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Chemical Mediators of Inflammation and Resolution in Post-Operative Abdominal Aortic Aneurysm Patients

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Abstract

Temporal–metabolomic studies of local mediators during inflammation and its resolution uncovered novel pathways and mediators, *e.g.*, lipoxins, resolvins, and protectins that stimulate key resolution responses. Since these studies were carried out with isolated human cells and in animal models, it is important to determine in humans whether temporal profiles between pro-inflammatory mediators and pro-resolving mediators are demonstrable *in vivo*. To this end, we examined patients undergoing abdominal aortic aneurysm (AAA) surgery. Profiles of mediators including eicosanoids were assessed in addition to pro-resolving mediators. The results demonstrate temporal relationships for local-acting peptides (*e.g.*, VEGF, IL-10, TGF β) and lipid mediators (leukotrienes and resolvins). In addition, profiles obtained for AAA patients divided into two groups based on their temporal profile: one group consistent with a pro-inflammatory and another with a resolving profile. Together, these translational metabolomic profiles demonstrate for the first time the temporal relationships between local mediators in humans relevant in inflammation resolution.

Keywords

metabolomics; resolution; DHA; EPA; leukotrienes; resolvins

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Conflict of Interest. The results reported herein were obtained by the authors in part prior to licensing of patents for clinical development of the resolvins. Charles N. Serhan is an inventor on patents assigned to Brigham and Women's Hospital and Partners HealthCare covering the composition of matter, uses, and clinical development of anti-inflammatory and pro-resolving lipid mediators. These are licensed for clinical development. Also, CNS retains founder stock in Resolvix Pharmaceuticals. All other authors have no conflict of interest.

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INTRODUCTION

The acute inflammatory response is a complex multicellular process that protects the host against infection and injury and is ideally self-limited, resolving on its own [1]. A body of recent evidence indicates that this protective mechanism resolves as a programmed temporal response that is biosynthetically active at the tissue level in experimental animal models, and permits the tissue to return to homeostasis [2]. Hence, excessive inflammatory responses may arise from the loss of endogenous resolution programs, and can lead to the progression of many chronic inflammatory diseases where uncontrolled inflammation plays a role such as in arthritis, Alzheimer's disease, and atherosclerosis [3,4]. The local acute inflammatory response is also initiated by surgery [1]. Surgical intervention triggers the release of local-acting chemical mediators including pro-inflammatory cytokines and lipid mediators, the clearance of which is critical for cessation of the acute inflammatory response and proper wound healing [5,6]. Recent evidence demonstrates that the biosynthesis and release of a new genus of endogenous specialized pro-resolving lipid mediators (SPM) promotes the active resolution of acute inflammation (reviewed in [7]).

Pro-inflammatory lipid mediators, such as the prostaglandins and leukotrienes, are well known for their roles in initiating the inflammatory response [8,9]. It is now clear from results in murine systems *in vivo* that a temporal class switch occurs in the inflammatory exudate during acute inflammation; leukotrienes and prostaglandins in the initial phase of inflammation, followed by arachidonic acid-derived lipoxins [7,10] and the novel families of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)-derived mediators, namely resolvins, protectins and maresins, during the latter phase [11–14]. These endogenous resolution agonists orchestrate the non-phlogistic clearance of apoptotic PMN (termed efferocytosis), reduce pro-inflammatory cytokines and lipid mediators, as well as enhance the removal of cellular debris in the inflammatory milieu [15]. Importantly, the SPM biosynthesized temporally during the transition from the initiation to resolution phase in turn prevent further neutrophilic infiltration into the tissue. Hence, SPM play pivotal roles both in limiting neutrophils and neutrophil-derived agents (*i.e.*, reactive oxygen species) at the inflammatory site or exudate, while enhancing clearance by macrophages.

Of the many currently available anti-inflammatory drugs, aspirin has the unique property to both block the formation of pro-inflammatory and prothrombotic prostaglandins and thromboxanes, and to jump-start resolution by initiating the biosynthesis of SPM, aspirin-triggered 15-epilipoxins and resolvins [11,12,16]. The biosynthesis and actions of these local mediators were identified *in vivo* during inflammation-resolution in animal disease models, where administration of an inflammatory stimulus takes place at a fixed time zero to initialize a self-limited inflammatory response [reviewed in ref. 7]. To address the temporal relationships of these responses in humans, a system with a fixed time zero is necessary to permit the analysis of temporal profiles of local chemical mediators before and after initiation. As in experimental animal models, the kinetics of leukocytic infiltration and the presence of biomarkers of biosynthetic pathways at specific time intervals within the inflammatory response provide determinants of resolution and serve as resolution indices *in vivo* [15].

Here, we investigated in patients undergoing open abdominal aortic aneurysm (AAA) repair the temporal relationships between chemical mediators of inflammation and its resolution, focusing on both lipid and peptide-derived local mediators. The chemical mediators profiled herein were selected for their established roles in animal models of inflammation and their functions as pro-inflammatory mediators and mediators of resolution with isolated human cell types and tissues. These results document the temporal relationship between local-acting mediators in humans using liquid chromatography-mass spectrometry (LC-MS)–MS to

identify key eicosanoids and biomarkers of E- and D-series resolvins and protectin pathway activation *in vivo*.

