Manifold beneficial effects of acetyl salicylic acid and nonsteroidal anti-inflammatory drugs on sepsis.

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Abstract.

Acetyl salicylic acid (ASA) and nonsteroidal anti-inflammatory drugs (NSAIDs) may have potential as adjunctive agents for sepsis. This review considers the large body of literature that indicates a basis for sepsis therapy with ASA and suggests an agenda for future intervention studies in sepsis prevention and treatment.

ASA and NSAIDs have beneficial effects in numerous experimental models of sepsis. Low doses of ASA of 100 mg/day or less trigger synthesis of lipoxins that are anti-inflammatory and aid in resolution of inflammation. Higher doses of ASA and NSAIDs act to reduce NF-kappaB stimulation and inhibit numerous septic pathways. While a previous randomised controlled trial of ibuprofen failed to show a reduction in mortality in sepsis it did reduce clinical manifestations of sepsis. More recent observational studies have shown reduction in sepsis or acute lung injury leading to lower mortality in ICU patients treated with ASA.

Low-dose ASA appears to be beneficial in prevention and treatment of sepsis and SIRS. If proven, this intervention would have a major, cost effective impact on sepsis care.

Introduction.

Acetyl salicylic acid (ASA) or aspirin has had a profound effect on human health since its discovery in 1897 [1]. This nonspecific cyclo-oxygenase (COX) inhibitor isone of the most widely used drugs in the world due to its potent vascular disease prevention [2].ASA contributes to systemic signalling in plants in response to parasitic invasion acting to limit infection severity[3].

ASA and other non-steroida lanti-inflammatory drugs (NSAIDs) have beneficial actions on inflammatory pathways contributing to sepsis. Low-doses of ASA (75-81 mg/day) trigger lipoxin synthesis [4], mediating both anti-inflammatory and inflammation-resolving effects [5]. Additionally, the NF-kappaB (NF-κB)cellular signalling pathway can be inhibited by ASA and NSAIDs [6].

The full range of therapeutic effects of ASA in sepsis is unknown. NSAIDs were shelved as agents for the therapy of sepsis because of the negative result of the single randomised controlled trial in this area [7]. ASA has activity against numerous cellular pathways and cytokine mediators of sepsis and demonstrated benefits in animal models with treatment after establishment of sepsis or tissue injury. Human pharmacokinetics show pharmacological levels achieved with safe doses. Finally, numerous observational studies suggest benefits of ASA in sepsis.

ASA triggers lipoxins, reducing and resolving inflammation. Figure 1.

Lipoxin A4 is a natural lipid mediator that actively alters a variety of inflammatory processes including nitric oxide production [8], the inhibition of superoxide production

by neutrophils, and prevention of neutrophil/endothelial interactions (reviewed in [5]). ASA triggered 15-epi-lipoxin A4 (ATL) has the same activity as lipoxinA4 [9]. ASA is the only NSAID that can directly acetylate the COX2 site in endothelial and epithelial cells to induce the formation of ATL.

Lipopolysaccharide (LPS) stimulated polymorphonuclear leukocytes (PMNs) exhibit delayed apoptosis contributing to ongoing inflammation in sepsis[10]. PMN apoptosis was reinstated by the addition of pharmacological concentrations of ASA and was mediated through both ATL production [11, 12] and NF-κB inhibition [13], resulting in a reduction in proinflammatory cytokines [11]. ATL also reduces the secretion of TNF-alpha by T lymphocytes [14]. Importantly, such ATL mediated actions are both anti-inflammatory and contribute to the resolution of the sepsis cascade. Recent studies also demonstrate that ASA triggers synthesis of resolvins, a novel class of lipid mediators with activities that resemble ATL [5].

Salicylate and NSAID effects on inflammatory pathways, particularly NFκB pathway modulation.

Investigators searching for potential antitumor effects of ASA determined its ability to inhibit NF-κB activation, an anti-inflammatory pathway additional to COX inhibition. NF-κB contributes to activation of genes involved in cell cycle control (cyclin-D1)[15], inflammation (TNF-alpha, IL-6, COX) [6], and coagulation (tissue factor, TF) [16]. For example, there is a dose-dependent reduction in NF-κB gene transcription seen in LPS-stimulated human monocytes treated with salicylates and NSAIDs [17].

