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Pro-resolving lipid mediators in the resolution of neointimal hyperplasia pathogenesis of in atherosclerotic diseases

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Abstract

Introduction: Despite advances in drug eluting technologies, neointimal hyperplasia (NIH) and restenosis still plagues endovascular therapy in atherosclerotic diseases. By appreciating atherosclerosis and NIH as complex inflammatory processes, specialized pro-resolving mediators (SPMs) are a superfamily of endogenous unsaturated fatty-acid derived lipids with the potential for inflammatory resolution.

Areas covered: Inquiry into SPMs in this context is a novel approach and is the focus of this review, with emphasis on our understanding with NIH. Prior mechanistic understandings of SPM deficiency with atherosclerosis has offered insight, as well as the complexity and diversity of the SPM superfamily. Therapeutic investigation using SPMs to combat NIH is also evaluated here.

Expert commentary: Endogenous deficiency of SPMs synthesis by 12/15-lipoxygenase underlies resolution deficits in atherosclerosis and NIH. Upstream PDGF inhibition by SPMs, most notably RvD1 and LXA4, confers a multifactorial attenuation of NIH that involves interconnected anti-inflammatory efforts, most notably switch pro-resolving smooth muscle cells (vSMCs) and macrophages. The ALX/ FPR2 is one receptor system identified on vSMCs that interacts with these SPMs to promote NIH resolution. Therapeutically, while shown to be promising with less stent burden or cytotoxicity, SPMs must be balanced by necessary mechanistic, pharmacokinetic and anatomical considerations.

Keywords

Atherosclerosis; Inflammation; Neointimal hyperplasia; Pro-resolving mediators; Resolvins; Lipoxins; Protectins

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1. Introduction

Atherosclerosis is a chronic inflammatory process that is most susceptible to occurrence in the intimal layer of arteries, particularly at bifurcation points of the blood vessel. The earliest events of atherosclerosis include activation of the endothelium by certain risk factors such as hypercholesterolemia.[1] In general, in atherosclerotic arteries, the clinical symptoms develop primarily due to two events: (a) The prototypical plaque formation that ensues within the inflammatory milieu results from oxidized lipids creating foam cells, a macrophage derivative.[3] Plaque instability and rupture are attributed to a weakening of its fibrous cap due to matrix degradation by further macrophage secretions (cytokines, chemokines, growth-factors, and disintegrins).[3–4] Subsequent vessel thrombosis can promote symptomatic stenosis or occlusion underlying the ischemic events seen in stroke of the brain, reduced coronary perfusion of the heart in patients with coronary artery disease (CAD), and limb claudication seen in peripheral arterial disease (PAD).[1], and (b) Following mechanical injury to the endothelium that commonly occur during interventional procedures, including balloon angioplasty and intravascular stenting, circulating leukocytes, monocytes, and T-lymphocytes attach and infiltrate the intima and release mediators to promote smooth muscle cell (SMC) migration towards lumen with fibrous cap formation, resulting in the development of neointimal hyperplasia and restenosis.[1–2]

2. Current therapeutic options to prevent neointimal hyperplasia

In the realm of atherosclerotic sequelae across various vascular beds, endovascular revascularization remains a viable option with minimally invasive options amongst medical management. The earliest forms of such intervention started in the 1970s and included angioplasty, where obligatory vessel injury is utilized to obliterate lesions and improve vessel patency with endothelial denudation.[5–6] However, employment of angioplasty alone can promote acute closure of vessels due to elastic recoil and post-injury endothelial denudation and sub-endothelial matrix exposure leading to platelet aggregation and infiltration of circulating cells into the intima.[7–8] Additionally, further closure is mediated by neointimal hyperplasia (NIH) secondary to balloon insult, an additional inflammatory event that lead to excess scar tissue growth and restenosis secondary to proliferation of vascular smooth muscle cells (Figure 1)[9]. In coronary intervention, issues with both acute recoil and dissection issues with angioplasty alone have been moderately alleviated with the development and improvement of stent devices. Drug-eluting stents are coated with paclitaxel, which disrupts microtubule organization, or sirolimus, which inhibits mammalian target of rapamycin (mTOR) for cell cycle arrest, with the release of these drugs controlling cell proliferation, intimal hyperplasia, and restenosis at the site of stent deployment.[6,10] However, delayed NIH remains an issue despite these advances in anti-proliferative and anti-inflammatory effects. In peripheral revascularization, the durability of stenting is even shorter and complicated by in-stent restenosis, with mixed evidence regarding the equivalency of femoropopliteal stenting to bypass in long-term patency outcomes.[11,12] In addition, peripheral revascularization is complicated by mechanical stress and torsion of the arteries in the lower limbs, illuminating the particular necessity of improved balloon intervention with drug-coated technology.[13] Nevertheless, paclitaxel-based drug coated balloons (DCBs) have revolutionized femoropopliteal revascularization in peripheral arterial

