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Review Series

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Pro-resolving lipid mediators in vascular disease

Michael S. Conte,¹ Tejal A. Desai,² Bian Wu,¹ Melinda Schaller,¹ and Evan Werlin¹

¹Division of Vascular and Endovascular Surgery, Department of Surgery, and Cardiovascular Research Institute, UCSF, San Francisco, California, USA. ²Department of Bioengineering and Therapeutic Sciences, UCSF, San Francisco, California, USA.

Unresolved inflammation is central to the pathophysiology of commonly occurring vascular diseases such as atherosclerosis, aneurysm, and deep vein thrombosis – conditions that are responsible for considerable morbidity and mortality. Surgical or catheter-based procedures performed on affected blood vessels induce acute-on-chronic inflammatory responses. The resolution of vascular inflammation is an important driver of vessel wall remodeling and functional recovery in these clinical settings. Specialized pro-resolving lipid mediators (SPMs) derived from omega-3 polyunsaturated fatty acids orchestrate key cellular processes driving resolution and a return to homeostasis. The identification of their potent effects in classic animal models of sterile inflammation triggered interest in their vascular properties. Recent studies have demonstrated that SPMs are locally synthesized in vascular tissues, have direct effects on vascular cells and their interactions with leukocytes, and play a protective role in the injury response. Early translational work has established the potential for SPMs as vascular therapeutics, and as candidate biomarkers in vascular disease. Further investigations are needed to understand the molecular and cellular mechanisms of resolution in the vasculature, to improve tools for clinical measurement, and to better define the potential for “resolution therapeutics” in vascular patients.

Resolution: clinical relevance to vascular diseases

Inflammation plays a central role in the pathogenesis of cardiovascular diseases such as atherosclerosis, aneurysm disease, and venous thrombosis. Acute events and chronic complications of these diseases are frequently driven by inflammatory exacerbations and/or unresolved injurious stimuli. This *JCI* Review series summarizes the current state of knowledge of the molecular drivers of resolution, their biochemical and cellular pathways, and translational implications across the spectrum of human disease. In this Review we will outline how vascular disease, and clinical vascular interventions, offer a unique opportunity to leverage the evolving pharmacobiology of resolution.

Over the last two decades, the identification of resolution as an active process with specific mediators, receptors, and downstream signaling pathways has provided a new framework for investigating mechanisms of disease as well as candidate therapeutic targets (1–3). Distinct families of specialized pro-resolving lipid mediators (SPMs), derived from polyunsaturated fatty acids (PUFAs), were identified using agnostic molecular profiling strategies in animal models of acute, self-limited inflammation (1–4). These SPM families, including the E- and D-series resolvins, protectins, maresins, and lipoxins, have been sequentially characterized by structure-function studies, and new members continue to be added (5–8). Total organic synthesis of SPMs and assessment of their physiologic effects across a range of disease and acute injury settings have followed (3, 9). Classic animal models of sterile inflamma-

tion, such as murine chemical peritonitis, have provided a useful starting point to elucidate SPM receptors and downstream signaling pathways, and to develop quantitative measures of resolution (2, 4, 8). Subsequent studies have demonstrated a lipid mediator “class switch” in the biochemical transition from inflammation to resolution, wherein early proinflammatory prostaglandins and leukotrienes are replaced by SPMs in exudates. The mechanisms of this shift in eicosanoid profile involve temporal changes in the expression, localization, and activity of key cellular enzymes such as 5- and 15-lipoxygenases (LOXs) (10–12). Once available, SPMs coordinate crosstalk between leukocytes and local cell populations, promoting an M1-M2 phenotypic transition in macrophages that is central to tissue repair (13). SPMs further promote resolution by positive-feedback effects on LOX activity and SPM receptor expression in leukocytes (11, 12, 14). Translation of these concepts into therapies for complex human diseases is the current challenge faced by investigators in this exciting, evolving field. A central hypothesis of these efforts is that a relative “resolution deficit” may contribute to disease progression or limit clinical responses to existing therapies, suggesting opportunities for monitoring and/or treatments that incorporate lipid mediators (15–17).