Cellular stimulation by various noxious stimuli, like tissue damage, infection or cytokines, releases NF-κB from binding to its inhibitory cytoplasmic protein complex, IκB, allowing NF-κB to translocate to the cell's nucleus and transcribe genes as above. Salicylates and NSAIDs inhibit NF-κB-activation by blocking ATP binding and phosphorylation of the cellular kinase IKK-β [18], preserving expression of IκB [6]. It has been shown that ASA is a less potent inhibitor of NF-κB activation than many NSAIDs and other unrelated drugs including tamoxifen and curcumin [15]. ASA's IC_{50} for inhibition of TNF-alpha-induced NF-κB activation is 5.67 mM, 10-fold less potent than indomethacin, and 500 times less potent than the anti-estrogen agent, tamoxifen [15].

The *in-vitro* ASA concentration required for COX inhibition is 1000-fold less than the dose required for NF-κB inhibition [15]. Previous authors considering the potential of ASA as an NF-κB inhibitor in critical care therapeutics have raised concerns that potentially toxic, conventional anti-inflammatory doses are required [19]. However, low-doses of ASA are required to produce ATL mediated anti-inflammatory effects [9].

Salicylatesblock some of the microbial mediators of sepsis.

Additional benefits of ASA therapy in sepsis may arise from demonstrated inhibition of prominent microbial mediators of sepsis particularly in gram-positive infection. Salicylic acid (SA), ASA's major metabolite, has been shown to exert its *in-vitro* effects on *S. aureus* virulence through hyper-activation of the stress response regulon, *sig B* [20, 21]. This results in reduced expression of at least two staphylococcal structural genes crucial to pathogenesis, *hla* (the α-toxin gene) and *fnbA* (a major fibronectin-binding adhesin)

[22, 23]. As ASA and NSAIDs reduce NF-κB activation these drugs may have profound effects in endotoxaemia due to gram-negative sepsis. Cell associated bacteria like rickettsia also activate NFκB which when blocked by ASA, attenuated vascular endothelium infection[24].

S. aureus infective endocarditis (IE) may be a condition for which ASA has specific effects on microbial pathogenesis leading to improved patient outcomes. Recent insights into IE pathogenesis suggest how the presence of ASA in the early stages of IE may reduce the extent of valvular and perivalvular infection in IE. ASA-mediated, platelet-dependent effects include a reduction in platelet aggregation, yielding smaller sterile vegetations, the platform upon which IE is initiated [25]. FnbA is a key determinant in both the initial vegetation colonization and persistence stages in IE [22, 23]. In contrast, hla is important in the post-colonization, progression phases of this infection [26].

In experimental animal models of staphylococcal-IE, improved microbiologic and embolic outcomes are seen, especially when ASA is provided to animals prior to their infectious challenge [27] or when *S. aureus* is pre-exposed to ASA prior to IV challenge [25].Importantly, improved microbiologic and embolic outcomes have also been seen in animals with staphylococcal-IE given ASA after the induction of experimental IE [21, 25]. Recent human cohort study data [28, 29] indicate the benefits of ASA in *S. aureus*-IE. Concerns relating to an increased risk of major bleeding in ASA treated patients with *S. aureus*-IE [30] are not proven to date

The role of activated platelets in sepsis.

Activated platelets contribute to sepsis pathogenesis with benefits inherent in ASA therapy. Early animal models of endotoxaemia showed prolonged survival time in ASA pretreated animals through antiplatelet affects [31]. Organ sequestration of activated platelets plays an important role in sepsis, and pretreatment with ASA 30 minutes before endotoxin challenge in sheep reduced accumulation in the lungs and liver [32]. Platelet sequestration was not prevented, however, by indomethacin pretreatment in endotoxin challenged rats [33]. The thrombocytopenia commonly seen in patients with sepsis may similarly be contributed to by sequestration of platelets as has been demonstrated in human lungs, liver and intestines [34, 35]. Sepsis induces changes in platelet aggregation although reported changes have been inconsistent, potentially as a result of the different models studied [36]. *In-vitro* examination of *Streptococcus pneumoniae* induced platelet aggregation showed that this was dependent on toll-like receptor 2 [37].

Antiplatelet agents other than ASA may also improve sepsis mediators as clopidogrel given 5 days before endotoxin challenge reduced TNF-alpha and IL-6 levels in rats [38] and prevented thrombocytopenia in a mouse polymicrobial sepsis model[39].

Isolated organ and whole animal models showing the benefit of ASA and NSAIDs on organ specific and general effects of sepsis.

