Aspirin triggered lipid mediators: novel inhibitors of leucocyte trafficking

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Despite nearly 100 years of wide usage, complete knowledge of the therapeutic impact of aspirin is still evolving and new beneficial effects are being uncovered in cardiovascular disease and cancer. The irreversible acetylation of cyclo-oxygenase (COX)-1 and its more recently discovered isoform COX-2 with inhibition of prostaglandins is well appreciated and explains some, but not all, of the pharmacological actions of aspirin.

Another series of bioactive lipid mediators on which aspirin has an impact are lipoxins (LX), and their C15 epimers (15R), denoted aspirin triggered lipoxins (ATL, fig 1), which are biosynthesised by separate routes involving transcellular circuits. Native LX in the nanomolar range inhibit the adhesion and transmigration of polymorphonuclear leucocytes (PMNs) and hence serve as counter-regulatory signals operative in the resolution of inflammatory sites. Not only do LX serve counter-regulatory roles, but specific enantio-merically modified LX (ATL) may also be actual effectors of well established anti-inflammatory therapeutic actions of aspirin. The impact of 5S,6R,15S-trihydroxy-7,9,13-tran-11-cis-eicosatetraenoic acid (LXA3) and ATL was investigated in tumour necrosis factor (TNFα) initiated PMN responses in vitro and in vivo using the metabolically more stable LX analogues 15R/S-methyl-LXA4, and 15-epi-16-(para-fluoro)-phenoxy-LXA4. These compounds represent subtle modifications to the native LXA3 and ATL structure that prevent rapid metabolic inactivation of the lipoxin and 15-epi-LX structures. These compounds are also potent novel inhibitors of TNFα driven PMN associated inflammatory events in vitro as well as in vivo, as shown in a murine model with end points relevant to pulmonary inflammation.

Methods

Six day air pouches were raised on the dorsum of 6–8 week old male BALB/c mice by subcutaneous injection of 3 ml sterile air and experiments were conducted on day 6. Individual air pouches were injected with either vehicle alone (0.1% ethanol), TNFα, ATL stable analogue, or TNFα + ATL, with each suspended in 1 ml of endotoxin free phosphate buffered saline (PBS; pH 7.45) immediately before injection into pouch cavities. Mice were sacrificed and individual air pouches were lavaged, and the cell exudates were measured and analysed for cytokines/chemokines.

Results

PMNs express and release several cytokines among which interleukin (IL)-1β is a potent proinflammatory agent. We have recently investigated the actions of native LXA3 and its stable analogues 15R/S-methyl-LXA3 in the presence of TNFα (25 000–50 000 activity units/ml) or vehicle alone. At a concentration of 100 nM, 15R/S-methyl-LXA3, inhibited approximately 60% of IL-1β release, a value which is comparable to that obtained with native LXA3. Inhibition of IL-1β by LXA3 and its stable analogue was, at least in part, the result of a down-regulation in gene expression, since the IL-1β messenger RNA levels in cells treated with TNFα (10 ng/ml) plus 15R/S-methyl-LXA3 (100 nM) were decreased by approximately 60% compared with cells treated with TNFα alone. Since IL-1β and TNFα are two cytokines considered to be important in inflammation, the inhibition of IL-1β suggested that ATL might exert an in vivo anti-cytokine action.

TNFα evokes leucocyte infiltration in a chemokine-dependent fashion in the murine six day air pouch. We therefore evaluated the impact of ATL stable analogues 15R/S-methyl-LXA3 and 15-epi-16-(para-fluoro)-phenoxy-LXA3 in this model to determine whether they also intersec the cytokine/chemokine axis in vivo and inhibit leucocyte infiltration. Murine TNFα gives a transient infiltration of leucocytes to the pouch cavity in a time-dependent fashion with maximal accumulation at four hours. At a concentration of 25 nmol inhibited the TNFα stimulated recruitment of leucocytes by approximately 62%. Inhibition was evident at one hour and was maximal at 2–4 hours. At these intervals a reduction in leucocyte infiltration of more than 60% was noted that remained significantly reduced at eight hours. Inflamma-
ATLM: regulators of leucocyte traffic and a cytokine/chemokine axis

PMNs, eosinophils, and basophils as well as six day air pouch cavity inhibited migration of and intravenous administration was as effective as administration into the air pouch (topical). This stable ATL analogue was also about 100 times more potent than aspirin in blocking PMN influx to the pouch.

Since macrophage inflammatory peptide (MIP)-2 is the major chemokine involved in recruiting PMNs to the pouch cavity following injection of TNFα, we determined the action of 15R/S-methyl-LXA₄ in this TNFα induced chemokine-chemokine axis. MIP-2 and IL-1β are important proinflammatory cytokines, and IL-4, IL-10, and IL-13 possess immunomodulatory properties. Exudates from selected time intervals were collected and cell-free supernatants assessed for the presence of these murine cytokines. 15R/S-methyl-LXA₄ (25 nmol)

inhibited TNFα stimulated MIP-2 and IL-1β release by 48% and 30%, respectively (fig 2). 15R/S-methyl-LXA₄ alone in the air pouch did not stimulate MIP-2 or IL-1β release. In sharp contrast, 15R/S-methyl-LXA₄ stimulated the appearance of IL-4 within the exudates. This stimulation of IL-4 was observed both with and without TNFα. Neither IL-10 nor IL-13 was detected within the pouch exudates. These results show that administration of 15R/S-methyl-LXA₄ dramatically modified the cytokine/chemokine axis in TNFα initiated acute inflammation and, interestingly, this re-orientation of the cytokine/chemokine axis paralleled the reduction in leucocyte infiltration.

Discussion

LXA₄ and ATL (generated by separate biosynthetic mechanisms in vivo; fig 1 and Serhan et al10–12), as evidenced by the actions of the metabolically stable analogue 15R/S-methyl-LXA₄, are both potent cytokine regulating lipid mediators that can also have an impact on the course of inflammation initiated by TNFα. In this exudate and skin wound model,13 15R/S-methyl-LXA₄ not only inhibited TNFα elicited PMNs, monocytes, basophils, and eosinophils as well as the appearance of IL-1β and MIP-2, but also stimulated IL-4 appearance within the pouch. These results provide the first evidence to indicate that lipoxins and ATL induce upregulation of potential “anti-inflammatory” cytokines such as IL-4.15 It is likely that both the inhibition of IL-1β and MIP-2 within exudates and IL-4 levels in the surrounding tissues by metabolically stable LX analogues may represent, in part, some of the in vivo impact of LXA₄ and aspirin triggered 15-epi-LXA₄. These findings provide a new understanding of the relationship between “anti-inflammatory” cytokines and lipid mediators and also open new avenues to investigate protective lipid and protein mediators in host defence.

In summary, these results suggest that LXA₄ and aspirin triggered LXA₄ appear to be involved in controlling not only acute inflammatory responses, but also mechanisms that can influence chronic inflammatory responses. The recent results reviewed here also support the notion that aspirin may exert its beneficial action in part via the biosynthesis of endogenous ATLMs such as 15-epi-LXA₄, that can, in turn, act directly on PMNs as well as affect the appearance of chemokines and cytokines. Thus, LX-ATL can protect host tissues via multi-level regulation of proinflammatory signals and may be relevant new lipid mediators of interest in airway diseases.

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3 Cronstein BN, Montesinos MC, Weissmann G. Salicylates and sulfasalazine, but not glucocorticoids, inhibit leukocyte accumulation by an adenosine-dependent mechanism that is independent of inhibition of prostaglandin synthesis and p105 of NFkB. Proc Natl Acad Sci USA 1999;96:3577–81.


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