Atherosclerosis is characterized by chronic unresolved inflammation, leading to plaque formation, progression, and downstream clinicopathologic consequences. The role of SPMs in atherogenesis is reviewed in detail elsewhere in this Review series (18). In murine and rabbit models, SPMs decrease atheroprogession and promote plaque stability (Table 1) (19–23). Peripheral arterial disease (PAD) is a common manifestation of atherosclerosis, wherein occlusive disease of the lower-extremity arteries leads to progressive disability, limb loss, and mortality. PAD is associated with aging, smoking, diabetes, dyslipidemia, and hypertension (24–28). PAD is increasing in prevalence around the globe and is currently estimated to afflict more than 200 million

Conflict of interest: MSC and TAD are co-inventors on a patent (US Patent and Trademark Office no. 9,463,177) assigned to Regents of the University of California and Brigham and Women's Hospital. MSC and TAD are cofounders of VasaRx. MSC has received research support from Metagenics Inc.

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Table 1. Summary of in vivo models demonstrating effects of SPMs in vascular injury/disease

Model type	Species	SPMs studied	Effects seen	References
Atherosclerosis	Mouse, rabbit	RvE1, RvD1, RvD2, MaR1, 15-epi-LXA ₄	Reduced atherosclerosis, enhanced plaque stability	19–23
Artery ligation	Mouse	RvD2, MaR1, 15-epi-LXA ₄	Decreased neointima	46, 86
Angioplasty	Rat, rabbit	RvD1, RvD2	Decreased neointima	45, 47
Vein bypass	Rabbit	RvD1	Reduced vein graft hyperplasia	48
Myocardial IR	Mouse, rat	RvD1, RvE1	Reduced infarct size, improved myocardial recovery	72–75
Cerebral IR	Mouse	15-Epi-LXA ₄ , LXA ₄ , RvD1, RvD2, MaR1	Reduced inflammation, mortality	64–69
Visceral IR	Mouse	LXA ₄ , RvD1–3, protectin D1	Reduced platelet-PMN aggregates, decreased leukocyte infiltration, preservation of renal function	70, 71, 118, 119
Hind-limb ischemia	Mouse	LXA ₄ , RvD2	Improved skeletal muscle recovery	62, 63
Aneurysm	Mouse	RvD1, RvD2	Decreased aortic diameter	50

individuals, a roughly 25% increase over the preceding decade (25). Patients with advanced PAD exhibit a marked chronic pro-inflammatory state, with elevated levels of circulating biomarkers such as C-reactive protein (CRP), IL-6, soluble adhesion molecules, and monocyte chemoattractant protein-1 (MCP-1) (29–32). In early studies, we identified that circulating levels of the SPM 15-epi-lipoxin A₄ (15-epi-LXA₄) were significantly lower in individuals with advanced PAD and correlated inversely with disease severity (15). Recent work from other investigators demonstrates that circulating levels of the SPM resolvin D1 (RvD1) or its precursor docosahexaenoic acid (DHA) are reduced in individuals with symptomatic carotid atherosclerosis (33). Thus, an imbalance between proinflammatory and pro-resolving signals may reflect or directly contribute to the progression of atherosclerotic vascular disease in humans (15, 20).

Surgical interventions for advanced PAD, such as angioplasty, stent placement, and bypass surgery, superimpose acute vascular injury on this chronic inflammatory substrate. The magnitude as well as temporal and spatial distribution of the ensuing inflammatory response in the vessel wall, and its resolution, are key drivers of vessel remodeling and downstream clinical outcomes (34–38). While often technically successful, the long-term outcomes of such procedures are limited by excessive vessel scarring or “restenosis” that may affect 50% or more of patients within 2 to 3 years (39–41). Elevated levels of proinflammatory cytokines have been associated with restenosis and adverse outcomes following peripheral interventions (42–44). These observations led us to hypothesize that augmentation of resolution via SPMs may mitigate the arterial injury response and reduce restenosis. Efforts in this arena have led to the preclinical development of candidate therapeutic strategies to deliver SPMs locally to arteries and bypass grafts, described in further detail below (45–48).