Bacterial virulence determinants contribute to depression of cardiac function in septic shock. Staphylococcal α-toxin is known to reduce myocardial function, with this effect being prevented by indomethacin (100 μmol/l) or ASA (500 μmol/l) [40]. When isolated rat hearts were exposed to LPS, increases in myocyte-derived TNF-alpha induced a reduction in cardiac contractility but not coronary perfusion. Indomethacin was able to partially reverse this TNF-alpha-related impairment in myocardial function [41].

Alveolar macrophages (AM) become activated during experimental septic shock with increase in TNF-alpha production via the NF-κB pathway. Macrophage inhibitory protein-2 (MIP) production is also increased in activated AM leading to increased PMN migration into the pulmonary interstitium. NF-κB inhibition reduced both TNF-alpha and MIP production by LPS stimulated rat AM's [42, 43]. Staphylococcal α-toxin also produces ventilation perfusion mismatch in perfused rabbit lungs [44]. This exotoxin-mediated process was inhibited by ASA. Importantly the vasculature changes resulting in pulmonary hypertension could also be potentiated by priming with endotoxin [45]. Activation of AM by LPS stimulation led to pulmonary vasoconstriction in a perfused rabbit lung model [46]. Here, rabbit lungs primed with LPS then exposed to arachidonic acid showed up to 3 fold increases in pulmonary artery pressure. This was completely reversed by pre-incubation with 1 μmol ASA. Resolution of LPS-induced acute lung injury (ALI) in mice was improved by treatment with ATL administered at the height of the inflammatory response [47]. ATL has been shown to be effective in treating as well as preventing ALI (carrageenan and LPS-induced) in mouse models [12, 47]. COX-2 expression has been shown to mediate recovery of ALI in mice via reduced leukocyte

recruitment and resolution of epithelial integrity. Selective COX-2 inhibition blocked these effects, while non-selective COX inhibition via ASA did not [48].

Many animal models of sepsis have shown beneficial effects of ASA or NSAIDs, particularly with ibuprofen [49-52]. Rat-endotoxemia models showed that pretreatment with ASA [51] substantially reduced mortality. In an ovine endotoxic shock model, ibuprofen given before and after endotoxin infusion reduced early stage hypovolemia and hypoxia without effecting late changes [53]. Another canine endotoxic shock model mirrored the findings of isolated rat hearts described earlier as ibuprofen was shown to protect against depression of the cardiac index [52]. Finally, in a rabbit Group B streptococcal shock model, ibuprofen significantly improved short-term survival [54].

Not all animal model data on NSAIDs in experimental sepsis indicate a beneficial role. COX-2 inhibition increased mortality in a rodent CLP model whereas it improved survival in an endotoxaemia model [55]. The murine CLP model has been shown to have a different cytokine profile to endotoxaemia models with more prolonged elevation of TNF-alpha, IL-6 and MIP-2 [56]. These differences may account for conflicting results with NSAID intervention in the different models described above.

Pharmacokinetic data relating to low-dose ASA.

The *in-vitro* concentrations required to produce NF-κB inhibition indicate that 100mg ASA/day cannot mediate sepsis outcomes via this pathway. However, multiple clinical

trials in healthy hosts have shown that 81 mg of ASA per day is sufficient to increase ATL [57, 58]. The ASA concentration required *in-vitro* to achieve 50% inhibition of NFκB is 5.67 mM [15]. The steady state ASA blood concentration in healthy human volunteers following seven days of 160 mg ASA daily is only 0.31mM [59]. Maximal concentrations after seven days of the same dose were 2.99 mM [59]. Maximal ASA concentrations seen after a single dose of 325mg enteric coated ASA were3.99 – 7.92 mM [60]. First order kinetics apply to ASA up to doses of 400mg [61]. The pharmacokinetics and pharmacodynamics of ASA in critically ill patients has not been defined as yet but a prospective trial is underway. (http://www.anzctr.org.au/trial_view.aspx?id=343088). As ASA absorption is rapidly achieved from the stomach, reduced splanchnic blood supply consequent on sepsis and hypotension will impact relatively minimally [62].

Studies of ASA and NSAIDs in inflammation in humans.