MATERIALS AND METHODS

Sample Collection

After institutional review board (IRB) approval of the study (IRB protocol #2006P000855 and BWH IRB Assurance #FWA00000484) and obtaining written informed consent, 15 recruited patients scheduled for surgical repair of AAA or aortoiliac bypass surgeries were studied. Patients with other inflammatory processes, as well as patients with congestive heart failure or renal failure, were excluded from the study. Patients had an average age of 69 years with a standard deviation of 9 years. Ten patients were male and five were female. All 15 patients had hypertension and two had diabetes mellitus. Ten patients were treated with statins, 12 with low-dose aspirin, and 14 with beta-adrenergic antagonists (Table 1).

An arm vein and one of the radial arteries were catheterized, and the thoracic epidural catheter was placed at the T7–T9 level under local anesthesia in all 15 patients. General anesthesia was induced with propofol, midazolam, and fentanyl titrated to loss of consciousness. Tracheal intubation was facilitated by a muscle relaxant, succinylcholine, or vecuronium. General anesthesia was maintained by a volatile anesthetic (desflurane or sevoflurane) and opioids (fentanyl or hydromorphone) titrated to maintain adequate depth of anesthesia. The central venous catheter was placed via right jugular internal vein after anesthesia had been induced.

During surgical repair of the AAA, the infrarenal aorta was clamped, and the segment of native aorta with the aneurysm was replaced by an allograft. The aorta was then unclamped and the abdominal surgical wound was closed.

During the perioperative period, 10 ml of blood was withdrawn prior to clamping the aorta, and 5 min, 30 min, 6 h, 24 h, 48 h, and 72 h after unclamping of the aorta. Blood samples were drawn from the central venous line (primary site) or an arterial line (secondary site). If the arterial line and central line had been discontinued in the intensive care unit before the last sample had been taken, the sample was drawn from a vein in the arm.

All medications that the patient received as well as any adverse clinical outcome (wound infection, graft failure, myocardial infarction, stroke, death, *etc.*) were recorded.

Standard care for anesthesia and surgery on the aorta was followed. Subjects were supposed to be removed from the study if there was significant hemorrhage due to the surgery (greater than 2,000 ml) or if the subject was hypotensive and not responsive to transfusion and vasoactive drug support. No patients needed to be removed from the present study.

Clinical Course and Sample Set

Fourteen patients underwent AAA repair and one had aorto-iliac disease and underwent aorto-iliac bypass. All 15 patients who underwent the surgery survived and did not have significant morbidity, except one patient who had a myocardial infarction on the second postoperative day; ST depression on ECG and an increase in concentration of troponin 1–25.6 ng/ml. This patient was treated with heparin. The concentration of troponin decreased and the ECG normalized. The patient was hemodynamically stable. On third postoperative day, the patient had another episode of ST depression. A cardiac catheterization revealed 90% stenosis of LAD and occlusion of RCA. Stents were placed and the following course was without complications.

Targeted LC-MS/MS-Based Lipidomics of AAA Samples

Plasma (~500 μ L) was collected and added to two volumes of cold methanol for extraction and workup [17]. LC-MS/MS identification was performed for select samples from the AAA sample set collected using an Agilent 1,100 series HPLC paired with an ABI Sciex Instruments 3200 Q TRAP linear ion trap quadrupole mass spectrometer. The column (Agilent Eclipse Plus C18, 4.6 mm $\times$ 50 mm $\times$ 1.8 μ m) was eluted at a flow rate of 0.4 ml/min with methanol/water/acetic acid (60/40/0.01; v/v/v) ramped to 80/20/0.01 (v/v/v) after 5 min, 95/5/0.01 (v/v/v) after 8 min, and 100/0/0.01 (v/v/v) after 14 min to wash the column. Data acquisition was performed using Analyst 1.4.2 software as in ref. [17]. The ion pair transitions from reported multiple reaction monitoring acquisition methods [17] were used in the present analyses for profiling and quantification. The criteria for lipid mediator identification included liquid chromatography retention time together with the matching of a minimum of six fragment diagnostic ions from the MS/MS spectrum matching those of synthetic standards for each of the targeted mediators in the present study. Deuterated standard (2 ng of d₈-5-HETE) was added before extraction as internal standard for calculating extraction recovery for each sample.

Cytokines and Eicosanoids

Plasma levels of cytokines (R&D, Minneapolis, MN, USA) and lipid mediators (Neogen Corporation, Lansing, MI, USA) were measured using a sandwich enzyme immunoassay, according to the manufacturer's protocol. The sensitivity of the LXA₄, 15-epi-LXA₄, LTB₄, TXB₂, monoclonal PGE₂, LTC₄/D₄/E₄, 6-keto-PGF1 α , sICAM-1/CD54, VEGF, IL-10, TGF- β 1, and sP-selectin assays were 0.03 ng/ml, 0.05 ng/ml, 0.1 ng/ml, 0.009 ng/ml, 0.12 ng/ml, 0.06 ng/ml, 0.05 ng/ml, 0.096 ng/ml, 9.0 pg/ml, 3.9 pg/ml, 4.61 pg/ml, and 0.5 ng/ml, respectively.

Data Analysis

The significance of difference between groups was evaluated using a two-tailed Student's *t* test. A *P* value < 0.05 was considered statistically significant.