Aortic aneurysm is a common and lethal vascular condition characterized by protracted inflammation of the aortic wall, elevated protease activity with progressive loss of structural integrity, and impaired resolution. Infiltration of lymphocytes and monocytes/macrophages into the aortic wall promotes matrix

degradation and vascular smooth muscle cell (VSMC) apoptosis. At present there is no available pharmacologic treatment to reduce aneurysm growth or rupture, and surgical repair is the only effective therapy. Several studies have examined the effects of omega-3 PUFA supplementation in animal models of aortic aneurysm, demonstrating protective effects mediated by attenuation of aortic inflammation and alterations in matrix metabolism (49–51). Recent studies demonstrate that the SPMs RvD1 and RvD2 attenuate aneurysm formation and progression in murine models, in association with a reduction in metalloproteinase activity and polarization of aortic wall macrophages toward a reparative M2 phenotype (50). Clinical relevance of

resolution biochemistry was also shown in the context of surgical repair of aortic aneurysm, where early postoperative changes in SPM pathways were measured (52).

Deep vein thrombosis (DVT) and its sequelae are common conditions with major public health implications (53). Resolution of the acute thrombosis is linked to downstream venous remodeling and subsequent clinical post-thrombotic syndrome, a chronic disabling condition characterized by pain, swelling, skin changes, and ulceration. We speculate that SPMs may modulate the magnitude of thrombo-inflammatory events in the vasculature and participate in their subsequent resolution. For example, maresin 1 (MaR1) has been shown to enhance hemostatic function of human platelets but suppresses their inflammatory function, suggesting that SPMs may play an important role in the resolution of thrombotic events (54). Resolvin E1 (RvE1) reduces platelet activation in response to agonists such as ADP (55, 56), and RvD2 treatment reduced dermal vessel thrombosis in a burn injury model (57). Early platelet-neutrophil interactions at sites of organ injury or thrombosis lead to the biosynthesis of SPMs such as MaR1, which may then stimulate the onset of resolution (58). Resolution of DVT requires phagocytosis of clot and apoptotic cells by macrophages, an activity that is increased by SPMs such as D-series resolvins and lipoxins (59). Moreover, recent work has defined a specific temporal pattern of SPM production that occurs during the formation of thrombus, which may be important to induce subsequent phases of clot resolution (60) and vessel wall remodeling (61). Collectively these data support an important role for SPMs in modulating the inflammatory response to thrombosis, and their potential to mitigate its downstream consequences.

Ischemia/reperfusion (IR) injury is a common complication of acute ischemic events, leading to ROS-dependent tissue damage. In settings such as stroke, myocardial infarction, and visceral or limb ischemia, IR injury can exert a dominant harmful effect on clinical outcomes. End-organ IR injury also affects solid organ transplants and autogenous vascular grafts. Studies in murine models of hind-limb (62, 63), cerebral (64–69), renal (70, 71), and myocardial IR (72–75) have demonstrated marked attenuation of