A recent human endotoxin challenge study showed that high dose ASA (425 mg bd) inhibited endotoxin-induced changes in platelet plug formation [63]. Other human-endotoxin challenge studies failed to show benefits of ASA on different aspects of the sepsis cascade. LPS-induced coagulation was not inhibited by ASA with no reduction in thrombin formation or TF production [63, 64]. Expression of endothelial cell adhesion molecules such as e-selectin and von Willebrand factor antigen that recruit inflammatory cells were not reduced by pre-dosing with 1000mg ASA [65]. Mean serum ASA levels in the experimental subjects were ~0.2mM at the time of endotoxin challenge [64, 65],

which is substantially lower than 5.67mM ASA concentration shown to block NF-κB activation *in-vitro* [15] but clearly in the range required for ATL activation. Interestingly, a placebo-controlled human endotoxaemia model study of ibuprofen use showed TNF-alpha and IL-8 responses were significantly higher in ibuprofen treated subjects [66].

Two randomised controlled studies have shown that low-doses of ASA trigger ATL. These studies have both been in healthy volunteers [57, 58]. The benefits of low-dose ASA (75mg) were recently illustrated using a human skin blister model of inflammation in healthy subjects [9]. In this model, low-dose ASA's anti-inflammatory effect was due to reduced neutrophil migration mediated by ATL synthesis and nitric oxide secretion. Here, ATL was increased in blister fluid 24 hours after ASA dosing [9]. Other septic cascade pathways affected by ATL have not been adequately explored.

Clinical experience of NSAIDs and ASA in sepsis. Table 1.

A large scale randomised controlled sepsis trial of ibuprofen was performed involving septic patients treated with 48 hours of intravenous ibuprofen [7]. In the study, ibuprofen treatment led to substantial reductions in sepsis-induced prostacyclin and thromboxane excretion. Reductions in temperature, lactic acid levels and oxygen consumption were also shown in the ibuprofen-treated group. There was no significant improvement in the incidence or duration of septic shock but a non-significant, 3% absolute reduction in mortality was found in ibuprofen treated patients [7]. In a post hoc, sub-group analysis of the small group of patients who entered the study with hypothermic septic shock and who

had the highest mortality, ibuprofen was associated with a survival benefit (36% absolute risk reduction) [67]. Ibuprofen treated patients in the sub-analysis were significantly younger than placebo treated controls as there had been no randomisation stratification for temperature [67].

The failure of the ibuprofen study in sepsis [7] predominantly relates to its small sample size based on exaggerated estimates of reduction in mortalityas it was powered to show an unrealistic 35% reduction in mortality. Subsequent trials showing small mortality improvements in sepsis have required far greater sample size [68]. The study groups were well matched for disease severity [7]. Adequate therapy for proven blood stream infection was provided in both groups but there is no quantitation of the suitability of treatment for non-blood stream infection, which may have introduced a bias between the study groups. The two-day ibuprofen regimen may also have been insufficient. As is a perennial issue in sepsis trials, the inability to identify patients early in their disease may have limited the effect of ibuprofen as the effects of the established sepsis cascade may have been too great to respond to cyclo-oxygenase inhibition. Lastly, with subsequent knowledge of ATL's inflammation resolving effects, it may be that ASA is a superior agent to ibuprofen for management of sepsis.

A number of recent, observational studies have shown potential benefits of ASA or anti-platelet drugs in patients with sepsis. Septic ICU patients with no increased bleeding risk were observed in a single centre study to have lesser mortality if they were treated with antiplatelet agents, most commonly ASA [69]. A smaller series of patients with

community-acquired pneumonia was studied by the same investigators, who showed reduced length of hospital stay in those treated with antiplatelet agents [70]. Fears that ASA or NSAIDs may predispose to severe sepsis do not seem to be borne out [71]. A large cohort study of ICU patients has shown an association between administration of ASA to patients within 24 hours of the onset of SIRS or sepsis and reduced mortality. These patients had been treated with ASA prior to hospitalisation [72]. These studies [69, 70, 72] showed that 25% to 37% of patients in the ICU's examined were administered ASA or antiplatelet agents.

Recent studies have concentrated on the possibility that ASA may prevent ALI in patients at high risk for this manifestation of sepsis or trauma. A population-based study involving a tightly defined group showed reduced ALI and ARDS in patients admitted to medical ICU who had been receiving ASA [73]. A substantially larger study of a more heterogeneous ICU population from 22 US and Turkish hospitals failed to confirm this beneficial association although a trend to reduced ALI remained in ASA treated patients [74]. The apparent beneficial effects of statins in preventing sepsis and ALI in another ICU cohort were both potentiated by concomitant ASA use [75]. Studies to date, including the ibuprofen trial [7], provide evidence of clinical equipoise for the effect of ASA in critically ill patients.