RESULTS

Temporal Response of Chemical Mediators: Signature Profiles

The temporal responses of known peptide-derived and lipid-derived chemical mediators (Fig. 1 and Table 2) during abdominal aortic aneurysm surgery were investigated in 15 patients undergoing AAA surgery at Brigham and Women's Hospital (Table 1). We evaluated both pro-inflammatory and pro-resolving local mediator levels at close intervals before and after surgical insult in order to assess temporal relationships in humans undergoing surgical stress as well as the potential for signature profiles and/or biomarkers that could emerge. To this end, relationships between the magnitudes of these mediators in the immediate post-operative period were ascertained. On average, the eicosanoids LXA₄, LTB₄, LTC₄/D₄/E₄, and the cyclooxygenase pathway products PGE₂ and 6-keto-PGF1 α each appeared to reach maximum levels 5 min after unclamping of the aortic cross-clamp, followed by a steep decrease by 6 h. Of interest, LXA₄, LTB₄, 15-epi-LXA₄, PGE₂, TXB₂, and LTC₄/D₄/E₄ levels rose to near maximum at 72 h (Fig. 2a–g). In Fig. 2d, PGE₂ levels increased significantly from preclamping to 5 min post-unclamping, and then decreased from 5 min to 24 h post-unclamping. From 24 to 72 h post-unclamping, PGE₂ significantly increased (Fig. 2d). This contrasts with the decline in prostacyclin levels (monitored as the 6-keto-PGF1 α metabolite), which remained low and appeared to decrease from 6 to 72 h (Fig. 2g). Although these levels did not prove to show statistically significant differences, they nonetheless gave consistent trends for each patient.

Inflammation and Resolution Profiles

On inspection of each patient's profile, the temporal profiles of AAA patients were clearly separable into two main groups or clusters based on the trends observed for their individual mediator response. Representative profiles of each group were selected to illustrate the general temporal trends. Overall, eight of the AAA patients cluster in group A and seven of the 15 AAA patients cluster in group B. In Fig. 3a, the representative patient from group A had higher initial levels of LXA₄ than the representative patient from group B, with a maximum at 5 min post-unclamping. In patient profile group A, LXA₄ levels declined to approximately 0.1 ng/ml, in contrast to the patient group B profile that showed a steady increase in LXA₄ levels, with a maximum at 72 h.

In Fig. 3, the representative patient for group A gave overall lower levels of the aspirin-triggered lipoxin 15-epi-LXA₄ in comparison to the representative patient from group B, who exhibited high levels at 5 min and 6 h post-unclamping. In Fig. 3, representative patient from group A also displayed initial high levels of thromboxane B₂, the biologically inactive marker of thromboxane A₂ (Table 2). The group B representative patient profile showed overall lower levels of TXB₂ with an increase at 72 h. Of interest, the representative patient A gave a dramatic LTB₄ response, a 5-LOX-derived product, immediately pre- and post-unclamping, with levels decreasing to approximately 0.1 ng/ml thereafter compared to the representative patient profile from group B that displayed consistently low levels of LTB₄ with a slight increase at 24 h. Here, the eicosanoid proinflammatory mediators such as leukotrienes (LTC₄/D₄/E₄, LTB₄) and anti-inflammatory-pro-resolving eicosanoids such as LXA₄ and 15-epi-LXA₄ [7,8] temporal changes defined two different responses of patients that underwent AAA repair.

Results in Fig. 3 indicate that sICAM-1 levels in both representative patients from groups A and B gave a similar trend, with the patient from group B profile exhibiting slightly higher levels. The group A patients reached a maximum IL-10 level (anti-inflammatory cytokine; see Table 2) at 5 min post-unclamping followed by a sharp decrease, while the profile of group B displayed overall lower levels of IL-10 (Fig. 4b). VEGF levels in both patient groups showed a similar trend in this time course (Fig. 3), although those in group B exhibited an overall higher level with a maximum at 30 min post-unclamping. TGFβ-1 levels from both groups A and B showed opposite trends: the patients in group A having higher levels initially followed by a decrease to levels below limits of detection at later time points, while those in group B had slightly higher levels at 48 and 72 h (Fig. 3). The soluble P-selectin levels were consistently higher for those in group A than in group B, whose level at 72 h reached below detection limits (Fig. 3).

Figure 4 reports the relationships between ω-6 polyunsaturated fatty acid arachidonic acid and its biosynthetic products and bioactive mediators, focusing on PGE₂, LTB₄, and LXA₄. The monohydroxy product of 15-lipoxygenase [7], namely 15-HETE, can serve as a pathway marker of lipoxin biosynthesis, and 12-HETE as a marker of platelet activation with release and conversion of arachidonic acid via the platelet 12-LOX. Results in Fig. 4 also demonstrate that the patient from Group B had higher levels of 5-, 12-, and 15-HETE compared to group A. Patient B also had higher levels of the bioactive eicosanoids themselves (Fig. 4). Figure 4b,c report representative mass spectra of 15-HETE and PGE₂ from LC-MS/MS metabolomics for their identification in samples from AAA patients.

Figure 5a reports the relationship between the ω-3 precursor DHA and its metabolic bioactive products, *e.g.*, D-series resolvins (resolvin D1) as well as maresin 1. In these LC-MS/MS-based analyses, both 17-HDHA and 14-HDHA serve as biomarkers for the metabolomic profiling and activation of these two different biosynthetic pathways *in vivo* (*e.g.*, resolvin *vs.* maresin pathways) with surgery. Figure 5b illustrates that representative

patient from group B had higher levels of both 17-HDHA and 14-HDHA, indicating the presence of pathways leading to the resolution agonists such as RvD1 and MaR1. Figure 5 also illustrates the relationships found for the ω -3 EPA-derived bioactive mediators RvE1 and RvE2. In Fig. 5 lower panel, the intermediate 18-HEPE, a marker of the E-series resolvins pathway [7], was identified in much higher amounts in the patient representative of group B compared to the levels from the patient in group A. Figure 5b,c report representative mass spectra for 17-HDHA and 18-HEPE, each identified in samples obtained from AAA patients identified based on their published criteria [17]. Hence, the pathway markers were identified in these patients whereas the bioactive resolvins and protectins were likely further metabolized and escape LC-MS-MS identification in these patient samples.

