation leads to the blockage of cell cycle exit, leading to hyperplasia and differentiation defects, i.e., extensive VSMC proliferation and phenotypic switch in the case of IH. The mevalonate pathway activates Hippo-Yap pathway through the prenylation of a small G-protein RhoA. The blocking of this prenylation reaction is the mechanism by which statins exert their antirestenotic effect (Ferri et al., 2007; Wang et al., 2018).

Protein kinase RNA-like endoplasmic reticulum kinase (PERK) pathway is an emerging target in restenosis prevention. Endoplasmic reticulum stress is a cell response to an overwhelming supply of new proteins and peptides, which require folding and processing, exceeding a cell's capabilities. A cell responds by activating PERK, which triggers the inhibition of protein translation and degradation of misfolded proteins, a response known as unfolded protein response. The inhibition of PERK in PDGF-induced VSMCs abrogates their phenotypic switch (Wang et al., 2020).

3.3. PI3K/Akt/mTOR/p70S6K pathway

A majority of GFs play a role in activating the phosphatidylinositol-3-phosphate kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR)/p70 ribosomal protein S6 kinase (p70S6K) pathway that induces VSMC proliferation, migration, and dedifferentiation (Martin et al., 2004). Blocking this pathway with an mTOR inhibitor, such as sirolimus, leads to cell cycle arrest in the G1-S phase. Furthermore, the PI3K/Akt pathway activates nuclear factor-kappa B (NF-κB), a transcription factor involved in the production of inflammatory cytokines IL-1, IL-6, and IL-8, tumor necrosis factor alpha (TNFα), reactive oxygen species (ROS), and adhesion molecules, such as intracellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM)-1, favorable for vascular remodeling (Mehrhof, Schmidt-Ullrich, Dietz, & Scheidereit, 2005).

3.4. TGF-β-mediated pathways

TGF-β, released by activated platelets, stimulates VSMC proliferation and dedifferentiation and proteoglycan synthesis through Smad-3 signaling by phosphorylating extracellular signal-regulated kinase (ERK)-mitogen-activated protein kinase (MAPK) in injury-induced hyperplasia (Dadlani, Ballinger, Osman, Getachew, & Little, 2008). It also upregulates MMP-2 and MMP-9 levels, contributing to ECM remodeling and VSMC migration. The inhibition of ERK-MAPK hinders the TGF-β-mediated stimulation of VSMC proliferation in culture (Suwanabol et al., 2012).

The onset and development of IH leading to restenosis occurs in successive stages over several months. At each stage, specific actors play a role and contribute to the final re-narrowing of the graft vessel with dramatic consequences for patients. Different pathways involved in this process are well identified, and pharmacological tools are available to prevent or reduce IH.

3.5. PCSK9

The proprotein convertase subtilisin-like kexin type 9 (PCSK9) plays a role in cholesterol metabolism: it destroys low density lipoprotein (LDL) receptors in the liver, controlling plasma LDL levels (Lagace et al., 2006). Several clinical reports suggested a correlation of increased carotid intima-media thickness with PCSK9 gene polymorphism and increased serum PCSK9 levels (Lee et al., 2013; Norata et al., 2010; Ruscica et al., 2017). In rat SMCs with overexpressed PCSK9 a decrease of the contractile phenotype marker and accelerated proliferation have been observed, suggesting the role of PCSK9 in the key features of IH. Moreover, the SMC^{PCSK9} have been partially resistant to antiproliferative and antimigratory effect of simvastatin (Lupo et al., 2021). Mice with a knock-out of PCSK9 and with collar-induced carotid neointimal lesions have had a significantly less intimal thickening compared to $PCSK9^{+/+}$ mice (Ferri et al., 2016). However, Guo et al. have reported opposite results, demonstrating senescence and apoptosis induced in human aortic SMC overexpressing PCSK9 (Guo et al., 2022). Interestingly, two recent case reports on the use of PCSK9 inhibitor show contradicting outcomes with no observed restenosis in case of coronary bypass graft (Abe et al., 2021) and in-stent restenosis in case of coronary artery stenting (Yonezawa et al., 2021). A natural alkaloid berberine is a potent PCSK9 inhibitor and has reduced IH in rat carotid artery injury model by 25% (Lee et al., 2006).

4. Prevention of intimal hyperplasia: Pharmacological approaches

The main events in IH development can be classified into five categories, with drugs specifically interacting at different stages (Fig. 2).

Depending on the drug and type of intervention, medications can be administered either systemically or locally, e.g., endovascularly or perivascularly. Local vascular delivery is an attractive approach for IH

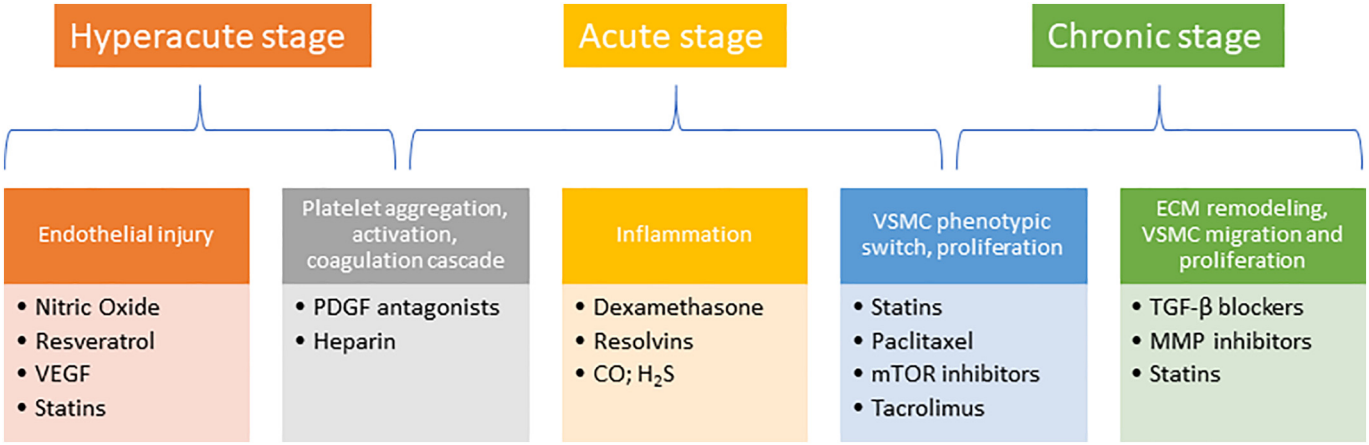

Fig. 2. The stages of intimal hyperplasia with the key pathophysiological events involved in its development. Each step and cellular events are presented with non-exhaustive lists of corresponding therapeutic strategies.
(CO indicates carbon monoxide; H_2S, hydrogen sulfide; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; NO, nitric oxide; PDGF, platelet-derived growth factor; TGF-β, transdermal growth factor-β; VEGF, vascular endothelial growth factor)

prevention, allowing drug application with reduced systemic side effects. Several types of perivascular and endovascular systems with different mechanical properties and release kinetics have been proposed; for review on perivascular systems see (Mylonaki et al., 2017). The active pharmaceutical ingredients (APIs) presented in the review and their effects on IH in animal models are summarized in Table 1.