disease (PAD) with long-term patency sustained at three years post-intervention, highlighting the yield of an anti-proliferative target.[14] Lastly, in-stent restenosis and NIH also plague those with cerebrovascular atherosclerotic disease undergoing carotid artery angioplasty with stenting, despite long-term follow-up at ten years for ischemic outcomes being equivalent to carotid endarterectomy.[15,16] Likewise, the advent and application of drug eluting techniques to limit restenosis in the carotid space are the least developed.

3. Pro-resolving lipid mediators: biosynthesis and subclasses

Given both the disease burden that atherosclerosis can ensue and the challenges of endovascular intervention with NIH, promotion of endogenous anti-inflammatory mediators is of therapeutic interest. By appreciating the link between an inflammatory state with both atherosclerosis and NIH, inquiry into the inflammation resolution program has identified a series of molecular and cellular effectors. One superfamily of interest are unsaturated fatty-acid derived lipid mediators referred to as specialized pro-resolving mediators (SPMs).[17] With earlier evidence of the localization of SPMs within human arterial cells, endogenous biosynthesis is regulated by lipoxygenases and cyclooxygenases that utilize polyunsaturated fatty acid precursors derived from omega-6 and omega-3 fatty acids.[18] These SPMs are further subdivided into four major classes of activators of potentially G-protein coupled receptors which include the lipoxins (LX), resolvins (Rv), protectins (P), and maresins (Mar), each of which attenuates inflammation and facilitates tissue repair.[17, 19,20] While the role of these SPMs with both atherosclerosis and NIH is not fully defined, exogenous delivery of these mediators and inquiry into endogenous mechanisms have uncovered few clues to their therapeutic potential. This review summarizes and critically evaluates the preclinical and interventional findings in attempts to elucidate the role of SPMs and their targeted mechanisms in atherosclerotic disease with a particular focus on NIH.

4. SPMs and atherosclerosis

4.1 SPM deficiency and the resolution deficit

The earliest evidence for the role of SPMs with atherosclerosis was found in CAD patients undergoing percutaneous transluminal angioplasty, which showed intracoronary release of peptidoleukotrienes and lipoxin A4 (LXA4) distal to the plaque site and after plaque rupture .[21] Additionally, it was found that patients undergoing aspirin (ASA) therapy had an increase in the appearance of these compounds. However, aspirin-induction of lipoxin levels has been shown to be reduced in CAD and PAD patients, highlighting a resolution deficit in counter-regulating platelet-derived growth factor (PDGF)-stimulated chemotaxis of SMCs. [22,23] The hypothesis that atherosclerosis results from an inflammatory resolution failure is further supported in animal models that emphasize a pertinent deficiency in local 12/15-lipoxygenase (12/15-LOX) pathways to upregulate LXA4, resolving D1 (RvD1), and protectin D1 (PD1) via oxygenation of free-form and complex fatty acid assemblies.[24,25] Conversely, it was shown prior to this that overexpression of 15-LOX and LXA4 dampened neutrophil-mediated inflammation with respect to bone and tissue loss in periodontitis and arthritis.[26] Importantly, as indicated by Sansbury et al. [27], mere supplementation of omega-3 polyunsaturated fatty acids (a substrate of SPMs) has failed to show improvement in

cardiovascular end points, indicating the possibility of altered downstreat mechanisms (e.g. SPM upregulation).