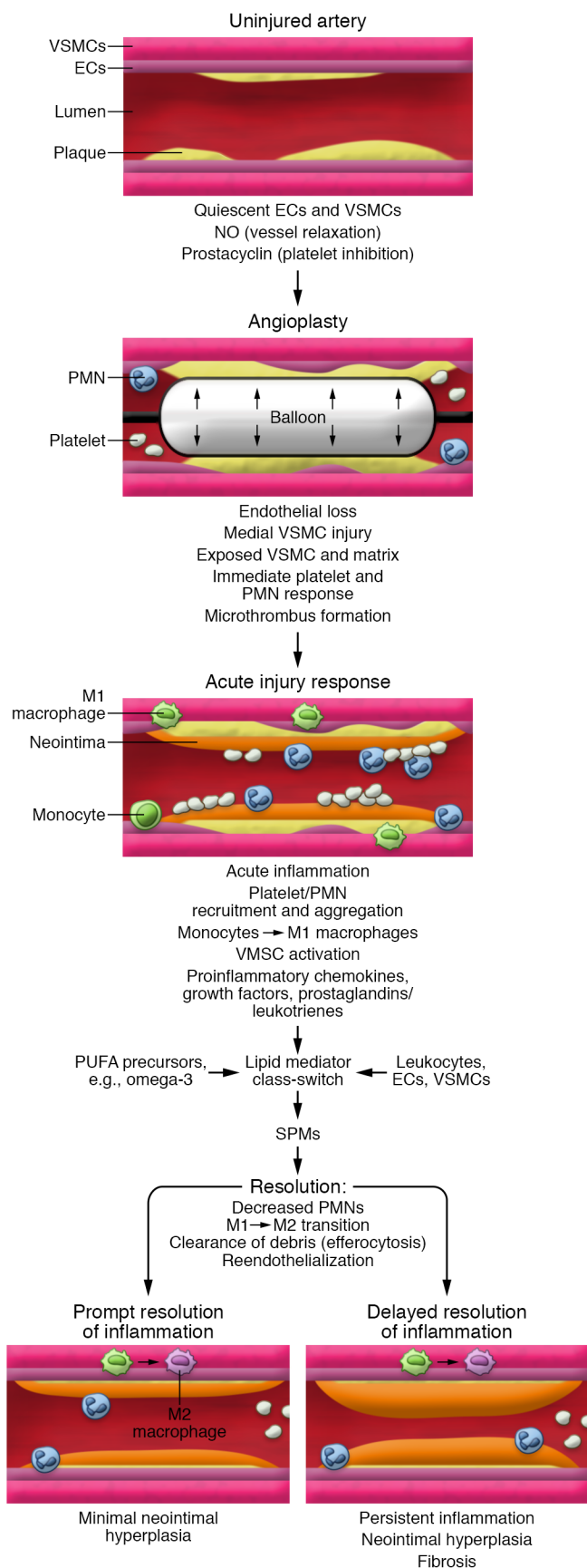


Figure 1. Resolution of vascular injury (angioplasty) and its regulation by locally derived SPM. The figure depicts injury-induced inflammation and immune response in the aftermath of arterial angioplasty. The efficiency of the resolution process that follows the acute response to vascular injury influences long-term vessel remodeling.

tissue injury by SPMs, with augmentation of cytoprotective pathways and reduced ROS signaling in a receptor-dependent manner.

SPM activities and biosynthesis in the vasculature

SPM effects on vascular cells and vascular cell-leukocyte interactions. Endothelial cells (ECs) form a continuous interface between circulating blood elements and the underlying vessel wall, regulating blood flow, platelet adhesion, and leukocyte and macromolecular egress. When ECs are disrupted or activated, a thrombo-inflammatory process ensues that is protective in settings of bacterial invasion or injury, but may have deleterious effects if recovery is impaired. Investigators have uncovered a range of effects of SPMs on ECs that collectively reduce their inflammatory activation and promote return to a quiescent state. For example, SPMs such as RvD1, RvD2, MaR1, LXA₄, and protectin D1 (in picomolar to nanomolar concentrations) reduce proinflammatory cytokine production, adhesion molecule expression, and leukocyte-EC adhesion in response to a variety of inflammatory stimuli in *in vitro* systems (61, 76–79). Mechanisms identified include downregulation of NF-κB activation (78), induction of nitric oxide and prostacyclin synthesis (80), reduction in ROS generation (81), and involvement of the cAMP/PKA (78, 82) and GSK-3β/C/EBPβ pathways (83). ECs express known SPM receptors including ALX/FPR2, GPR32, and GPR18 (79, 81, 84, 85). Thus the effects of SPMs on ECs may have direct relevance to chronic atherosclerosis progression and/or recovery from acute vessel injury.

VSMCs constitute the bulk of the vessel wall, providing biomechanical stability and vasomotor activity and contributing importantly to vessel wall healing. When endothelium is completely denuded (as in angioplasty injury), exposed VSMCs are also a substrate for platelet and leukocyte adhesion/recruitment. Normally quiescent VSMCs are highly organized within the arterial media, surrounded by extracellular matrix proteins. In acute vessel injury, VSMCs rapidly assume a promigratory, inflammatory, and proliferative phenotype in response to an array of locally available cytokines and growth factors, such as TNF-α, IL-1, IL-6, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and angiotensin II. These early events are central to the formation of neointima that leads to vessel wall thickening and subsequent reductions in lumen caliber. RvD1, RvD2, and MaR1 treatment reduces monocyte adhesion to cytokine-activated VSMCs *in vitro*, associated with downregulation of the expression of adhesion molecules (e.g., ICAM-1, VCAM-1) and proinflammatory genes (45, 78). SPMs such as LXA₄, RvD1, RvD2, and RvE1 attenuate VSMC migration to a range of relevant motogens, including PDGF, thrombin, and angiotensin II (45, 47). These effects are receptor-dependent and involve NF-κB downregulation as well as activation of the cAMP/PKA pathway. We and others have identified modest antiproliferative effects of SPMs such as RvD1, RvD2, and MaR1 on VSMCs both *in vitro* and *in vivo* (45–48, 86, 87).