4.1. Targeting endothelial injury

Healthy endothelium produces NO, well-known for its ability to relax vascular tissues and inhibit VSMC proliferation. Below, we review the therapeutic options that either target endothelial healing or antagonize the subsequent stages of IH by mimicking healthy endothelial activity.

4.1.1. VEGF

VEGF is a selective endothelial cell (EC) mitogen that helps to heal the damaged endothelial layer in IH. The restored endothelium can exert its anti-IH effects through prostacyclins and NO generation.

Luo et al. pre-incubated vein grafts with recombinant human VEGF solution for 20 min before implantation as jugular vein-to-common carotid artery interposition graft and reported a 23% reduction in intimal area in rabbits 28 days later (Luo et al., 1998). However, the perivascular application of VEGF worsened IH by inducing adventitial angiogenesis, providing an incentive to avoid this application of VEGF (Bhardwaj et al., 2005).

4.1.2. NO-generating and -releasing agents

In the vascular system, NO is a product of the metabolization of L-arginine, nicotinamide adenine dinucleotide (NADH), and oxygen, catalyzed by eNOS, which is expressed by the endothelium. In injured arteries, the ability of the endothelium to express eNOS and generate NO from L-arginine is impaired, especially in the early stages of IH development.

IH is driven by NO deficit. Indeed, the effects of NO in an IH setting include the stimulation of EC proliferation, restoration of the endothelial layer, and reduction of inflammatory chemotaxis, which lead to a decrease in VSMC proliferation and migration (Allagnat et al., 2016). This indicates a sound strategy for IH prevention, focused on endothelium rather than or complementary to conventional approaches aiming at smooth muscle cells and antiproliferative action against them.

The challenge associated with this strategy is the instability and short half-life of NO, as it rapidly binds to hemoglobin. Another limitation is that systemic exposure to NO through inhalation and administration of NO donors or agents that favor arginine accumulation through arginase inhibition has a vasodilating action on the entire vascular system, leading to side effects. Once these limitations are overcome, the local delivery of NO-releasing molecules or NO promoters is a reasonable and feasible option.

The local delivery of NO-donors from stents and coated balloons successfully inhibited IH in various animal models. Upon implantation in the pig carotid artery, the expanded polytetrafluoroethylene (ePTFE) stent coated with sodium nitroprusside, an NO donor, reduced neointimal formation by 72% compared to the uncoated control (Hou et al., 2005).

NO donors with extremely short half-life contribute to IH reduction. The periadventitial and endoluminal application of NO donors belonging to the group of diazeniumdiolates, also called NONOates, with a half-life as short as 1.8 s, reduced IH in balloon-injured rat carotid arteries (Havelka et al., 2013). The reduction in IH achieved by the application of NO donors just after vascular injury, despite their short half-life, proves that addressing endothelial healing is critical in the hyperacute stage of IH.

Another way to raise NO levels at the site of potential IH is to upregulate endogenous NO through the transfer of the inducible NO synthase (iNOS) encoding gene to the vascular tissue. Wang et al. performed a local adenoviral iNOS encoding gene transfer with infiltrator delivery catheters to achieve a 37% IH reduction in the stent-injured porcine coronary arteries (Wang et al., 2003).

Several studies recently investigated catalytic NO generation. At least two groups reported the use of polydopamine-selenocystamine coatings for controllable NO generation on stent surface (Wang et al., 2020; Yang et al., 2018). Furthermore, copper ion is another catalyst of NO release from endogenous donors. By grafting copper-loaded nanoparticles on polydopamine-coated stent surface, Fan et al. reported controlled NO generation and IH reduction in rats (Fan et al., 2019). This effect was also reported by Jiang et al. in rabbits (Jiang et al., 2020).

The availability of catalytic technologies for controlled NO generation may allow a better control over IH progression. On the other hand, using NO donors with short half-lives provides a fast, immediate action. Both of these modalities have potential in IH prevention and should be investigated in larger animals.

4.1.3. Resveratrol

Resveratrol, a naturally derived polyphenolic compound found in grapes and red wine, is well known for its vascular protective effects.

Table 1
APIs and their effects on IH reduction in animal models.