4.2 Dichotomy of the 12/15-LOX pathway and association with 5-LOX

Unlike the predominantly established pro-atherogenic mechanisms of the 5-LOX pathway, evolution of these findings within the 12/15-LOX pathway have led to the postulation that its activation can confer protective vascular effects via PPARγ with increased nitric oxide (NO) production and reduction in IL-12p40.[25, 28] However, deletion of 12/15-LOX has also been shown to promote atherosclerosis, with specific evidence that increased 15-LOX-2 (an isoform of 15-LOX) expression is seen in human monocyte to macrophage differentiation.[29] Nevertheless, with an array of metabolites exist for 12/15-LOX pathway, catalytic action may be attributed to be tissue- and species-specific.[30] Additionally varying cell types offer a different intracellular redox state that would preferentially result in a pro-atherogenic state or an anti-inflammatory state. For example, evidence exists for 15-LOX-2 products in macrophages within human carotid plaques with increased hypoxia.[31] In coronary atherosclerosis, heterozygote carriers with a null T560M allele in 15-LOX-1 had an increased risk, highlighting a genetic polymorphic component as well.[32] Furthermore, a sequential action of LOX isoforms has been identified, such as 15-LOX with 12-LOX or 5-LOX, and may allow for bypass mechanisms that should be elucidated and targeted to explain atherogenic or anti-inflammatory effects towards atherosclerosis.[30, 33–34] The common substrate for lipoxygenases is arachidonic acid (AA), where 5-LOX alone can create leukotriene A4 (LTA4) from the transformation of AA.[35] Inquiry into the mechanism of macrophages promoting inflammatory plaque progression has shown that nuclear 5-LOX may upregulate a bypass via LTA4 hydrolase.[36.37] Contrary to this and utilizing the same AA substrate, the sequential action of 5-LOX in association with 12/15-LOX creates anti-inflammatory lipoxins, a type of SPM. To this effort, non-nuclear 5-LOX is seen to localize near 12/15-LOX, facilitating the conversion of arachidonic acid to SPMs potentially via an efferocytosis receptor, MerTK.[38,39]

4.3 Plaque stability: central role of LOX-produced SPMs in atherosclerosis

The specific importance of SPMs produced by the LOX pathways primarily centers around plaque stability and underlies the therapeutic potential of SPMs in atherosclerosis. In addition to SPM deficiency conferring a resolution deficit that promotes plaque formation, the vulnerability to subsequent plaque rupture was identified in human carotid plaques where the ratio of RvD1 to LTB4 was significantly decreased.[40] Similarly, in aortic lipid mediator profiling of *ApoE* −/− mice, increased leukotriene B4 (LTB4) and prostaglandin E2 (PGE2) and decreased SPM levels (RvD2 and Mar1) were associated with increasing vulnerability of the plaque rupture.[41] To appreciate this to be secondary to the resolution deficit, RvD2 and Mar1 were shown to shift the macrophage profile to secondarily stimulate collagen synthesis in SMCs and inhibit expansion of the necrotic core in the plaque. Interestingly in this same study, therapeutic delivery of the two SPM types was successful in halting advanced atherosclerosis. Considering the atherogenic potential of nuclear 5-LOX alone, further inquiry into advanced plaque prevention showed that RvD1 is reciprocally capable of preventing nuclear localization of 5-LOX to promote SPM synthesis in macrophages and inhibit LTB4.[38] Likewise, RvD1 administration in fat-fed *Ldlr* −/− mice

improved the RvD1:LTB4 ratio to that of less advanced plaques, with greater stability.[40] The lipoxin subset of the SPM family has also been shown to have importance with plaque stability. Aspirin-triggered LXA4 (ATL) is seen to provide athero-protection via stimulation of formyl peptide receptor 2 (FPR2) receptor, reducing macrophage and apoptotic cell infiltration to halt advanced plaque necrosis.[42]