SPM effects on vascular contractility. Vessel tone is continuously modulated by a balance of locally produced factors, most

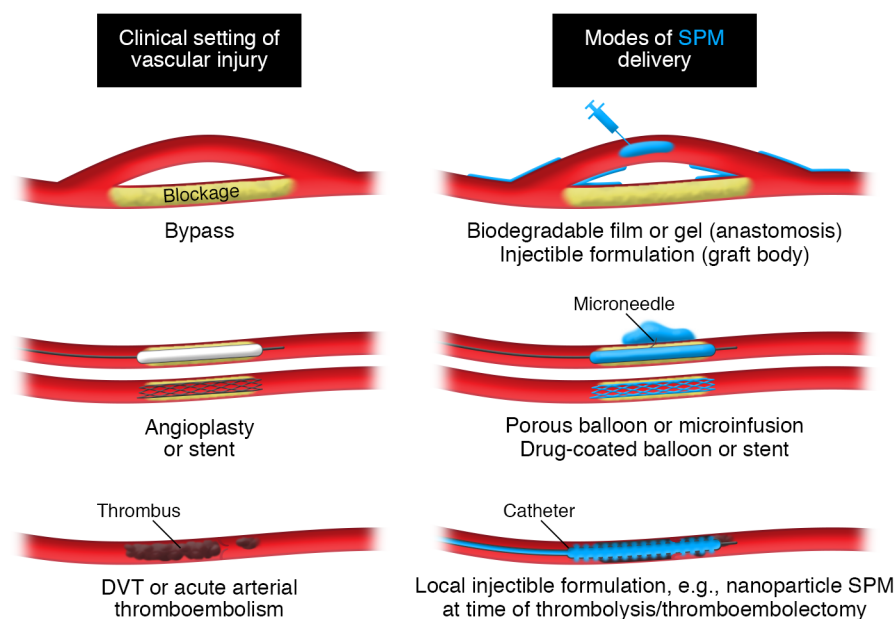


Figure 2. Potential strategies for delivering pro-resolving therapeutics in clinical settings of acute vascular injury or intervention. Top: SPM-loaded films, gels, or injectable formulations can be delivered to the site of anastomosis or bypass graft. Middle: Drug-coated balloons and stents or microneedle infusions into the vascular wall can enable slow release in a site-specific manner. Bottom: Local injections via a catheter can deliver SPMs to the site of venous or arterial thrombosis concomitant with thrombolysis or thrombo-embolctomy procedures, to promote clot resolution.

critically nitric oxide, which is generated by quiescent ECs and induces VSMC relaxation via soluble guanylate cyclase. In settings of injury or local inflammation, initial vasodilatory responses are important to enhance local perfusion and provide a route for neutrophils and substrates to reach target tissues. However, generation of potent vasoconstrictors in the inflammatory milieu, such as thrombin, angiotensin II, and endothelin, can promote further ischemic injury and vessel thrombosis. Recent studies have identified that certain SPMs, notably RvE1, RvD1, and RvD2, attenuate vasoconstrictive responses of human pulmonary artery and rat aortae to agonists such as thromboxane and mitigate hyperresponsiveness to extracellular calcium (88, 89). These responses may be important to protect against secondary ischemic insults during the phase of resolution and tissue repair. They could also be relevant to clinical settings of vasospasm that complicate surgical repair in acute traumatic injury or thromboembolic events.

Vascular injury and intimal hyperplasia. In settings of acute vessel injury such as angioplasty or bypass grafting, loss of endothelial integrity and damage to underlying VSMCs initiates a rapid response of platelets, coagulation proteins, and leukocytes, particularly neutrophils and monocytes (34–36, 38). These elements attach to and invade the vessel wall, elaborating an array of growth factors, proteases, cytokines, and vasoactive substances that induce secondary proinflammatory gene cascades in the surviving VSMCs and adventitial cells. Collectively these signals amplify early leukocyte recruitment and activate cellular repair programs. VSMCs undergo dramatic phenotypic transformation to an activated, migratory, and proliferative state that leads to vessel healing and neointima formation. In the early stages after injury, monocyte-derived macrophages (MDMs) in the vessel wall display an M1 phenotype, elaborating chemokines such as MCP-1 that promote further cellular recruitment. When these local inflammatory and VSMC phenotypic changes are protracted, the resultant neointimal hyperplasia is exacerbated and final lumen caliber may be reduced. In this overly simplified model (Figure 1), a resolution phase may be described during which EC regeneration occurs, VSMCs return to a quiescent state, and MDMs convert to an M2

phenotype to enhance clearance of apoptotic cells and initiate adaptive remodeling of the vessel wall. We have hypothesized that locally available SPMs are central to the resolution of vascular injury and may constitute an important homeostatic mechanism (3, 9, 61, 90).