Active pharmacological ingredient	Drug administration route/local drug delivery device	Animal injury model	% reduction of intimal area (unless otherwise indicated) compared to drug-free control	P values	Reference
Targeting endothelial injury					
VEGF					
Recombinant human $VEGF_{165}$	Vein conduits immersed in VEGF solution before implantation	Rabbit vein graft jugular to common carotid artery interposition	23%	$p < 0.05$	Luo, Asahara, Tsurumi, Isner, & Symes, 1998
Adenoviral vectors encoding VEGF-A, VEGF-B, VEGF-C, VEGF-$C^{\Delta N\Delta C}$, VEGF-D and VEGF-$D^{\Delta N\Delta C}$	Periadventitial delivery	Rabbit silastic collar carotid artery injury	No effect (VEGF-B, VEGF-C and VEGF-C $C^{\Delta N\Delta C}$)[b] Opposite effect, increase (VEGF-A, VEGF-D and VEGF- $D^{\Delta N\Delta C}$)[b]	$p > 0.05$ $p < 0.05$	Bhardwaj, Roy, Heikura, & Ylä-Herttuala, 2005
NO-generating and -releasing agents					
Sodium nitroprusside	ePTFE stent coated with sodium nitroprusside	Porcine carotid artery	72%	$p < 0.05$	Hou et al., 2005
PROLI/NO	Periadventitial (dry powder)	Rat carotid artery	46%	$p < 0.05$	Havelka et al., 2013
	Endoluminal (solution) via permeable balloon catheter	Rat carotid artery	64%	$p < 0.05$	
Polydopamine-selenocystamine	Endoluminal via stainless steel coated stent	Rabbit iliac artery	54%	$p < 0.05$	Yang et al., 2018
Polydopamine-copper ion	Endoluminal with titianium oxide wires	Rat abdominal aorta	58%[a]	$p < 0.05$	Jiang et al., 2020
	Endoluminal, metal organic frameworks on Ti wires and stainless steel stents	Rat abdominal aorta (wire)	35%[a]	$p < 0.05$	Fan et al., 2019
		Rabbit femoral artery and iliac artery (stent)	28%[a]	$p < 0.05$	
Resveratrol	Periadventitial pluronic gel	Rat carotid artery	42%[a,b]	$p < 0.05$	Zhu et al., 2017
	Oral	Mouse carotid artery	86%[a]	$p < 0.05$	Khandelwal, Hebert, & Dugas, 2010
	Oral	Rat carotid and aortic arteries	50%	$p < 0.05$	Breen et al., 2012
	Resveratrol coated balloon	Porcine peripheral arteries	2%[c]	$p > 0.05$	Speck et al., 2018
		Porcine coronary arteries	9% insignificant opposite effect, increase[c]	$p > 0.05$	
	Resveratrol coated balloon	Porcine peripheral arteries	25% insignificant opposite effect, increase[c]	$p > 0.05$	Kamann et al., 2019
		Porcine coronary arteries	25%[c]	$p > 0.05$	
Targeting platelets aggregation, activation, coagulation cascade					
PDGF antagonists					
PDGFRβ-specific tyrphostin AG-1295	Periadventitial matrix	Rat carotid artery balloon injury model	35%[e]	$p < 0.05$	Fishbein et al., 2000
PDGFR antagonist CT52923	Oral	Rat carotid artery balloon injury model	27%[b]	$p < 0.05$	Yu et al., 2001
Imatinib	Oral	Rat carotid artery balloon injury	89%[a,b]	$p < 0.05$	Makiyama et al., 2008
	Drug coated stent	Porcine coronary artery balloon injury	No significant effect	$p > 0.05$	Hacker, Griffin, Guttormsen, Stoker, & Wolff, 2007
	Drug nanoparticle-eluting stent	Porcine coronary artery stent	40%[a]	$p < 0.05$	Masuda et al., 2011
Heparin					
Heparin	i/v	Baboon peripheral artery balloon injury	No significant effect	$p > 0.05$	Geary, Koyama, Wang, Vergel, & Clowes, 1995
Targeting inflammation					
Resolvins					
RvD2	Intra-luminal infusion	Rabbit femoral artery balloon injury	28%[a]	$p < 0.05$	Miyahara et al., 2013
RvE1	i.p. injection	Mouse femoral artery wire injury	62%[a,b]	$p < 0.05$	Liu et al., 2018
RvD1	Perivascular gel	Rat carotid artery balloon injury	52%	$p < 0.05$	Wu et al., 2017
	Perivascular wrap		45%	$p < 0.05$	
	Perivascular gel	Rabbit vein graft	63%	$p < 0.05$	Wu et al., 2018
	Perivascular film		50%	$p < 0.05$	
	Oral	Rat carotid artery balloon injury	No significant effect	$p > 0.05$	Mottola et al., 2020

(continued on next page)

Table 1 (*continued*)

Active pharmacological ingredient	Drug administration route/local drug delivery device	Animal injury model	% reduction of intimal area (unless otherwise indicated) compared to drug-free control	P values	Reference
CO-, H_2S- generating and releasing formulations					
CO	Inhalation	Porcine iliac artery balloon injury	52%	$p < 0.05$	Raman et al., 2006
		Rat aortic segment transplantation	61%	$p < 0.05$	Otterbein et al., 2003
		Rat balloon injury	74%	$p < 0.05$	
H_2S	Oral	Mouse femoral wire injury	59%[a]	–	Longchamp et al., 2018
	Ex vivo treatment of human vein segments with H_2S releasing gel		61%[a]	$p < 0.05$	Longchamp et al., 2019
Targeting VSMC phenotypic switch, proliferation					
Statins					
Atorvastatin	Perivascular gel	Mouse carotid artery ligation model	68%[b]	$p < 0.05$	Mylonaki et al., 2016
Cerivastatin	Drug eluting stent	Porcine coronary artery stenting	55%	$p < 0.05$	Miyauchi et al., 2008
Simvastatin	Perivascular microparticles	Arteriovenous fistula mouse model	59%	$p < 0.05$	Zhao et al., 2020
PERK inhibitor					
GSK2606414	Endovascular nanocarriers	Rat carotid artery balloon injury	70%[b]	$p < 0.05$	Wang et al., 2020
	Perivascular hydrogel		80%[b]	$p < 0.05$	
mTOR inhibitors					
Rapamycin	i/v nanoparticles	Rat carotid artery balloon injury	88%	$p < 0.05$	Feng et al., 2016
	Perivascular gel	Rat carotid artery balloon injury	80%	$p < 0.05$	Chen et al., 2017
	Adventitial injection	Porcine femoral artery injury	90%	$p < 0.05$	Ang et al., 2020
Tacrolimus					
Tacrolimus	Drug eluting stent	Minipigs coronary artery stent	42%	$p < 0.05$	Prunotto et al., 2009
	Drug eluting stent	Swine coronary artery stent	33%	$p < 0.05$	Hamada et al., 2010
	Intramuscular injections	Venous graft implantation into rat infrarenal aorta	61%	$p < 0.05$	Varga, Matia, Lodererova, & Adamec, 2012
Targeting ECM remodeling, VSMC migration					
TGF-β blocking strategies					
Ribozyme oligonucleotides targeted to TGF-β gene	Incubation of the injured segment with ribozyme solution	Rat carotid artery balloon injury	77%	$p < 0.05$	Yamamoto et al., 2000
Anti-TGF- β1-loaded nanoparticles	Endoluminal catheter delivery	Rat vein graft to ipsilateral carotid artery	57%	–	Sun et al., 2012
Anti-TGF-β adenoviral solution	Intramural injection	Porcine coronary artery percutaneous transluminal angioplasty	44%[a,d]	$p < 0.05$	Kingston et al., 2001
MMP inhibitors					
Doxycycline	Oral	Mouse femoral artery cuff	68%	$p < 0.05$	Lardenoye, 2005
	Oral	Mouse murine vein graft interposition mode	35%	$p < 0.05$	

[a] Value calculated based on Image J software measurement of bar graphs in pixels, if values are not reported in the paper.
[b] I/M ratio.
[c] I + M.
[d] Luminal loss.
[e] Luminal stenosis.

In the vasculature, resveratrol activates eNOS, enhancing NO production by ECs (Breen et al., 2012).

Zhu et al. demonstrated a 42% intima/media ratio reduction 14 days after balloon injury in a rat carotid injury model and confirmed the resveratrol-mediated reversal of VSMC dedifferentiation in vitro (Zhu et al., 2017). The mechanism of action appeared to involve the eNOS pathway as systemically administered resveratrol failed to inhibit IH in eNOS genetically deficient mice or in rats and mice co-administered with eNOS inhibitor (Breen et al., 2012; Khandelwal et al., 2010). Both these studies have shown a significant IH reduction with oral resveratrol administration in wild type rats and mice. However, direct effect on IH was not observed with resveratrol-coated balloons in injured porcine arteries (Speck et al., 2018; Kamann et al., 2019;). The primary interest behind the action of resveratrol on endothelial healing and replacement therapy is its action in the early stages of the disease and its mechanism of action, compensating for impaired endothelium.