Taken together, the importance of SPMs in a resolution deficit with atherosclerotic disease is well-supported in both animal and human studies. While the synthesis of SPMs via LOX pathways is critical, the diversity and sequence activation of LOX isoforms with plaque stability or atherogenic process must be elucidated individually. Additionally, changes in the microenvironment and genetic implications may play a role. Nevertheless, the 12/15-LOX pathway with nuclear exclusion of 5-LOX is proven to be important. Lastly, future therapeutic application of SPMs therapeutically in atherosclerosis may be two-fold, revolving around addressing both the formation and stability of plaques. However, considering both aspects, the mechanisms of consumption or degradation of SPMs in those that are deficient must be further considered and may emphasize targets specific to a LOX isoform. The consideration of plaque regression with SPMs is also an area of interest.

5. SPMs and neointimal hyperplasia (NIH)

5.1 Pathophysiology of NIH

The consequences of an unhindered atherosclerotic plaque instability can lead to acute thrombotic events seen in various vascular pathologies. As discussed above, an endovascular approach with vessel injury is often attempted for revascularization but is currently complicated in the long-term due to the development of NIH. NIH involves fibroblast and SMC proliferation in the intima layer of arteries and veins with deposition of extracellular matrix (ECM).[43] The thickened intimal layer reduces the luminal area of the vessel. A phenotypic switch in SMC type from a differentiated contractile type to a dedifferentiated high proliferation type is believed to underlie the expansion of NIH.[44] The phenotypic switch is associated with increased migration activity, proteolytic activity, and decreased levels of contractile and cytoskeletal proteins. However, the pathways and factors involved in this switch are less well known, with platelet derived growth factor-BB (PDGF-BB) and basic fibroblast growth factor (bFGF) believed to play a multifactorial role.[45] The progression of migration and differentiation of these dedifferentiated SMC in NIH is perpetuated by downstream signal transduction, involving the Ras-MAPK and PI3K-Akt pathways.[46] Downstream activation of NF-kB by MAPK signaling has previously been implicated in NIH with recent evidence of attenuated NF-kB activity and decreased medial cell proliferation upon exogenous delivery of another SPM subclass, protectins (specifically PD1).[47–49] However, these signaling cascades also promote ECM synthesis, the second component of the vascular remodeling that occurs with NIH. ECM remodeling, as seen in the MMP/TIMP system, involves a harmony between degradation and synthesis, the former of which involves matrix metalloproteinases (MMPs) and the latter of which involves MMP tissue inhibitors.[50] These components of NIH physiology are summarized in a simplified theoretical sequence (Figure 1). However, while the pathophysiology of NIH continues to be elucidated, therapeutic considerations of combating NIH might not involve a single

downstream target, a belief indicated earlier by Collins et al. with the failure of the PREVENT trials.[43] Secondary induction of NIH may only be bypassed by upstream targets, such as prevention of phenotypic switching.