There may be deleterious effects of salicylates or NSAIDs in sepsis. Renal impairment is a common and serious side effect of NSAID use [76]. Increases in bleeding due to salicylates and ASA are also of major concern although they are not shown to increase following low-doses of ASA. For instance, there was no overall increase in gastrointestinal bleeding risk in large-scale primary prevention studies involving ASA in

participants taking ≤70 mg/day [77]. It is difficult to be precise about the risk of bleeding in ASA-treated critically ill patients due to a paucity of data.

Potential treatment strategies for prevention or treatment of sepsis

The substantial body of literature reviewed from cellular, animal models and the trends from human studies suggest that ASA and NSAIDs may have beneficial roles in sepsis, and that further study is warranted. Sepsis prevention and reduction of infectious disease mortality may be shown in planned analysis of current ASA primary prevention studies [78]. Targeted prevention could also be considered in high-risk populations such as hospital inpatients with the aim of reducing the frequency and severity of nosocomial sepsis. Finally, ASA may be shown in future interventional trials to be beneficial in treating established sepsis. The demonstrated impact of low-dose ASA on human models of inflammation [9] suggest that this agent, rather than other NSAIDs is probably the best agent to consider for sepsis interventions. ASA alone stimulates ATL's with their anti-inflammatory and pro-resolution effects on sepsis [5], avoiding the greater toxicity of NSAIDs in the critically ill and potential deleterious, pro-inflammatory effects of selective COX-2 inhibition [48]. Properly targeted treatment with low-dose ASA could hold promise as a relatively safe, extremely cheap agent to use in sepsis even if it is shown to have only modest overall clinical benefit.

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Table 1.

Study design	Comparison	Sample size	Outcomes associated with ASA/NSAID/antiplatelet treatment.	Reference
Randomised controlled trial.				
Multicentre, ICU trial, USA. Patients with sepsis.	Indomethacin 10 mg/kg IV qid vs. placebo for 48 hours	455	No significant reduction in 30-day mortality. No adverse effects of ibuprofen	[7]
Observational studies.				
Single centre, Germany Consecutive ICU admissions.	Antiplatelet agents (25% of cohort) vs. none. ASA< 160mg/day was antiplatelet agent in 80%	615	Reduction in death during ICU admission. Odds ratio 0.19 (95% CI 0.12 to 0.33) on multivariable analysis	[69]
Single centre, Germany. Consecutive community acquired pneumonia hospital admissions.	Antiplatelet agents (20% of cohort) vs. none. ASA in 84%	224	Reduced hospital length of stay. Trend to reduced need for ICU admission	[70]
Single centre, Australia. Consecutive ICU admissions.	ASA vs. none. 37% cohort on ASA, <150mg in 96%	7945	Propensity analyses showed reduced mortality among patients with SIRS -6.2% (-9.5 to -3.5%); with sepsis -14.8%, (-18.9 to -8.6%). Increased risk of renal injury 3.3% (2.5 to 5.0%)	[72]
Single centre, USA. Consecutive ICU admissions.	Antiplatelet (49% of cohort) agents vs. none. ASA in 90%	161	Reduced acute lung injury in patients with at least one major risk factor for condition. Odds ratio 0.34 (0.13 – 0.88)	[73]
Multicentre, USA and Turkey. Consecutive ICU admissions.	ASA (25% of cohort) vs. none.	3855	No reduction in acute lung injury after propensity analysis for ASA use performed	[74]
Multicentre, USA. Consecutive ICU admissions.	Statin and ASA vs. ASA alone	575	ASA potentiated effect of statins in reducing acute lung injury and sepsis	[75]

Table and Figure headings.

Table 1. Summary of clinical studies on associations between SIRS / sepsis outcomes and acetyl salicylic acid (ASA) / NSAID / antiplatelet agent use. The observational studies all involved patients who had been taking the agents listed prior to hospitalisation.