4.2. Targeting platelets aggregation, activation, coagulation cascade

4.2.1. PGDF antagonists

Given the important role of PDGF released by activated platelets in IH development, it is an obvious target for IH prevention.

Blocking PDGF signaling with PDGF-β specific inhibitor with sustained perivascular delivery resulted in IH reduction in balloon-injured rats (Fishbein et al., 2000). Yu et al. demonstrated IH inhibition in rats with oral administration of a PDGF receptor antagonist (Yu et al., 2001).

Orally administered imatinib, a non-selective PDGF inhibitor, reduced IH in a balloon-injured rat IH model as well as in hypercholesteraemic rabbits (Makiyama et al., 2008). Hacker et al. confirmed the inhibition of VSMC migration in culture by imatinib, while no EC effect was reported (Hacker et al., 2007). They also investigated the local delivery of imatinib from a polymer-coated stent. They did not observe any impact on IH in a porcine model of arterial injury. This result may be related to the hydrophilic nature of imatinib and its subsequent low local tissue retention and fast washout from the tissues. However, when delivered in a sustained manner from a nanoparticle-coated stent, imatinib successfully inhibited IH in a porcine coronary artery stent model (Masuda et al., 2011), indicating its potential for sustained local delivery.

4.2.2. Heparin

Heparin is an indirect thrombin inhibitor. Thrombin, a potent activator of platelet adhesion, increases VSMC proliferation and inflammatory response through NF-κB activation and decreases EC proliferation (Maruyama et al., 1997). It also stimulates VSMC migration by activating ROS-sensitive p38 MAPK and by upregulating VEGF, a chemoattractant for VSMCs (Wang, Castresana, & Newman, 2004). Indirectly, thrombin increases VSMC proliferation through its effect on platelet activation and subsequent pro-inflammatory and mitogenic effects of activated platelets. Despite the critical role of thrombin in IH development, heparin has not shown promising results in IH inhibition (Geary et al., 1995). Heparin, covalently bound through PEG to an electrospun synthetic graft has prevented platelet deposition. It also showed comparable effect to an autologous artery graft in rabbits, however, the study had no control with the same synthetic graft in the absence of heparin (Zhu et al., 2021).

Systemic antithrombotic therapy in revascularization management is ubiquitously prescribed in a wide range of combinations and doses. However, a local treatment demonstrating clinical outcomes for restenosis patients has not yet been identified (Zavgorodnyaya, Knight, Daley, & Teixeira, 2020). In addition, approaches targeting the release of mitogenic GFs by platelets appear more efficient than standard anti-platelet therapy (Masuda et al., 2011).

4.3. Targeting inflammation

Inflammation is at the center of IH development causally linked to the tissue healing mechanism after surgical injury. In response to cell membrane injury and thrombin upregulation arachidonic acid, is releasedinducing the production of inflammation mediators, including prostaglandins, pro-inflammatory leukotrienes, and TXA_2. In response, leukocytes are recruited to the site of the injury. In the acute stage of IH, leukocytes infiltrate the vascular wall and release pro-inflammatory cytokines and GFs, contributing to the creation of overall stimulatory environment for VSMC proliferation and migration.

4.3.1. Dexamethasone

Before the development of limus drugs and paclitaxel, dexamethasone-eluting stents were used in the clinics. Controversial reports have been published on the effects of dexamethasone on IH from the early laboratory investigations to the patient outcome analyses. For example, the promising results of a swine study with 45% I/M ratio reduction were not confirmed in humans (Hoffmann et al., 2004). Interestingly, a recent DANCE (Dexamethasone to the Adventitia to Enhance Clinical Efficacy after Femoropopliteal Revascularization) trial with perivascular administration of dexamethasone reported its effectiveness and safety in peripheral artery disease (Razavi, Donohoe, D'Agostino Jr., Jaff, & Adams, 2018), suggesting the importance of the perivascular route of administration for an anti-inflammatory approach to IH prevention.

4.3.2. Resolvins

Resolvins are products of the enzymatic oxygenation of omega-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These acids transform into anti-inflammatory, pro-resolving, and cytoprotective lipid mediators: *E*-series resolvins from EPA and D-series resolvins from DHA (Serhan & Petasis, 2011). In addition, there are aspirin-triggered resolvins (AT-Rv), which are synthesized from polyunsaturated fatty acids and aspirin-acetylated cyclooxygenase (COX)-2.

As inflammation became an emerging target in cardiovascular diseases and surgery, resolvins have received attention for their ability to resolve inflammation and promote tissue recovery from injury, as reviewed in (Wu, Mottola, Schaller, Upchurch, & Conte, 2017). As small lipophilic molecules, resolvins have a high aptitude for tissue retention. They have been encapsulated in biodegradable polymer wraps, gels, and films and investigated for their effects on IH in various animal models after local application. In addition to their anti-inflammatory effect, resolvins present anti-migratory effects: PDGF-BB-induced migration was attenuated by resolvins in VSMCs (Liu et al., 2018). Miyahara et al. reported the reduction of IH with locally delivered D-series resolvin in balloon-injured rabbit arteries (Miyahara et al., 2013). Wu et al. demonstrated the inhibition of IH with perivascular delivery of D-series resolvin from poly(lactic-*co*-glycolic acid) (PLGA) film in rat (Wu, Mottola, Chatterjee, et al., 2017) and rabbit (Wu et al., 2018) models of carotid angioplasty. When given orally to rats, low effect was observed on carotid injury-induced IH (Mottola et al., 2020). This is likely linked to the low systemic exposure related to the lipophilicity of resolvins.

In summary, the anti-inflammatory effects of resolvins and their lipophilic nature make them promising candidates for local drug delivery systems for IH prevention. Despite the extensive investigations of resolvins and reports of its effectiveness for IH prevention in animal models, no clinical trials have been conducted to determine the effect of resolvins on patients with a risk of restenosis. Since resolvins have shown promises in clinical trials to fight inflammation, further investigation is warranted to demonstrate the capacity of resolvins to improve vessel patency outcomes after vascular interventions.

4.3.3. Formulations generating and releasing CO and H_2S

CO is an endogenous product of heme degradation. At low concentrations, it modulates inflammatory cytokine production and cell

proliferation, the satellites of IH. Inhaled at low concentrations before carotid artery angioplasty, it reduced IH in rat and porcine models by up to 61% (Otterbein et al., 2003; Raman et al., 2006).