5.2 ALX/FPR2-mediated signaling with SPMs

The developing role of SPMs with atherosclerosis previewed its therapeutic potential lying upstream in the pathophysiology of the closely related process of NIH after vascular insult. The earliest inquiry into this topic stemmed from the detection of the A type LX (ALX) receptor/ FPR2 as a receptor not only for LXA4 and ChemR23 as a receptor for RvE1 has been identified in human vascular SMCs (vSMCs).[22] Within these findings in human vSMCs related to peripheral arterial atherosclerosis, the catalytic action of LXA4 and RvE1 attenuated PDGF-stimulated vSMC migration and PDGF receptor phosphorylation. However, it was uncertain whether differential expression of LXA4 and RvE1 receptors mediates this process. The first inquiry of these findings with NIH involved a rabbit model with *in vivo* arterial angioplasty insult.[51] Upregulation of D-series resolvins (RvD1 and RvD2) exhibited a dose-dependent inhibition of vSMCs proliferation and migration, the culminating events of NIH. Additionally, increased expression levels of these SPMs after vessel injury highlighted endogenous biosynthetic pathways with its receptor presence known but poorly localized. Furthermore, successful attenuation of NIH with the exogenous delivery of RvD2 provided the earliest evidence for SPM based therapy. Subsequent to this, and after atheroprotective findings of ATLs via FPR stimulation were found, ATL signaling through the ALX/FPR2 receptor in vSMCs was explored after carotid artery ligation in a mouse model.[52] It was seen that vSMCs had a significantly higher rate of proliferation in ALX/FPR2 knockout (KO) compared to wild type (WT) mice. Additionally, *in vitro* and *in vivo* administration of ATL to the ALX/FPR2 KO vSMCs did not alleviate this, emphasizing the role of the ALX/FPR2 receptor for ATL inhibition of PDGF-induced migration. Both of these initial studies with SPMs in NIH provides strong evidence for the upstream involvement of the ALX/FPR2 signaling in vSMCs but is best evidenced with RvD1 and LXA4.

5.3 SPM-induced phenotypic switch in macrophages and vSMC

Further inquiry into other SPM subtypes showed success with the exogenous delivery of maresin as an additional therapeutic agent in attenuating NIH.[53] In an *in vivo* mouse model with carotid artery ligation, omega-3 polyunsaturated fatty acid-derived SPMs (Mar1 and RvD2) significantly inhibited NIH compared to vehicle via decreased cell proliferation, decreased neutrophil and macrophage recruitment, and increased polarization of M2 macrophages. The latter component augments the resolution phase of the inflammation with NIH by switching the dominant phenotype of macrophages from a pro-inflammatory state (M1) to a pro-resolving state (M2), a process that requires decreased PMN infiltration.[17] Likewise, the ability for Mar1 and RvD2 to enable this phenotypic switch in macrophages was the first evidence of its kind in vascular injury and NIH resolution. Evidence for a SPM-induced macrophage phenotype switching to M2 is provided in a most recent study by Liu et al., where exogenous and endogenous RvE1substantially reduced NIH after femoral artery injury in mice.[54] Similarly, the phenotypic switch occurred in the background of diminished vascular PMN infiltration and vSMC migration. Interestingly, Liu et al.[54] also found that

RvE1 attenuated T-cell trafficking by reducing chemokine [C-C motif] ligand 5 (CCL5) secretion from vSMC, thereby reducing leukocyte recruitment. In the *in vitro* part of the study by Akagi et al. [53], Mar1 and RvD2 treatment inhibited SMC migration secondary to a PDGF gradient and reduced responsiveness to TNF-α, facilitating both beneficial alterations in leukocyte-vessel wall interactions and the phenotypic switch to vSMC with reduced migratory capability. Nevertheless, unlike the elucidation of the RvD1 and LXA4 receptor (ALX/FPR2), ChemR23 for RvE1, and SRV2/GPR18 for RvD2,[55] the receptor type is unclear for Mar, although a GPCR may be speculated to be the type. Furthermore, linking these elucidated receptors with the attenuation of PDGF signaling requires further evaluation of these downstream mechanisms.