DISCUSSION

The present report describes the results of the first study that addresses the temporal relationships between local chemical mediators of inflammation and resolution in patients undergoing AAA repair. We focused on both lipid- and peptide-derived chemical mediators of interest and their relationship to well-appreciated pro-inflammatory mediators, including vasoactive eicosanoids and cytokines important to tissue repair [see Table 2 and refs. 8,18,19 for reviews].

AAA is a relatively frequent disease (approximately 10 per 100,000 persons/year) [20]. The most appropriate treatment of this disease is surgical repair. The overall post-operative mortality is between 3% and 7% [21–23]. The pathophysiological basis for the disease is quite complex and includes chronic inflammation [24,25]. The relatively high rate of complication and mortality after this surgery results from many different factors including older age, multiple comorbidities, and medications that treat the comorbidities; but one of the many reasons for the high rate of complications and mortality is the severe inflammatory response to surgical intervention associated with ischemia-reperfusion injury, either to tissues subjected directly to temporary ischemia followed by reperfusion (all organs and tissues distal to the aortic clamp), and/or injury to distant organs (such as the lungs, kidneys, and others) initiated by activated neutrophils and pro-inflammatory mediators released by reoxygenated tissues [6,26–30]. Along with and apparently in response to the release of pro-inflammatory mediators, the formation and release of anti-inflammatory cytokines and mediators is also increased. The latter eventually leads to the inflammatory process to subside and/or resolve. Since it is now apparent from results of *in vivo* experiments with laboratory animals that resolution is an active process that involves the newly uncovered local mediators, namely lipoxins and resolvins [7,11], excessive inflammatory responses may arise from the loss of endogenous resolution programs, and can lead to the progression of a chronic inflammatory state [2]. The complete resolution of acute inflammation is critically important in preventing tissue injury, auto-immunity, chronic inflammation, and the return to homeostasis. The AAA patients studied here gave mediator profiles that grouped into two main profiles denoted as group A and B. The profile, magnitude, and relationship between the local mediators monitored herein based on their known potent bioactions (Table 2) suggests that those in the group A profile fit a pro-inflammatory status throughout the time course and those in group B displayed a pro-resolving mediator profile. These two broad categories may reflect an early resolver population and a delayed resolver population since all patients in both groups recovered. Future translational metabolomic studies are needed to relate these pathways and bioactive mediators to individual patient outcomes (*vide infra*).

Recently, with samples from humans, Wu *et al.* reported that the time course of resolution of streptococcal glomerulonephritis is accompanied by increased levels of lipoxin A₄ and

decreases in leukotrienes in peripheral blood and urine as the infection resolves [31,32]. Also, Gilroy *et al.* found that low-dose aspirin increased 15-epi-lipoxin-A₄ (the aspirin-triggered form of lipoxin A₄) in skin blisters in humans, reducing the neutrophil infiltration [33,34]. The mechanisms for the aspirin-triggered lipoxins as well as resolvins shorten the resolution time in many experimental animal models and include their ability to counterregulate cytokine formation and actions [14]. Along these lines, tumor necrosis factor- α , which is well appreciated as a cytokine in inflammation and infection, has also recently been shown to play a pivotal role in postoperative cognitive decline [35]. Since resolvins, in particular RvE1 and RvD1, counterregulate TNF- α formation and actions, the levels of resolvins may be critical in preventing surgery-induced cognitive decline. Also, recently, aspirin-triggered lipoxin and RvE1 were found to modulate smooth muscle phenotype, which can correlate with the degree and magnitude of atherosclerosis [36]. These findings suggest that the temporal biosynthesis of these mediators following surgery and particularly following AAA repair can have an acute local impact as well as initiate long-range responses in AAA patients that are relevant to long-term effects of surgical intervention and tissue remodeling. Since lipoxins and resolvins can regulate growth factor responses [37], it is likely that the ability of an individual to generate a pro-resolving profile can have a long-range impact on the outcome of AAA surgery. These findings are also consistent with published literature on LXA₄ inhibition of VEGF and stimulation of IL-10 [38]. Thus, in view of the present results, in order to enhance the endogenous profile, it is important to consider the nutritional availability of essential fatty acids such as arachidonic acid that can be converted to local mediators including the lipoxins and aspirin-triggered lipoxins, as well as the omega-3 essential fatty acids DHA and EPA, which can be converted to resolvins and protectins. The nutritional status upon surgery and the circulating and tissue levels of these fatty acids can have a far-reaching impact on the response to surgical intervention.