H_2S is another gaseous molecule with protective and anti-inflammatory properties, making it a candidate molecule for IH prevention. To our knowledge, its antirestenotic potential has been studied only by Longchamp et al., who demonstrated the inhibition of IH by an H_2S-releasing peptide hydrogel in human vein segments and a mouse model of femoral artery wire injury (Longchamp et al., 2018; Longchamp et al., 2019).

As inflammation governs some of the central mechanisms underlying neointimal expansion, an anti-inflammatory approach seems an interesting strategy to prevent IH. Despite several mixed reports concerning the efficacy of anti-inflammatory agents, we believe that the success of such a therapy is a question of dose and route of administration. Perivascular delivery has shown great potential in anti-inflammatory restenosis prevention therapy (Longchamp et al., 2018; Razavi et al., 2018; Wu et al., 2018). Thus, perivascular administration route should be the route of choice in future studies and clinical trials evaluating anti-inflammatory drugs.

4.4. Targeting VSMC phenotypic switch and proliferation

4.4.1. Statins

3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, also known as statins, inhibit HMG-CoA reductase. Thus, they block cholesterol synthesis and exert pleiotropic effects by downregulating the nonsteroidal downstream impacts on the mevalonate pathway (Corsini et al., 1999).

An essential advantage of statins for IH prevention is that owing to their pleiotropic mode of action, they cover the disease progression from the hyperacute to the chronic stage. Indeed, statins primarily targeting VSMCs also upregulate NO in the early stages and MMP-induced ECM breakdown during the chronic phase. Statins inhibit VSMC proliferation, phenotypic switch, and migration through prenylation inhibition of the small G-proteins RhoA and Rac1, as recently discovered, by suppressing the Hippo-YAP pathway (Ferri et al., 2007; Wang et al., 2018). Besides, statins downregulate MMPs involved in IH through matrix degradation (Porter & Turner, 2002). Fluvastatin blocked TGF-β-mediated effects on VSMC proliferation and migration by blocking the p38 MAPK pathway (Sakamoto, Murata, Chuma, Hori, & Ozaki, 2005). The inhibition of geranylgeranyl pyrophosphate induces the upregulation of eNOS, favorable for endothelial healing (Kureishi et al., 2000). It would be beneficial to investigate the therapeutic dose window that allows a significant action on VSMC and ECM breakdown while sparing the endothelium. Statins are widely prescribed as oral medication to patients undergoing stenting or vascular grafting. However, to further reduce the impact on endothelium in local drug delivery devices, they may be applied perivascularly rather than endovascularly.

In porcine coronary arteries, cerivastatin-eluting stent reduced IH (Miyauchi et al., 2008). More recently, a perivascular atorvastatin-loaded microparticle-hydrogel delivery system has reduced intimal thickening in a mouse carotid artery ligation model of IH (Mylonaki et al., 2016). Interestingly, the biphasic system combining the fast and slow release of atorvastatin demonstrated a 68% IH reduction in a carotid ligation mice model. In contrast, the controls receiving only fast- or only slow-release formulation did not significantly affect IH. This study demonstrates that to inhibit IH effectively, statins have to act both in the initial hyperacute/acute phase as well as in the long term during at least the first four weeks of the chronic phase. This drug delivery system was later evaluated in a porcine model of arteriovenous patch (Mylonaki et al., 2018). In a larger model, atorvastatin failed to prevent IH, which could be explained by the suboptimal dose in the formulation. Moreover, Zhao et al. showed 59% reduction of neointimal area after periadventitial application of simvastatin-loaded microparticles in an arteriovenous fistula mouse model (Zhao et al., 2020).

To our knowledge, no clinical trial assessing statins for their effect on restenosis has evaluated local application. However, based on preclinical data, convincing reports demonstrating the benefit of IH reduction show that the local administration of statins can be an effective and safe therapeutic option.

4.4.2. PERK inhibitor

Recently, Wang et al. have established the role of PERK in IH and VSMC phenotypic switching (Wang, Xu, et al., 2020). They induced a VSMC phenotypic switch with PDGF-BB and saw a consistent abrogation of this effect on cells pre-treated with a PERK inhibitor, GSK2606414. Furthermore, in vivo, they observed 80% reduction of I/M ratio and rescue of impaired endothelium in balloon-injured rat carotid arteries after perivascular and 70% after endovascular administration of GSK2606414.

4.4.3. Paclitaxel

Cell proliferation plays a crucial role in the progression of IH. The uncontrollable division of VSMCs is the primary driver of intimal thickening, thus the cell cycle has become one of the most 'wanted targets' in IH prevention. The obvious limitation of this approach is the lack of selectivity of these compounds. Indeed, the cell cycle arrest intended to inhibit synthetic VSMCs proliferation is deleterious toward EC, which are crucial for vessel healing.

Paclitaxel, one of the most-used drugs in IH prevention, targets cell division by stabilizing tubulin and inhibiting microtubule formation, arresting the cell cycle in the mitotic phase. When delivered to the vessel wall after endovascular application, it binds to hydrophobic sites in the arterial parenchyma and then diffuses across the arterial walls for weeks, accumulating primarily in the neointimal tissue in solid drug reservoirs (Li et al., 2016). Adverse reactions of paclitaxel, such as infections (Panthier, Warein, Cochennec, Desgranges, & Touma, 2019), have been reported. Furthermore, paclitaxel has recently received a safety warning from the United States Food and Drug Administration (FDA) because of a suspected increase in long-term risk of death (Katsanos et al., 2018). These safety concerns remain to be confirmed to decide the future of paclitaxel in the cardiovascular field. Since then, the real-world analysis of paclitaxel-eluting devices has shown no evidence of mortality associated with it for 11 years (Freisinger et al., 2019), keeping the debate open.

4.4.4. mTOR inhibitors

Inhibition of the mTOR pathway is a conventional strategy to prevent restenosis, as this signaling pathway is central in regulating the replication of mammalian cells. mTOR inhibitors have been investigated and used in clinics for restenosis prevention since the early 2000s. Rapamycin (also known as sirolimus), discovered in Easter Island soil samples, is the first drug of this class. Its analogs, called rapalogs or limus drugs, show improved solubility and aptitude for local delivery. Zotarolimus and biolimus A9 were explicitly designed for drug-eluting stents.

mTOR inhibitors act by associating with the immunophilin FK506 binding protein 12 (FKBP-12) and inhibiting mTOR kinase activity. By interfering with the PI3K/Akt/mTOR/p70S6K pathway, mTOR inhibitors arrest the cell cycle in the G1-S phase and hinder VSMC proliferation, migration, and dedifferentiation (Martin et al., 2004).