5.4 Pharmacokinetics of potential SPM therapeutics in NIH

With some success with the exogenous delivery of SPMs to attenuate NIH in animal models, consideration is also given to the pharmacokinetic challenge of drug elution kinetics that has also complicated current treatment options in vascular disease. In addition to further investigating the role of RvD1 with NIH, Wu et al. [56] were the first to evaluate the safety and efficacy of perivascular delivery of SPMs (RvD1) through thin biodegradable three-layered poly (lactic-co-glycolic-acid) (PLGA) wraps or 25% Pluronic F127 gels in a rat model with carotid angioplasty. The therapeutic rationale of these delivery mediums is the ability for biodegradability in the case of PLGA, or the novelty of the Pluronic gels from proof-of-concept studies.[57–58] Consistent with a previous study discussed here, exogenous RvD1 was shown to attenuate NIH processes of proliferation, migration, and ECM deposition via PDGF inhibition after carotid vessel insult without any hazardous cytotoxic evidence. However, reduced leukocyte recruitment was not seen as noted in the previous studies that attributed macrophage phenotype switching to it. Likewise, the delivery apparatus is believed to have had a role in this. A similar reduction in NIH was seen with both the gel and the PLGA wrap with no thrombotic or infectious events noted, or death. This safety profile demonstrates a useful property of SPM therapeutics along with having no cytotoxicity. Despite equivalent evidence, it is speculated that the PLGA wrap may be more suitable for translation to NIH attenuation in larger animals or humans due to prolonged kinetics and is less sensitive to temperature and positional sensitivity similar to Pluronic gels. Furthermore, previous evidence with a paclitaxel-loaded PLGA device was troubled by high infection rates, an issue Wu et al. did not see with their RvD1 apparatus.[59] Finally, a therapeutic option that can be delivered via a biodegradable device like the PLGA wrap would also address the issue of in-stent restenosis that currently plagues stenting in vascular disease, in addition to stent fracture.

6. Expert commentary

The pathophysiology of NIH is complex, involving multiple signaling pathways, phenotypic switching of vSMCs, and matrix deposition. However, upstream PDGF inhibition was consistently demonstrated in elucidating a multifactorial therapeutic potential of SPMs in the preliminary animal studies discussed here. Furthermore, the endogenous or exogenous involvement of SPMs in the resolution of NIH through PDGF inhibition, is mostly mediated by GPCRs in vSMCs. The best understood one to date is the ALX/FPR signaling via RvD1

and LXA4, which facilitated decreased leukocyte and macrophage recruitment, decreased proliferation, and a switch in macrophages and vSMCs to a proresolving phenotype. Additionally, further anti-inflammatory efforts identified in these studies included diminished TNF-α sensitivity, NF-kB activity, and CCL5 secretion, which all may be SPM amplifiers of NIH resolution. A proposed early mechanism that incorporates these parts is provided (Figure 2). While other SPMs showed evidence for these effects in NIH, such as other resolvin types, maresins and protectins, their direct interactions with PDGF are more poorly understood.

7. Five-year view

It will be pertinent to further characterize SPM receptor types to optimize pharmacokinetics with exogenous delivery of SPMs. Likewise, understanding differential expression for SPM receptors, such as ALX/FPR2 receptor along the arterial wall will be a pertinent therapeutic consideration in deciding exogenous delivery mechanisms with excipients. Mechanistically, understanding the mode of signaling (ex. paracrine or autocrine) would facilitate understanding of how an endogenous SPM balance is kept. These practical considerations to the therapeutic application of SPMs warrants even further investigation given the benefits described. The first study of its kind discussed here is promising for delivery mechanisms that have less vessel burden and cytotoxicity. However, long term patency remains the primary goal with any SPMs or combination of SPMs, thus requiring considerations in addition to a delivery vehicle such as PLGA. Finally, our understanding of the importance of SPMs in a resolution deficit with atherosclerosis offers additional diagnostic and therapeutic routes in the context of endogenous SPM biosynthesis through the 12/15-LOX pathway with nuclear exclusion of 5-LOX. Evidence of the sequence activation described presents an area of additional inquiry into their biosynthesis with dual properties that are both anti-inflammatory and atherogenic. Diagnostically, the RvD1:LTB4 ratio may serve to show an underlying deficit in biosynthesis and SPM resolution. However, the feasibility of assessing such a biomarker is not well-understood. It should be stressed from the varying success of vascular intervention in relation to the type of vascular bed (e.g. peripheral vs. coronary), that macro- and microenvironments and vessel bed differences are important considerations as well with further inquiry of SPMs. The interaction of even the same subclass of SPMs within different vessel bed microenvironments may confer a differing response that could also have a genomic determinant underlying the varied response, as appreciated in atherosclerotic processes.[60]

Thus, the elucidation of the endogenous role of SPMs in atherosclerotic disease has offered valuable insight into NIH, an intimately related process stemming from its treatment. The therapeutic investigation of SPMs is a worthy endeavor in NIH, but their biosynthetic and resolution events require further evaluation. Likewise, the exogenous delivery of SPM treatment may offer an efficacious but also safe way to address atherosclerotic vascular disease.