In the present study, in addition to monitoring the anti-inflammatory and pro-resolving mediators such as lipoxin A₄, its relationship to the aspirin-triggered epimer was also obtained. Along these lines, it is of interest to point out that the production of lipoxin A₄ was recently documented in peripheral blood of women during parturition [39]. Thus, lipoxin A₄ levels could increase in peripheral blood during pregnancy; however, it is unlikely that any of the patients undergoing the AAA repair procedure in the present study was pregnant. Hence, the lipoxin A₄ levels monitored were more likely to reflect the status of the surgical patient to be able to mount an endogenous anti-inflammatory cascade. Two widely used drugs, aspirin and the statins such as lovastatin, increase the production of 15-epi-LXA₄ [40,41], which has a potent pro-resolving and anti-inflammatory action. Thus, the profiles obtained for 15-epilipoxin A₄ documented in the present patients may serve as new means to gauge the ability of the AAA patients to completely resolve and to initiate repair. Along these lines, Gilroy *et al.* recently identified and defined early resolvers and delayed resolvers as two phenotypes that emerge in healthy subjects challenged in a controlled experimental setting with an inflammatory stimulus [33]. In these healthy subjects on cantharidin-challenge and skin blister formation, lipoxin A₄ and 15-epilipoxin A₄ time course of formation within blister exudates defined the early and delayed resolver phenotypes. Also, DiGennaro *et al.* recently demonstrated in human AAA patients increased expression of leukotriene C₄ synthesis and cysteinyl-leukotriene production in tissue samples from these patients [42]. Together, these recent reports and results from the present emphasize the potential value for monitoring lipoxygenase pathway products and their temporal metabolomics in human samples obtained on challenge in experimental settings and/or in surgery to assess future outcomes associated with excessive leukotriene production and inflammation *versus* pro-resolving mediators (*e.g.*, resolvin, protectin), resolution, and the return to homeostasis.

The inflammatory processes associated with surgical AAA repair are relatively well-established [6,30,43]. The processes critical in the resolution of inflammation as it concerns AAA repair have not been studied earlier. Therefore, it is reasonable to hypothesize from the present results that the knowledge of these mediators (Table 2) and resolution potential in these patients should help to better understand the nature of inflammation and its timely resolution, and to serve as a basis for rational and effective future therapeutics that would improve the outcome of this disease and surgery. In order to test such possibilities, information concerning the time course of changes in pro-, anti-inflammatory mediators, and pro-resolving mediators as well as their relationships was obtained. The results herein thus suggest that surgical patients that have undergone AAA fall into two main groups of temporal profile(s) for local chemical mediator(s): those with an essentially pro-inflammatory profile such as patients in group A or a potential resolving profile as those profiles obtained and noted for patients in group B. Whether these translational metabolomic profiles of local mediators in inflammation and resolution can be used to determine patient outcomes and/or pinpoint their response to surgery or progress to recovery remains for future studies. Nonetheless, the present results identify chemical mediators and metabolomic pathway markers that show temporal changes on surgery and recovery in AAA patients. They also establish the feasibility of using lipidomic metabolomic profiles to gauge initial responses to surgical interventions and their potential outcomes. Together, these results suggest that enhancing the patient profile of endogenous anti-inflammatory and pro-resolving mediators may shorten recovery times, resolution, and improve outcomes in these patients.

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ABBREVIATIONS

17-HDHA	17S-hydroxy-docosa-4Z, 7Z, 10Z, 13Z, 15E, 19Z-hexaenoic acid
18-HEPE	18-hydroxy-eicosapentaenoic acid
12-HETE	12-hydroxyeicosatetraenoic acid
15-HETE	15-hydroxyeicosatetraenoic acid
LOX	lipoxygenase
LTB₄	leukotriene B ₄ 5S, 12R-dihydroxy-eicosa-6Z, 8E, 10E, 14Z-tetraenoic acid
LXA₄	lipoxin A ₄ 5S, 6R, 15S-trihydroxy-eicosa-7E, 9E, 11Z, 13E-tetraenoic acid
15-epi-LXA₄	5S, 6R, 15R-trihydroxy-eicosa-7E, 9E, 11Z, 13E-tetraenoic acid
PGE₂	prostaglandin E ₂ , 9-oxo-11a,15S-dihydroxy-prosta-5Z,13E-dien-1-oic acid
Rv	resolvin
RvE1	resolvin E1 (5S,12R,18R-trihydroxy-eicosa-6Z,8E,10E,14Z,16E-pentaenoic acid)