The use of mTOR inhibitors in the clinics to prevent restenosis encountered limitations related to the relatively low tissue absorption and short tissue retention times. However, novel drug delivery technologies have emerged to overcome this issue. Several sirolimus-eluting devices have recently received the 'breakthrough device designation' from the United States FDA. In these devices, lipophilic molecules are assembled into amphipathic lipid carriers bound to the balloon surface that stays in the artery wall, releasing the drug in a sustained manner.

Formulations less advanced in terms of clinical application present a great interest and potential. Feng et al. formulated rapamycin nanoparticles, responsive to inflammatory environment markers such as mildly

acidic pH and high ROS concentration (Feng et al., 2016). They observed IH attenuation to a greater extent compared to non-responsive controls after intravenous injection in a rat model of arterial restenosis, supporting systemic delivery of targeted drugs as an alternative to local drug delivery. Rapamycin-loaded nanoporous α-Fe_2O_3 nanotubes on a pure iron surface have demonstrated higher cell viability of ECs compared to VSMCs (Li et al., 2017). This is attributed to the effect of iron ions, essential for oxygen transport and metabolism. A similar observation was reported with rapamycin-loaded polylactic acid (PLA) nanoparticles in vitro, inducing a more pronounced inhibition of human VSMC proliferation than human ECs (Zhao et al., 2018), a feature sought after for IH prevention. Rapamycin micelles attenuated neointima formation in balloon-injured rats for three months after application (Chen et al., 2017). Adventitial injection of rapamycin nanoparticles (Nanolimus) into swine balloon-injured arteries significantly reduced the neointimal area (Ang et al., 2020).

mTOR inhibitors have been the therapeutic strategy of choice, particularly in the coronary field, but their poor tissue retention is still a limitation. New avenues to improve their delivery and pharmacokinetics through novel drug delivery technologies are being explored with convincing results and acknowledgment from the regulatory authorities.

4.4.5. Tacrolimus

Tacrolimus is a macrolide antibiotic, as rapamycin, but not an mTOR inhibitor. This lipophilic molecule triggers different effects downstream to FK binding protein 12. It inhibits VSMC proliferation in vitro less potently than rapamycin and is relatively innocuous toward ECs (Matter et al., 2006), suggesting beneficial selectivity toward VSMCs. With tacrolimus-eluting stents, Hamada and colleagues demonstrated a 33% reduction in the neointimal area in swine (Hamada et al., 2010), and Prunotto et al. reported a 42% reduction in IH in minipigs (Prunotto et al., 2009). Varga et al. reported a 61% reduction in rats with vein grafts on the abdominal aorta (Varga et al., 2012).

Cell cycle arrest in IH prevention is a double-edged sword: it is effective due to the hyperplasic nature of the disease, but, cytotoxic drugs are harmful to the surrounding cells, some of which are crucial for the restoration of tissue homeostasis and vessel healing. However, these drugs are commonly used in the clinics as they are the only registered options for clinicians. With the acceptance that this strategy has gained, it will remain in place for the future. Still, mitotic inhibitors would have to yield the way to more selective drugs such as statins or limus-based devices with improved pharmacokinetic characteristics.

4.5. Targeting ECM degradation, arterial remodeling

4.5.1. TGF-β blocking strategies

In balloon-injured rats, the intravenously injected anti-TGF-β antibodies blocked ECM accumulation in the lesions and reduced intimal area by 33% compared to the control rats (Wolf, Rasmussen, & Ruoslahti, 1994). In contrast, in rabbit vein grafts, it did not influence neointima formation but prevented local thrombogenicity (Kapur et al., 2011), possibly because of the lower dose and less frequent regimen, i.e., biweekly vs. daily in the former study.

Yamamoto and colleagues developed ribozyme oligonucleotides to cleave the RNA encoding the TGF-β gene (Yamamoto et al., 2000). Having incubated the murine catheter-injured vessel segments with liposomes carrying ribozyme, they observed a marked reduction in collagen levels and a 77% reduction of IH compared to the control at 2 weeks. In porcine catheter-injured arteries injected with an anti-TGF-β adenoviral solution, IH was reduced by 44% compared to controls (Kingston et al., 2001). The nanoparticle-delivered antisense TGF-β reduced IH by 57% compared to the control in rat vein grafts (Sun et al., 2012).

4.5.2. MMP inhibitors

During the acute and chronic stages of IH, MMPs responsible for ECM degradation are upregulated, contributing to intimal thickening. Doxycycline, an antibiotic of the tetracycline group, is a non-selective MMP inhibitor potentially useful as an anti-IH drug. Lardenoye et al. reported 81% inhibition of IH in doxycycline-treated veins, 68% reduction in IH in cuffed femoral arteries of mice, and 35% reduction in vein graft thickening with daily doxycycline orally administred in drinking water. They also demonstrated a reduction in vascular MMP expression (Lardenoye et al., 2005). Another group implanted a highly dosed doxycycline-loaded pellet in balloon-injured rats. Interestingly, the vascular MMP did not decrease sufficiently, and they did not observe subsequent significant IH reduction (Mountain et al., 2019). These results may be attributed to the differences in the bioavailability and release of the drug from the implanted pellet compared to the oral administration.

The drugs targeting ECM degradation and arterial remodeling target the later stages of the IH process. Therefore, it may not be successful unless associated with another approach targeting earlier stages of IH. Systemic delivery has been explored but with controversial results; hence, a dose-escalation study would be beneficial. In contrast, with the possibility of local delivery, the strategy to target the chronic stage of IH is challenging, as the device has to stay in place for weeks to months before the drug can find its targets, and the end-point of the study should be carefully selected to allow maximal effectiveness of the drug.

4.6. Single drug strategies summary

Currently, clinicians rely mostly on systemic administration of drugs, such as anti-platelet drugs, anticoagulants, and statins, and local endovascular administration of paclitaxel or rapalogs via drug-eluting stents or balloons. IH develops in consecutive stages, and drugs interfering with each step are available. Many drug candidates have demonstrated outstanding potential; however, inconclusive preclinical results have been reported.

Besides the nature of an active compound, drug developers face multiple challenges, such as delivering the drug at the right place with the right timing. With the currently available technologies for formulating drugs into biodegradable scaffolds with release control, local perivascular administration is an attractive option.

However, as monotherapy may not prevent all IH stages, striking on multiple targets with a combinatorial approach may bring new insight into the development of preventive therapy for restenosis. A combination could address various mechanisms of IH development in parallel and at different stages.

5. Drug combination strategies for the prevention of intimal hyperplasia

IH is a complex multifactorial process resulting from the imbalance between inhibitory and stimulatory factors, inflammation mediators, and disrupted coagulation cascade. Such complexity requires a corresponding pharmacological approach. The drugs described in the first part of this review act on different single pathways: VSMC phenotypic switch, VSMC proliferation, VSMC migration, platelet aggregation, inflammation, leukocyte recruitment, endothelial healing, arterial remodeling. The unique benefit of drug combinations is the possibility of attacking the disease through the multiple pathways presented in Fig. 2. The following section of the manuscript will review drug combinations delivered via intravenous injections, oral gavage, and locally delivered drugs.