8. Key issues

- Atherosclerosis is a chronic inflammatory process that is most susceptible to occur in the intimal layer of arteries, particularly at the bifurcation points of the blood vessel.
- In the realm of atherosclerotic sequelae across various vascular beds, endovascular revascularization is complicated by the development of NIH and restenosis.
- One superfamily of endogenous mediators for resolution of inflammation is unsaturated fatty-acid derived lipid mediators referred to as specialized pro-resolving mediators, or SPMs.
- Atherosclerosis results from inflammatory resolution failure from deficiency in local 12/15-lipoxygenase (12/15-LOX) pathways that upregulate SPMs.
- Non-nuclear 5-LOX is seen to localize near 12/15-LOX, facilitating the conversion of arachidonic acid to SPMs. Other LOX isoforms may exist.
- Importance of SPMs produced by the LOX pathways centers around plaque stability in atherosclerosis.
- NIH involves fibroblast and smooth muscle cell (SMC) proliferation in the intimal layer of arteries and veins with deposition of extracellular matrix (ECM). The thickened intimal layer reduces the luminal area of the vessel.
- A phenotypic switch in SMC type from a differentiated contractile type to a dedifferentiated high proliferation type is believed to underlie the expansion of NIH.
- Several signal transduction pathways may be implicated in NIH and a sole target may not be sufficient, but PDGF signaling is well described.
- Detection of the ALX/FPR2 as a receptor not only for LXA4, but also for RvD1 in human vSMCs stemmed inquiry into SPM involvement with NIH.
- Each of these SPM receptor types (potentially all GPCRs) may be modulated by PDGF phosphorylation but are not fully investigated like ALX/FPR2.
- SPMs inhibits NIH via decreased cell proliferation, decreased leukocyte, and increased polarization to M2 macrophages.
- Therapeutic delivery mechanisms of SPMs may offer less vessel burden (vs. intravascular stenting) and less cytotoxicity. However, long-term vessel patency with SPMs is not understood.

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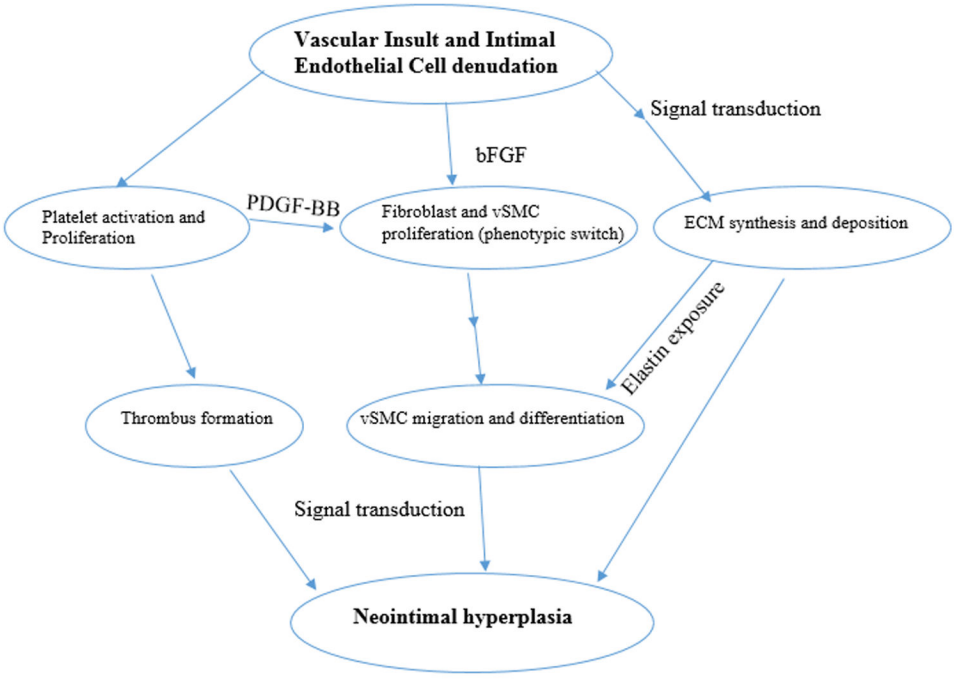