References

1. Kumar, V.; Abbas, AK.; Fausto, N.; Robbins, SL.; Cotran, RS. Robbins and Cotran pathologic basis of disease. 7. Philadelphia: Elsevier Saunders; 2005.
2. Serhan CN, Brain SD, Buckley CD, Gilroy DW, Haslett C, O'Neill LA, Perretti M, Rossi AG, Wallace JL. Resolution of inflammation: State of the art, definitions and terms. *The FASEB Journal*. 2007; 21:325–332. [PubMed: 17267386]
3. Perry VH, Cunningham C, Holmes C. Systemic infections and inflammation affect chronic neurodegeneration. *Nature Reviews Immunology*. 2007; 7:161–167.
4. Hansson GK, Libby P. The immune response in atherosclerosis: A double-edged sword. *Nature Reviews Immunology*. 2006; 6:508–519.
5. Laffey JG, Boylan JF, Cheng DC. The systemic inflammatory response to cardiac surgery: Implications for the anesthesiologist. *Anesthesiology*. 2002; 97:215–252. [PubMed: 12131125]
6. Gelman S. The pathophysiology of aortic cross-clamping and unclamping. *Anesthesiology*. 1995; 82:1026–1060. [PubMed: 7717537]
7. Serhan CN. Resolution phase of inflammation: Novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annual Review of Immunology*. 2007; 25:101–137.
8. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: Structures, biosynthesis, and biological effects. *Science*. 1987; 237:1171–1176. [PubMed: 2820055]
9. Yedgar S, Krinsky M, Cohen Y, Flower RJ. Treatment of inflammatory diseases by selective eicosanoid inhibition: A double-edged sword? *Trends in Pharmacological Sciences*. 2007; 28:459–464. [PubMed: 17692394]
10. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: Signals in resolution. *Nature Immunology*. 2001; 2:612–619. [PubMed: 11429545]
11. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL. Resolvins: A family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *The Journal of Experimental Medicine*. 2002; 196:1025–1037. [PubMed: 12391014]
12. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *The Journal of Experimental Medicine*. 2000; 192:1197–1204. [PubMed: 11034610]
13. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M. Maresins: Novel macrophage mediators with potent antiinflammatory and proresolving actions. *The Journal of Experimental Medicine*. 2009; 206:15–23. [PubMed: 19103881]
14. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: Dual anti-inflammatory and pro-resolution lipid mediators. *Nature Reviews Immunology*. 2008; 8:349–361.
15. Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature*. 2007; 447:869–874. [PubMed: 17568749]
16. Claria J, Serhan CN. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proceedings of the National Academy of Sciences of the United States of America*. 1995; 92:9475–9479. [PubMed: 7568157]
17. Yang R, Chiang N, Oh SF, Serhan CN. Metabolomics-lipidomics of eicosanoids and docosanoids generated by phagocytes. *Curr Protoc Immunol*. 2011 in press.
18. Samuelsson, B. Les Prix Nobel: Nobel Prizes, Presentations, Biographies and Lectures. Almqvist & Wiksell; Stockholm: 1982. From studies of biochemical mechanisms to novel biological mediators: prostaglandin endoperoxides, thromboxanes and leukotrienes; p. 153-74.
19. Nathan C, Ding A. Nonresolving inflammation. *Cell*. 2010; 140:871–882. [PubMed: 20303877]
20. Clouse WD, Hallett JW Jr, Schaff HV, Gayari MM, Ilstrup DM, Melton LJ 3rd. Improved prognosis of thoracic aortic aneurysms: A population-based study. *JAMA*. 1998; 280:1926–1929. [PubMed: 9851478]
21. Lederle FA, Wilson SE, Johnson GR, Reinke DB, Littooy FN, Acher CW, Ballard DJ, Messina LM, Gordon IL, Chute EP, Krupski WC, Busuttill SJ, Barone GW, Sparks S, Graham LM, Rapp

- JH, Makaroun MS, Moneta GL, Cambria RA, Makhoul RG, Eton D, Ansel HJ, Freischlag JA, Bandyk D. Immediate repair compared with surveillance of small abdominal aortic aneurysms. *The New England Journal of Medicine*. 2002; 346:1437–1444. [PubMed: 12000813]
22. Pronovost PJ, Jenckes MW, Dorman T, Garrett E, Breslow MJ, Rosenfeld BA, Lipsett PA, Bass E. Organizational characteristics of intensive care units related to outcomes of abdominal aortic surgery. *JAMA*. 1999; 281:1310–1317. [PubMed: 10208147]
 23. Ernst CB. Abdominal aortic aneurysm. *The New England Journal of Medicine*. 1993; 328:1167–1172. [PubMed: 8455684]
 24. Lamblin N, Ratajczak P, Hot D, Dubois E, Chwastyniak M, Beseme O, Drobecq H, Lemoine Y, Koussa M, Amouyel P, Pinet F. Profile of macrophages in human abdominal aortic aneurysms: a transcriptomic, proteomic, and antibody protein array study. *J Proteome Res*. 2010; 9(7):3720–3729. [PubMed: 20513153]
 25. Abdul-Hussien H, Hanemaaijer R, Kleemann R, Verhaaren BF, van Bockel JH, Lindeman JH. The pathophysiology of abdominal aortic aneurysm growth: Corresponding and discordant inflammatory and proteolytic processes in abdominal aortic and popliteal artery aneurysms. *Journal of Vascular Surgery*. 2010; 51:1479–1487. [PubMed: 20488324]
 26. Brand JM, Kirchner H, Poppe C, Schmucker P. The effects of general anesthesia on human peripheral immune cell distribution and cytokine production. *Clinical Immunology and Immunopathology*. 1997; 83:190–194. [PubMed: 9143381]
 27. Barry MC, Kelly C, Burke P, Sheehan S, Redmond HP, Bouchier-Hayes D. Immunological and physiological responses to aortic surgery: Effect of reperfusion on neutrophil and monocyte activation and pulmonary function. *The British Journal of Surgery*. 1997; 84:513–519. [PubMed: 9112905]
 28. Mayers I, Johnson D. The nonspecific inflammatory response to injury. *Canadian Journal of Anaesthesia*. 1998; 45:871–879. [PubMed: 9818111]
 29. Franke A, Lante W, Fackeldey V, Becker HP, Thode C, Kuhlmann WD, Markewitz A. Proinflammatory and antiinflammatory cytokines after cardiac operation: Different cellular sources at different times. *The Annals of Thoracic Surgery*. 2002; 74:363–370. discussion 70-1. [PubMed: 12173814]
 30. Norwood MG, Horsburgh T, Bown MJ, Sayers RD. Neutrophil activation occurs in the lower-limbs of patients undergoing elective repair of abdominal aortic aneurysm. *European Journal of Vascular and Endovascular Surgery*. 2005; 29:390–394. [PubMed: 15749040]
 31. Wu SH, Liao PY, Yin PL, Zhang YM, Dong L. Elevated expressions of 15-lipoxygenase and lipoxin A₄ in children with acute poststreptococcal glomerulonephritis. *The American Journal of Pathology*. 2009; 174:115–122. [PubMed: 19095947]
 32. Wu SH, Liao PY, Yin PL, Zhang YM, Dong L. Inverse temporal changes of lipoxin A₄ and leukotrienes in children with Henoch-Schönlein purpura. *Prostaglandins Leukotrienes and Essential Fatty Acids*. 2009; 80:177–183.
 33. Morris T, Stables M, Colville-Nash P, Newson J, Bellingan G, de Souza PM, Gilroy DW. Dichotomy in duration and severity of acute inflammatory responses in humans arising from differentially expressed proresolution pathways. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:8842–8847. [PubMed: 20421472]
 34. Morris T, Stables M, Hobbs A, de Souza P, Colville-Nash P, Warner T, Newson J, Bellingan G, Gilroy DW. Effects of low-dose aspirin on acute inflammatory responses in humans. *Journal of Immunology*. 2009; 183:2089–2096.
 35. Terrando N, Monaco C, Ma D, Foxwell BM, Feldmann M, Maze M. Tumor necrosis factor- α triggers a cytokine cascade yielding postoperative cognitive decline. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:20518–22. [PubMed: 21041647]
 36. Ho KJ, Spite M, Owens CD, Lancero H, Kroemer AH, Pande R, Creager MA, Serhan CN, Conte MS. Aspirin-triggered lipoxin and resolvin E1 modulate vascular smooth muscle phenotype and correlate with peripheral atherosclerosis. *The American Journal of Pathology*. 2010; 177:2116–2123. [PubMed: 20709806]