Figure 1. Pathways and effects of aspirin-triggered lipoxin synthesis. Aspirin promotes the generation of 15*R*-hydroxyeicosatetraenoic acid (HETE) from arachidonic acid via the acetylation of COX-2. HETE is rapidly metabolized through the action of 5-lipoxygenase (5-LOX), leading to production of 15-epi lipoxin A4. This aspirin-triggered lipoxin (ATL) pathway mirrors classic lipoxin synthesis and function. ATL then mediates anti-inflammatory effects via reduced proinflammatory cytokines formed directly by stimulated lymphocytes as well as effects on phagocytes that contribute to additional pro-resolution effects on inflammation. Inhibition of plasma derived growth factor (PDGF), epidermal growth factor (EGF) and leukotriene D4 (LTD4) mediated signalling in neutrophils leads to reduced migration and promotes apoptosis while increasing phagocytosis in macrophages. The combined effects promote resolution of inflammation.

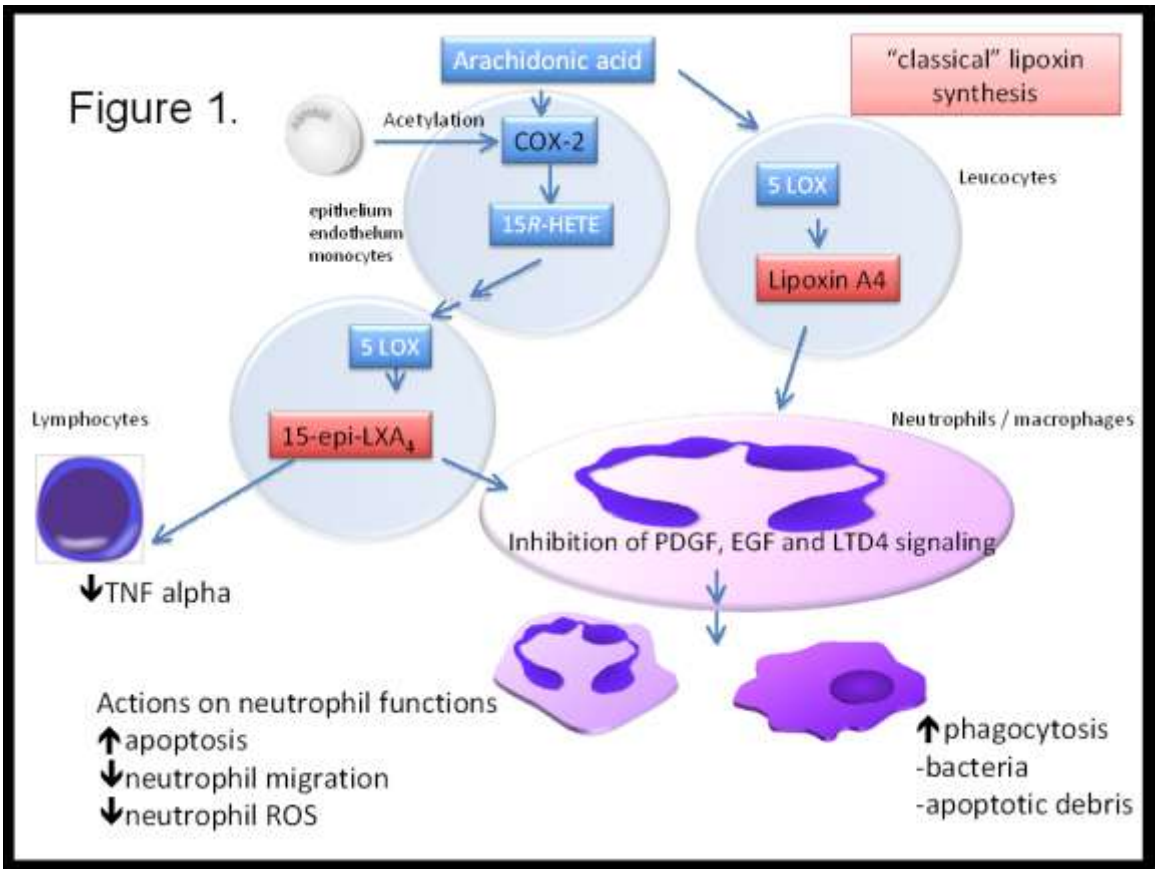
Figure 1.
Arachidonic acid
"classical" lipoxin synthesis
Acetylation
COX-2
epithelium
endothelium
monocytes
15R-HETE
5 LOX
Leucocytes
Lipoxin A4
5 LOX
Lymphocytes
15-epi-LXA4
Neutrophils / macrophages
Inhibition of PDGF, EGF and LTD4 signaling
TNF alpha
Actions on neutrophil functions
apoptosis
neutrophil migration
neutrophil ROS
phagocytosis
-bacteria
-apoptotic debris