Figure 1: Event sequence in the pathophysiology of neointimal hyperplasia (NIH):

Therapeutic insult creates obligatory vessel injury and inflammation, which activates platelet. Recruited platelets induce thrombus formation and release PDGF-BB, which promotes fibroblast proliferation in addition to a downstream switch in vSMC to the dedifferentiated proliferating type (potentiated by bFGF release from subendothelial injury). Additionally, endothelial injury of the vessel promotes ECM remodeling with an imbalance of matrix metalloproteinases potentiating vSMC migration via exposure of elastin components.

EC endothelial cell, ECM – extracellular matrix, PDGF-BB – platelet derived growth factor-BB; bFGF- basic fibroblast growth factor

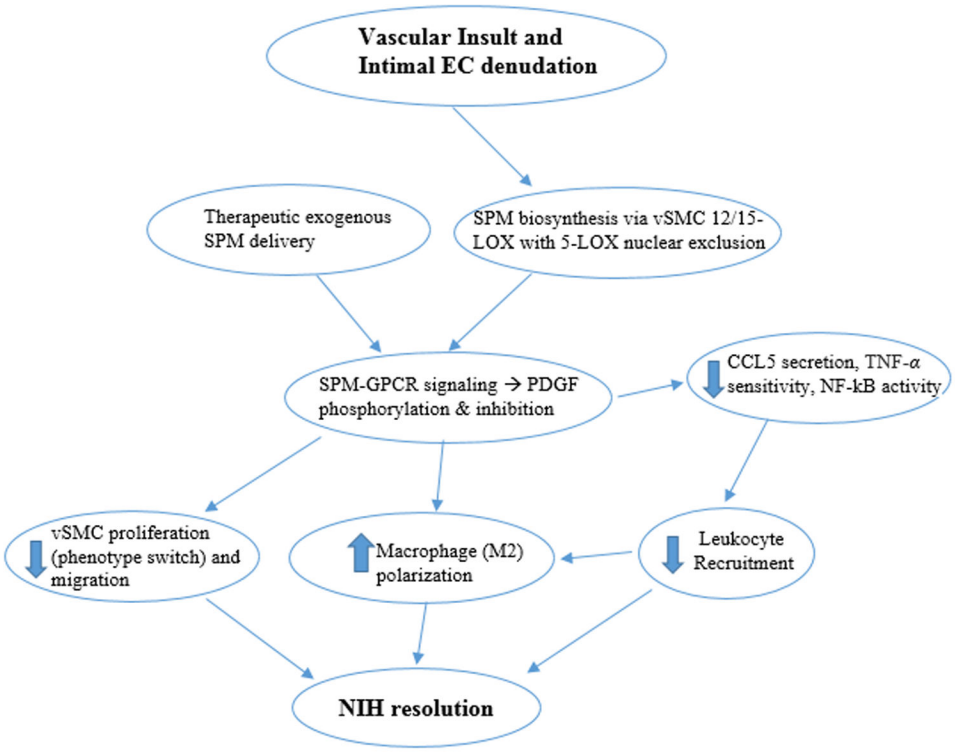

Figure 2: Proposed mechanism of neointimal hyperplasia (NIH) resolution with SPMs:
Therapeutic insult creates obligatory endothelial injury and inflammation, which promotes SPM biosynthesis. Subsequently, synthesized SPMs bind to GPCRs on vSMCs to inhibit PDGF, creating direct and amplifying anti-inflammatory effects, in addition to promoting NIH resolution phenotypes within macrophages and vSMCs (differentiated).