37. Zhang F, Yang H, Pan Z, Wang Z, Wolosin JM, Gjorstrup P, Reinach PS. Dependence of resolvin-induced increases in corneal epithelial cell migration on EGF receptor transactivation. *Investigative Ophthalmology and Visual Science*. 2010; 51:5601–5609. [PubMed: 20538990]
38. Baker N, O'Meara SJ, Scannell M, Maderna P, Godson C. Lipoxin A4: Anti-inflammatory and anti-angiogenic impact on endothelial cells. *Journal of Immunology*. 2009; 182:3819–3826.
39. Maldonado-Pèriz D, Golightly E, Denison FC, Jabbour HN, Norman JE. A role for lipoxin A₄ as anti-inflammatory and proresolution mediator in human parturition. *FASEB J*. 2010; 24:1096–1100. [PubMed: 20101096]
40. Birnbaum Y, Ye Y, Lin Y, Freeberg SY, Huang MH, Perez-Polo JR, Uretsky BF. Aspirin augments 15-epilipoxin A₄ production by lipopolysaccharide, but blocks the pioglitazone and atorvastatin induction of 15-epi-lipoxin A₄ in the rat heart. *Prostaglandins & Other Lipid Mediators*. 2007; 83:89–98. [PubMed: 17259075]
41. Planagumà A, Pfeffer MA, Rubin G, Croze R, Uddin M, Serhan CN, Levy BD. Lovastatin decreases acute mucosal inflammation via 15-epi-lipoxin A₄. *Mucosal Immunol*. 2010; 3:270–279. [PubMed: 20130564]
42. Di Gennaro A, Wågsäter D, Mäyränpää MI, Gabrielsen A, Swedenborg J, Hamsten A, Samuelsson B, Eriksson P, Haeggström JZ. Increased expression of leukotriene C4 synthase and predominant formation of cysteinyl-leukotrienes in human abdominal aortic aneurysm. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:21093–21097. [PubMed: 21078989]
43. Galle C, De Maertelaer V, Motte S, Zhou L, Stordeur P, Delville JP, Li R, Ferreira J, Goldman M, Capel P, Wautrecht JC, Pradier O, Dereume JP. Early inflammatory response after elective abdominal aortic aneurysm repair: A comparison between endovascular procedure and conventional surgery. *Journal of Vascular Surgery*. 2000; 32:234–246. [PubMed: 10917982]
44. Chiang N, Hurwitz S, Ridker PM, Serhan CN. Aspirin has a gender-dependent impact on antiinflammatory 15-epi-lipoxin A₄ formation: A randomized human trial. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2006; 26:e14–e17.
45. Chiang N, Bermudez EA, Ridker PM, Hurwitz S, Serhan CN. Aspirin triggers antiinflammatory 15-epi-lipoxin A₄ and inhibits thromboxane in a randomized human trial. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:15178–15183. [PubMed: 15471991]
46. Dona M, Fredman G, Schwab JM, Chiang N, Arita M, Goodarzi A, Cheng G, von Andrian UH, Serhan CN. Resolvin E1, an EPA-derived mediator in whole blood, selectively counterregulates leukocytes and platelets. *Blood*. 2008; 112:848–855. [PubMed: 18480426]
47. Vane, JR. Les Prix Nobel: Nobel Prizes, Presentations, Biographies and Lectures. Almquist & Wiksell; Stockholm: 1982. Adventures and excursions in bioassay: the stepping stones to prostacyclin; p. 181–206.
48. Massagué J. The transforming growth factor-beta family. *Annual Review of Cell Biology*. 1990; 6:597–641.
49. Ferrara N. Vascular endothelial growth factor: Basic science and clinical progress. *Endocrine Reviews*. 2004; 25:581–611. [PubMed: 15294883]
50. Serbina N, Jia T, Hohl T, Pamer E. Monocyte-mediated defense against microbial pathogens. *Annual Review of Immunology*. 2008; 26:421–452.
51. Andre P. P-selectin in haemostasis. *British Journal Haematology*. 2004; 126:298–306.
52. DiStasi MR, Ley K. Opening the flood-gates: How neutrophil-endothelial interactions regulate permeability. *Trends in Immunology*. 2009; 30:547–556. [PubMed: 19783480]

Fig. 1. Lipid mediator metabolome. Arachidonic acid-, EPA- and DHA-derived mediators and pathway markers studied in the AAA patients. Also see Table 2 for functional annotations and peptide mediators.