In murine models of carotid artery ligation, neointimal hyperplasia is rapidly induced as a low-shear response in the adjacent artery. This process was markedly attenuated by systemic (i.p.) delivery of either RvD2 or MaR1, accompanied by a reduction in neutrophil and macrophage recruitment, and reduced VSMC proliferation (46). Similar observations were made with subcutaneous delivery of 15-epi-LXA₄, while no effect was seen in mice lacking the LXA₄ receptor, ALX/FPR2 (86). In a rat model of carotid artery angioplasty, we found that local perivascular application of RvD1 (200 ng) using either pluronic gel or a biodegradable scaffold delivery strategy reduced neointimal hyperplasia by 50% to 60% (47). Protective effects were also demonstrated when RvD2 was delivered directly into the lumen of rabbit femoral arteries immediately after balloon angioplasty, despite highly unfavorable pharmacokinetics for this approach (45). Collectively these studies provide proof of concept for SPMs as a potential new class of candidate antirestenosis therapeutics.

Vascular biosynthesis of SPMs. Current concepts of SPM biosynthesis focus on local generation and activity as for classic autacoids. The PUFA precursors, arachidonic acid (AA), eicosapentaenoic acid (EPA), DHA, and docosapentaenoic acid (DPA), made available via local edema and in tissue exudates, are serially converted by LOXs (especially 5-LOX and 15-LOX) and cyclooxygenase (COX) pathways into bioactive lipid mediators including SPMs. These enzymatic activities may involve direct transcellular exchange of biochemical intermediates between leukocytes, platelets, and resident cells such as ECs or VSMCs. AA-derived mediators such as prostaglandins dominate the early response, and a lipid mediator class switch is hypothesized to account for the subsequent elaboration of SPMs derived from AA, EPA, DHA, and DPA (9, 91). These mediators act locally and may be rapidly inactivated by ubiquitous enzymes such as eicosanoid oxidoreductases and prostaglandin dehydrogenases (e.g., 15-prostaglandin dehydrogenase) (92).

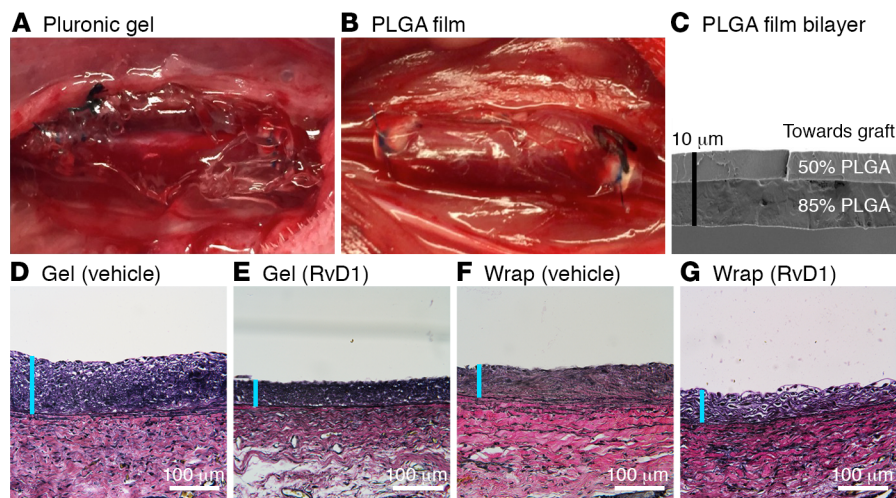


Figure 3. Perivascular delivery of RvD1 to rabbit vein grafts. (A and B) Images depict two methods of perivascular delivery of RvD1 to rabbit vein grafts (carotid interposition) using either a pluronic gel (A) or a PLGA film device (B). (C) PLGA films (“wraps”) were constructed using a thin bilayered design and loaded with 1 μ g of RvD1; representative scanning electron microscopy of a film is shown. (D–G) Perivascular delivery of RvD1 via gel or wrap attenuated vein graft hyperplasia 28 days after implantation, whereas perivascular application of vehicle-loaded gels and wraps had no effect (elastin-stained sections). Adapted with permission from ref. 48.