The APIs combinations presented below and their effects toward IH in animal models are summarized in Table 2.

5.1. Combinations with growth factors

5.1.1. PDGF-BB and tacrolimus

PDGF-BB induces both EC and VSMC proliferation, while tacrolimus restrains their proliferation, with VSMCs being more sensitive than

Table 2
API combinations and their effects on IH reduction in animal models.

Active pharma-cological ingredient	Drug administration route/local drug delivery device	Animal injury model	% reduction of neointimal area (unless otherwise indicated)	P values	Reference
PDGF-BB and tacrolimus	Permeable balloon catheter	Rabbit carotid artery balloon injury	19% vs tacrolimus 46% vs PDGF-BB 31% vs control[a,b]	$p < 0.05$ combination vs control $p < 0.05$ combination vs tacrolimus	Ma et al., 2017
VEGF and paclitaxel	Nanoparticle coated stent	Mini-swine coronary artery stenting	11% vs VEGF 54% vs paclitaxel 54% vs control[a,d]	$p < 0.05$ combination vs control $p < 0.05$ VEGF vs control	Yang et al., 2013
VEGF and rapamycin	Hierarchical capillary coated stent	Minipigs coronary artery stenting	>1% vs rapamycin 88% vs control[a]	$p < 0.05$ combination vs control	Wang, Xu, et al., 2020
VEGF and imatinib	Imatinib: orogastric sond VEGF: local drug delivery catheter	Rabbit femoral artery injury	54% vs imatinib 40% vs VEGF 55% vs control[a,b]	$p < 0.05$ combination vs control	Leppänen et al., 2004
Imatinib and rapamycin	Oral	Rat iliac artery balloon injury	78% vs rapamycin 81% vs imatinib 81% vs control[c]	$p < 0.05$ combination vs rapamycin $p < 0.05$ combination vs imatinib	Vamvakopoulos et al., 2006
	Oral	Rat aortic denudation injury	63% vs rapamycin 75% vs imatinib 82% vs control	$p < 0.05$ combination vs control $p > 0.05$ imatinib vs control $p < 0.05$ rapamycin vs control	Tigerstedt, Aavik, Lehti, Hayry, & Savolainen-Peltonen, 2009
	Oral	Rat carotid artery balloon injury	5% vs rapamycin 11% vs imatinib >1% vs control	$p > 0.05$ combination vs drugs alone $p > 0.05$ combination vs control	Park, Min, Min, Kim, & Ha, 2015
Rapamycin and heparin	Dual nanoparticle drug-eluting stent	Porcine coronary artery explants injury	78% vs control	$p < 0.05$ combination vs control	Betala, Bae, Langan, LaBerge, & Lee, 2020
Acetylsalicylic acid and paclitaxel	Dual nanofibers drug-eluting stent	Rabbit aorta balloon injury	>95% vs control	–	Lee et al., 2014
Resveratrol and quercetin	Dual drug-eluting nanocomposite stent	Rat carotid artery stenting	64.6% vs control[e]	$p < 0.05$ combination vs control	Kleinedler, Foley, Orchard, & Dugas, 2012
	Oral	Mouse carotid artery injury	94.6% vs control	$p < 0.05$ combination vs control	Khandelwal et al., 2012
Tannic acid and bivalirudin	Drugs grafted on the stainless steel stent	Rabbit iliac artery stenting	66% vs bare stent	$p < 0.05$ combination vs control	Qiu et al., 2021

'Control' refers to drug-free control.
[a] Value calculated based on Image J software measurement of bar graphs in pixels, if values are not reported in the paper.
[b] I/M ratio.
[c] Mean intimal cell count.
[d] Luminal loss.
[e] Luminal stenosis.

ECs (Matter et al., 2006). Ma et al. found a therapeutic window, allowing the promotion of EC proliferation and inhibition of VSMC proliferation with a combination of PDGF-BB and tacrolimus in vitro and in vivo with the common carotid artery injury in rabbits (Ma et al., 2017). The combination showed a higher reduction of the I/M ratio than tacrolimus alone.

5.1.2. VEGF and selenocystamine

Topical delivery of VEGF improved endothelialization and reduced IH in rabbits (Luo et al., 1998). To target endothelial healing, Wang et al. grafted selenocystamine and VEGF on an artificial ePTFE vessel and saw the formation of a complete surface monolayer at day 10 in cell culture with human coronary artery ECs (Wang, Xu, et al., 2020). This in vitro proof of concept deserves further in vivo investigations.

5.1.3. VEGF and paclitaxel

Yang et al. proposed a stent with a dual release system based on nanoparticles loaded with paclitaxel and coated with VEGF plasmid (Yang et al., 2013). They hypothesized that the early release of VEGF encoding gene would promote early re-endothelialization, and the subsequent release of paclitaxel would inhibit VSMC proliferation during 7 days after the surgery, which was confirmed upon implantation in mini-swine coronary arteries; the restenosis rate was reduced by 54% compared to the commercial paclitaxel-eluting stent.

5.1.4. VEGF and rapamycin

Hu et al. developed a multiple drug-eluting stent loaded with these two molecules as well as a platelet membrane glycoprotein IIIa monoclonal antibody to inhibit thrombosis. This device was tested in rabbit carotid arteries (Hu et al., 2017). However, in-stent restenosis was compared to bare stents, and no control was included with either of these agents alone, making it difficult to assess the added value of each formulation constituent. Wang et al. developed a stent coated with rapamycin in the core layer and VEGF in the top layer (Wang, Xue, et al., 2020). The combined delivery promoted endothelial regeneration and reduced neointimal thickness by over 90% compared to the bare-metal stent in porcine coronary arteries, but not significantly compared to rapamycin. The added benefit of VEGF-mediated re-endothelialization has not provided significant results in terms of intimal expansion, making the validity of this combination questionable.

5.1.5. VEGF and imatinib

Combination therapy is not limited to the local administration of multiple drugs. Orally administered imatinib and locally delivered VEGF encoding gene have shown acceleration of re-endothelialization and a 55% (vs. no-treatment control) reduction of the neointimal lesion in balloon-injured rabbits, while neither of the agents alone had a significant effect (Leppänen et al., 2004). Promotion of endothelial healing by VEGF with inhibition of PDGF and its effects on VSMC proliferation had the desired effect of neointima reduction.

The complementary combination of endothelium-healing VEGF with several VSMC-targeting drugs has been beneficial in many studies. In these studies, advanced drug delivery technologies, such as hierarchical multiple-layer coatings, nanoparticles, gels, or combination of oral drug administration with local delivery, were vital to prevent restenosis.