Fig. 2.

Temporal analysis of lipid mediators. Time course of **a** LXA₄, **b** LTB₄, **c** 15-epi-LXA₄, **d** PGE₂, **e** TXB₂, **f** LTC₄/D₄/E₄, and **g** 6-keto-PGF1α. Samples from AAA patients on aspirin were collected before clamping of aortic artery, and 5 min, 30 min, 6 h, 24 h, 48 h, and 72 h after unclamping. Plasma was obtained and levels of lipid mediators determined (see “MATERIALS AND METHODS” section). Results represent mean±SEM ($n=7-15$, * $p<0.05$).

Fig. 3. Time course of chemical mediators before and after AAA surgery: AAA patient inflammation-resolution profiles representative of two main groups: group A and group B, *upper and lower panels*. Plasma was obtained and levels of lipid mediators and cytokines were determined (see “MATERIALS AND METHODS” section).

Fig. 4. Generation of eicosanoids and their precursors: (*upper left*) arachidonic acid and downstream bioactive products. Representative plasma samples from patients with differing inflammation–resolution profiles 24 h after unclamping of the aortic artery were extracted for targeted lipidomics (see “MATERIALS AND METHODS” section), and levels of LOX-derived products/markers and eicosanoids (*lower right*) were analyzed and quantitated by LC-MS/MS. Representative mass spectra (MS–MS) are shown ($n=4-6$) for **b** 15-HETE and **c** PGE₂.

Fig. 5.

Generation of resolvins, protectins and related biosynthesis markers: *upper left* DHA and DHA metabolome for mediators. Representative plasma samples obtained from patients with different inflammation-resolution profiles at 24 h after unclamping of the aortic artery were extracted for targeted lipidomics, and *upper right* levels of D-series resolvins and maresin biomarkers were identified and quantitated by LC-MS/MS. (*lower left*) EPA and related intermediates. *Lower right* E-series resolvins biomarker 18-HEPE was identified and quantitated by LC-MS/MS. Representative mass spectra ($n=4-6$) are shown for **b** 17-HDHA and **c** 18-HEPE.

Table 1**AAA Patients**

	<i>n</i> (%)
Demographic	
Age (mean±SD)	69.1±9.6
Male: <i>n</i> (%)	10 (73)
Comorbidities	
Hypertension	15 (100)
Diabetes mellitus	2 (13)
Renal insufficiency	0 (0)
Medications	
Statins	10 (73)
Insulin	0 (0)
Aspirin	12 (80)
Beta adrenergic antagonists	14 (93)

All surgeries were elective

Table 2**Functional Annotation for Chemical Mediators**

Mediator/marker	Pathway	Function	Ref.
Pro-resolving mediators			
Lipoxin A ₄	Marker of 15-LOX-5-LOX or 5-LOX-12-LOX pathways (transcellular biosynthesis)	Stimulation of macrophage phagocytosis of apoptotic PMN and cellular debris. Mediates platelet-neutrophil interactions. Stimulated IL-10 production during phagocytosis	[7,14]
Aspirin-Triggered-LXA ₄	Aspirin treatment, aspirin-triggered epimer of LXA ₄	Block leukocyte adhesion to endothelium, inhibits thromboxane	[16,44,45]
Pro-inflammatory mediators			
LTB ₄	Marker of 5-LOX LT pathway activation	Stimulates PMN, T cell chemotaxis	[8]
LTC ₄ /D ₄ /E ₄	Marker of 5-LO pathway	Increase vascular permeability, smooth muscle contraction, bronchoconstriction	[8]
PGE ₂	Arachidonic acid-COX-derived product	Bronchoconstriction, vasodilation, inflammatory cell activation, pain sensitization, signals switch from pro to anti-inflammatory mediator production	[9]
Markers of hemostasis/vascular function			
TXB ₂	Inactive marker of TXA ₂	Potent activator of platelet aggregation	[46]
6-keto-PGF1 α	Stable product and biomarker of PGI ₂	Prostacyclin induces vasodilation and inhibits platelet aggregation	[47]
Cytokines			
TGF β 1	Anti-inflammatory/pro-resolving	Promotes tissue remodeling, wound healing, atheroprotective	[48]
VEGF	Angiogenesis	Stimulates IL-6, TNF- α , IFN- γ and IL-8 secretion	[49]
IL-10	Anti-inflammatory/pro-resolving	Anti-Inflammatory cytokine; suppresses the production of prostaglandin E ₂ , TNF- α , IL-1, IL-6, and IL-8.	[50]
Adhesion Molecules			
sP-Selectin	P-selectin on surface of activated platelets and endothelial cells	Recruitment of leukocytes to site to inflammation, leukocyte rolling	[51]
sICAM/CD54	Marker of endothelial activation	Leukocyte endothelial transmigration, signal transduction in pro-inflammatory pathways	[52]