Specific sources of SPM synthesis within the vasculature remain incompletely defined. Brezinski et al. identified intraluminal LXA₄ following coronary angioplasty in humans (93). Using liquid chromatography–tandem mass spectrometry analysis, we identified RvD1, RvD5, MaR1, and LXB₄ in rabbit femoral arteries with a trend of increased SPM production early after balloon angioplasty (45). SPMs have been identified in atherosclerotic lesions of mice (20, 94). In recent work, we demonstrated that isolated, ex vivo human artery segments and primary cultured human vascular cells generated D-series resolvins and maresins when the relevant fatty acid precursors (e.g., DHA, 17-hydroxydocosahexaenoic acid [17-HDHA]) were made available, and in the absence of leukocytes (95). Furthermore, conditioned media from DHA-supplemented vascular cells blunted leukocyte adhesion to cytokine-activated ECs, in a receptor-dependent fashion (i.e., ALX/FPR2 and GPR32). These studies suggest that endogenous production of SPMs within the vessel wall may represent an important paracrine pathway to actively counterregulate vascular inflammation. Further studies are needed to characterize the regulation of SPM bioavailability in blood vessels, and its influencing by dietary PUFA intake and commonly used medications such as aspirin, NSAIDs, and statins.

Translation: developing a platform for pro-resolving vascular therapies

Local delivery of SPMs is a potentially attractive approach to modulate vascular injury and circumvent the challenges of systemic pharmacokinetics and biodistribution. Although atherosclerosis is a systemic disease, surgical and interventional treatments are lesion- or vessel-focused. Based on the findings from small-animal models described above, we have sought to develop a platform for pro-resolving vascular therapeutics leveraging polymer and device technologies (Figure 2). Using poly(lactic-co-glycolic acid) (PLGA) as a biodegradable scaffold, we described thin-film devices capable of directional elution of bioactive RvD1 over several weeks (47, 48, 96). Others have described similar approaches using biomaterial scaffolds to locally deliver SPMs to subcutaneous tissues (97, 98) and bone (99). RvD1-releasing PLGA films were tested in rat carotid angioplasty (47) and rabbit vein bypass graft (48) models, demonstrating reduced early leukocyte recruitment,

reduced cellular proliferation, and attenuation of downstream intimal hyperplasia (Figure 3). Injectable nanoparticle-based SPM formulations (100) may also be highly relevant, particularly if they can be targeted to localized regions of vascular damage or surgical/interventional procedures.

Clinical studies of PUFA supplementation in vascular disease

Evidence linking dietary intake of omega-3 PUFAs to cardiovascular health has been the subject of many epidemiologic and cohort studies (101–103). The elucidation of SPM biosynthetic pathways provides a new potential mechanism for these effects. However, randomized clinical trials directly testing the “omega-3 hypothesis” in patients with cardiovascular disease or risk factors have yielded conflicting results (104, 105), and few studies have included subjects with PAD. Challenges in the interpretation of these trials include variability in formulations and dosing, as well as understanding the individual balance between individual omega-3 and omega-6 fatty acid intake and its relationship to proinflammatory versus pro-resolving lipid mediator production. Most of these trials have used lower dosing regimens of marine oil supplements, focused on downstream major clinical events such as myocardial infarction, stroke, and mortality.

Oral supplementation with omega-3 fatty acids or diets rich in their marine sources increase plasma and cell membrane levels of EPA and DHA (106, 107). However, the relationship between PUFA levels (or surrogate markers such as the omega-3 index [O3I]) and downstream SPM pathways is poorly understood. The physiologic impact of oral supplementation of omega-3 PUFAs is sensitive to a multiplicity of factors, including prior dietary intake and hereditary metabolic factors (108). The OMEGA-PAD-I Trial examined the effects of short-term omega-3 fatty acid supplementation on SPMs and their biosynthetic pathways in PAD patients (107). Among subjects randomized to receive 4.4 g of fish oil daily (2.6 g of EPA and 1.8 g of DHA) for 1 month, a significant increase was observed in the plasma levels of SPM pathway markers such as 5-hydroxyeicosapentaenoate (5-HEPE), 15-HEPE, 18-HEPE, and 4-HDHA. Secondary analyses illuminated a direct relation-

ship between changes in the O3I and these SPM pathway markers (109). However, downstream bioactive SPMs were sparsely detected in plasma, and measures of endothelial function and systemic inflammation were unchanged following the 1-month intervention. Ongoing studies seek to examine these relationships using novel marine oil fractions and broad profiling of lipid mediator pathways and leukocyte and macrophage functions in similar populations of PAD patients (OMEGA-SPM studies; ClinicalTrials.gov identifier NCT02719665).