For such combinations to have the expected effect, the GF required for endothelial healing has to be delivered at the right time to allow the endothelial layer restoration, while a VSMC-targeting drug should be delivered once the endothelium is healed.

5.2. Combinations of two drugs

5.2.1. Imatinib and rapamycin

The combination of imatinib and rapamycin is expected to provide a complex effect on IH by inhibiting PDGF activity and blocking mTOR-mediated cell replication. At least three groups have investigated this combination after oral administration with contradicting conclusions. Tigerstedt et al. and Vamvakopoulos et al. reported a synergistic IH reduction (Tigerstedt et al., 2009; Vamvakopoulos et al., 2006), while Park et al. reported the failure of this therapy in rats, which may be explained by a 2-week delay between injury and drug administration (Park et al., 2015). As a PDGF inhibitor, imatinib may have been more beneficial in the first hours and days after the surgery. Testing this hypothesis with controlled drug release kinetics could provide more insights into the synergistic mechanisms.

5.2.2. Rapamycin and heparin

While rapamycin acts directly to block VSMC proliferation and phenotypic switch, heparin inhibits the generation and mitogenic effects of thrombin. Su et al. developed dual heparin-rapamycin-eluting stents (Su, Chen, & Chen, 2013). In a recent study, the effect of polymeric nanocarriers with dual drug delivery of rapamycin and heparin was tested on cultured rat aortic VSMC and ex vivo on balloon-injured porcine coronary artery segments. The group reported a reduction in VSMC proliferation and collagen deposition and an increase in the patency of arteries treated with heparin and rapamycin. However, there were no single drug controls (Betala et al., 2020).

5.2.3. Aspirin and paclitaxel

While paclitaxel attenuates VSMC proliferation, the role of aspirin in this combination is to limit thrombosis. Aspirin- and paclitaxel-loaded biodegradable polymeric stents with sequential sustained release of aspirin and paclitaxel for more than 1 and 2 months, respectively, were prepared (Lee et al., 2014). The drug combination increased EC development and reduced VSMC proliferation in vitro and in vivo in balloon-injured rabbits; the dual drug-loaded stent reduced IH and promoted endothelial recovery compared to the bare stent, although there was no comparison with either of the drugs alone.

5.2.4. Resveratrol and quercetin

Quercetin is a plant polyphenol with numerous properties, including anti-inflammatory and anti-atherosclerotic activities (Deng, Li, Fang, Chen, & Xue, 2020). Both resveratrol and quercetin inhibit VSMC proliferation, platelet aggregation, and promote EC function (Deng et al., 2020; Zhu et al., 2017). Kleinedler et al. have investigated this combination on coated stents placed in rat carotid arteries (Kleinedler et al., 2012). Unfortunately, the only control was a bare-metal stent, and there was no control with resveratrol and quercetin alone. Nevertheless, the authors claimed a synergistic effect based on their previous in vitro findings (Kleinedler et al., 2011). The same group has tested oral administration of this combination in mouse carotid injury model and demonstrated a more-than-additive combined effect compared to the drugs alone (Khandelwal et al., 2012).

5.2.5. Tannic acid and bivalirudin

Tannic acid, another plant polyphenol, has five times more phenolic hydroxyl groups than quercetin, providing a higher degree of antioxidant properties, as well as a high aptitude for adhesion to various materials (Andrade et al., 2005). Grafted to the surface of a stainless steel vascular stent, it provides binding sites for linking a thrombin inhibitor, bivalirudin. The coated stent had anti-inflammatory and antithrombotic properties, facilitating endothelialization and reducing thrombi formation. Implanted in rabbit iliac arteries, the stent reduced the neointimal area by 66% compared to a bare stent (Qiu et al., 2021).

5.2.6. Atorvastatin and fenofibrate

Both atorvastatin and fenofibrate are lipid-lowering drugs. Their use in combination has been studied extensively in systemic drug delivery, with beneficial effects on vascular endothelial function (Koh et al., 2005). In studies with human VSMC and human umbilical vein endothelial cells (HUVECs), a 1:1 ratio of atorvastatin and fenofibrate was identified as safe for EC but with slightly reduced VSMC viability

(Roopmani & Krishnan, 2018). The combination had a more pronounced anti-inflammatory action on macrophage activation and platelet adhesion than the individual drugs. The presence of fenofibrate allowed to reduce the atorvastatin dose to spare the endothelium while keeping the VSMC proliferation low. Testing this combination in animals would be the next logical step for in vivo proof of concept.

5.3. Combination strategies summary

In summary, combination therapy reports are encouraging, showing a dual action of the administered drugs and demonstrating the possibility of decreasing drug concentration, thereby reducing adverse effects. Some drug combinations can play a double role as APIs and as coating excipients or linkers for other drugs, which is an attractive and feasible strategy for endovascular stents (Qiu et al., 2021).

The literature about drug combinations is relatively poor compared to monotherapies. Some of these reports compare the advantage of the combination to the absence of treatment and not to the drugs alone (Kleinedler et al., 2012); thus, it is difficult to clearly assess the benefit of having one or the other drug/growth factor in the combination. Some of the reports reviewed above have shown efficient IH prevention; particularly of interest are the temporally-coordinated release of GF to favor endothelial healing and cell cycle-affecting drugs targeting VSMC proliferation (Leppänen et al., 2004; Yang et al., 2013), which provides an incentive to combine drugs with GFs. Another noteworthy combination includes one active compound and two release profiles: initial burst release and sustained release over four weeks, which can be another approach to combination therapy with multifaceted drugs that tackle IH at different stages (Mylonaki et al., 2016). Despite regulatory complications associated with combination therapy, it has demonstrated a potential to improve monotherapy outcomes and needs to be pursued further.

6. Conclusions and perspectives

Rethinking the concepts for preventing IH and subsequent restenosis is a much-needed exercise. The complexity of IH can be approached from different angles, using drugs that selectively target cells and GFs rather than antimitotic agents that do not distinguish between ECs and VSMCs. Another essential aspect is the localized delivery of the drug(s), which may dispense them right to the vascular/diseased/pathological site at an effective dose to prevent restenosis without eliciting adverse effects.

One approach that could improve post-surgery and post-angioplasty outcomes is combination therapy. As this strategy is gaining popularity, there are more and more reports of successful combination therapy for IH prevention in the laboratory setting, but these devices have not yet made it to the clinically approved use in humans.

Considering the available possibilities of formulation and modulation of drug release kinetics, for instance, using biodegradable materials, local administration of active ingredients, alone or in combination, has significant therapeutic potential for the effective control of the IH and improvement of vascular intervention outcomes.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

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