There have been few studies examining the effects of dietary marine oil intake and venous thromboembolism (VTE). In a population-based Norwegian cohort study, high fish consumption was associated with reduced risk of VTE, and this effect was strengthened by the use of fish oil supplements (110). Bonutti et al. reported on a prospective, nonrandomized study of orthopedic patients undergoing total knee arthroplasty ($n = 850$) receiving aspirin plus a pulsatile compression stocking, rivaroxaban, or a combination of aspirin and fish oil. The occurrence of DVT was significantly lower in the aspirin plus fish oil group versus aspirin plus stocking (0.33% vs. 7%; odds ratio 0.045; $P < 0.05$), and not different from that in the rivaroxaban group (1%) (111). Larger prospective, randomized, and double-blind studies are needed to determine whether omega-3 supplements have a beneficial impact on VTE.

These studies highlight a number of challenges in pursuit of the nutritional approach to augment resolution pathways. Formulations need to be standardized in terms of content of free fatty acids and downstream metabolites. Substrate utilization and biosynthesis pathways for omega-3 and omega-6 PUFA-derived mediators are complex and involve competition for the available enzymes (3, 9, 112–116). Production of different mediator isomers can have varying potency or conflicting biologic effects. Aging and other risk factors (e.g., smoking) may alter the biosynthetic pathways of lipid mediators (117). In addition to the complexities of SPM biosynthesis, little is known about how SPM degradation pathways or SPM receptors are regulated in a tissue-specific fashion or how they may be altered in disease.

Current limitations and future directions

Based on the above summary of cell biology, animal models, and early-stage clinical investigations, the relevance of SPMs and their related pathways to vascular disease has become established and is growing. Further studies in arterial injury and restenosis, aneurysm disease, and venous thrombosis will provide much-needed insight into the mediators and processes that govern resolution in

the vasculature. There are several key areas to address along the road to clinical translation.

Greater understanding of SPM receptor expression, selectivity, and downstream signaling in vascular tissues is needed to provide a stronger foundation for therapeutic targeting and pharmacokinetic studies. Rational selection of the optimal SPM and formulation for different clinical settings hinges on such efforts. Improvements in medicinal chemistry and scalability of organic synthesis are already taking place and will be pivotal to further development of SPM-based therapeutics. Locally delivered SPMs or synthetic SPM analogs may be an optimal approach for certain clinical settings, given available degradation pathways in the circulation. Nutritional supplementation with marine oil formulations enriched for SPM precursors and bioactive mediators holds promise, but will require rigorous clinical studies in vascular patients. To date, specialized instrumentation and analytics are needed to accurately measure bioactive SPMs and their precursors in tissue samples; efforts to develop more standard bench top (e.g., ELISA) assays will allow for cheaper, repeatable, and more broadly available measurement tools. Surrogate biomarkers of resolution, similar to biomarkers of inflammation (e.g., CRP, IL-6), need to be developed and validated in various disease settings to enhance preclinical and clinical study designs.

Despite these limitations, the basis for investigating SPM-based therapeutics in acute settings of vascular injury and inflammation is strong. With bioactivity in picomolar to nanomolar concentrations, a lack of discernible toxicity, and a broad profile of antiinflammatory and resolution-enhancing effects, SPMs appear particularly well suited for targeted vascular delivery across a range of clinical settings. Surgical and endovascular intervention, aneurysm disease, and acute ischemic/thrombotic events are prime targets for resolution therapeutics.

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Address correspondence to: Michael S. Conte, Department of Surgery and Cardiovascular Research Institute, University of California, San Francisco, 555 Mission Bay Boulevard South, San Francisco, California 94143, USA. Phone: 415.353.4366; Email: Michael.conte2@ucsf.edu.

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