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Angiogenesis and lipoxins

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Abstract

Angiogenesis, the growth of new capillaries from pre-existing ones, occurs through dynamic functions of the endothelial cells (EC), including migration, proliferation and maturation, which are essential to achieve an organized formation of the vessel sprout. Aspirin-triggered lipoxins (ATL), the 15*R* enantiomeric counterparts of native lipoxins, are endogenous lipid mediators generated within the vascular lumen during multicellular responses, which display potent and well-described immunomodulatory actions. Here we present some of the findings regarding the inhibition of EC responses in vitro and in vivo by these novel compounds and the modulation of fundamental steps of the angiogenic process, identifying previously unappreciated vascular actions of locally generated ATL and their longer acting synthetic analogs.

1. Introduction

Angiogenesis, the sprouting of new blood vessels from pre-existing endothelium, is an important component of embryonic vascular development, organ regeneration and wound healing, including the recovery from myocardial ischemia and from peptic ulcer. Conversely, it also contributes to the progression of pathologies that depend on neovascularization, including diabetic retinopathies, rheumatoid arthritis, and tumor growth [1,2].

The angiogenic process is controlled by the net balance between molecules that have positive and negative regulatory activity and this concept had led to the notion of the 'angiogenic switch', depending on an increased production of one or more of the positive regulators of angiogenesis [3]. Numerous factors can impact this balance, including mitogenic factors such as fibroblast growth factor 1 and 2 (FGF), transforming growth factor-α (TGF-α) and vascular endothelial growth factor (VEGF) [4,5]. The nonmitogenic factors include selected cytokines, CXC chemokines and angiopoietins [4,6]. More recently, internal peptide fragments of larger polypeptides, angiostatin and endostatin, have been identified as potent angiogenesis inhibitors [7].

Endothelial cells (EC) are normally quiescent, becoming activated during the angiogenic response. The sprouting process consists of several consecutive steps induced and regulated by a network of mitogens and cytokines [4,8]:

- local degradation of the basement membrane;
- migration of EC toward the angiogenic stimulus;
- formation of a lumen and EC proliferation;
- loop formation by connection of individual sprouts;
- vessel wall maturation (alignment of pericytes and smooth muscle);
- formation of new basement membrane.

2. Eicosanoids and angiogenesis

Arachidonic acid (AA)-derived metabolites have potent biologic actions on vascular EC that can

Abbreviations: ASA, aspirin; ATL, aspirin-triggered 15-epi-lipoxins; ATL-1, 15-epi-16-(*para*-fluoro)-phenoxy-lipoxin A_4; COX, cyclooxygenase; EC, endothelial cells; LO, lipoxygenase; LX, lipoxin; VEGF, vascular endothelial growth factor.

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synthesize various eicosanoids, including the 12-lipoxygenase (LO) product 12(*S*)-hydroxyeicosatetraenoic acid (12(*S*)-HETE), which is a signaling molecule with effects on cell proliferation, motility, and angiogenesis [9]. 12(*S*)-HETE was found to activate Erkl/2 kinases in LNCaP cells [10] and to induce the degradation of IkB by the S6 proteasomal pathway thus activating NF-kB in PC-3 cells [11]. Overexpression of 12-LO and 15-LO-1 in prostate cancer cells stimulates tumor angiogenesis and growth, suggesting a facilitative role for these enzymes in tumor progression [10].

A recent report showed that 5-LO products, namely, 5(*S*)-HETE and leukotriene A_4 (LTA_4), but not LTB_4, potently up-regulate VEGF transcription in a human malignant mesothelioma model [12]. Since VEGF is a potent proangiogenic factor and neo-angiogenesis is crucial for tumor growth and invasion, 5-LO can promote tumor development by potentiating the proangiogenic response. Along these lines studies using molecular and pharmacological approaches have found that cyclooxygenase-2 (COX-2) and 12-LO, when overexpressed in carcinoma cells, enhance their angiogenic potential and stimulate tumor growth [13].

The proangiogenic effects of COX-2 are mediated primarily by three products of AA metabolism: thromboxane A_2 (TXA_2), prostaglandin E_2 (PGE_2), and prostaglandin I_2 (PGI_2). Downstream proangiogenic actions of these eicosanoid products include: (1) production of VEGF [14–16]; (2) promotion of vascular sprouting, migration, and tube formation [17,18]; (3) enhanced endothelial cell survival via Bcl-2 expression and Akt signaling [19,20]; (4) induction of matrix metalloproteinases (MMP) [21]; (5) activation of epidermal growth factor receptor-mediated angiogenesis [22]; and (6) suppression of interleukin-12 production [23].

Various well-documented clinical and experimental studies have confirmed the effects of nonsteroidal anti-inflammatory drugs, such as aspirin (ASA), in the prevention of certain types of human cancer, including lung, colon, and breast cancers. The mechanism by which ASA acts to reduce the incidence and risk of these cancers is not clear but is thought to result from the reduction of angiogenesis [24,25].

3. Lipoxins and angiogenesis?

ASA's well-known therapeutic mechanism of action includes inhibition of COX-2-derived prostanoids [26]. In addition, when acetylated by ASA, COX-2's ability to generate prostanoids is blocked, but remains active in EC to induce the biosynthesis of new products termed aspirin-triggered-15-epi-lipoxins (ATL) [27]. These novel endogenous lipid mediators are the carbon 15 epimers of the native lipoxins (LX) that carry their 15 alcohol in the *R*-configuration compared with their native LX counterparts and mimic some of their bioactivities [28].

ATL are generated in vivo during cell–cell interactions, that can involve, for example, EC–neutrophils [29], and display potent inhibitory actions in several key events in inflammation, serving as local counterregulatory mediators of leukocyte adhesion and diapedesis in the low nanomolar to subnanomolar range [28]. In this manner, these compounds regulate important initial steps in tissue inflammation and its resolution [30]. A recent work has documented ATL generation in a randomized human trial, pointing to the importance of a local 15-epi-lipoxin A_4 biosynthetic circuit within the vasculature [31].

In view of the rapid transformation and inactivation of LX and ATL, it was highly desirable to design analogs that would resist metabolism and maintain their structural integrity. To that end, LX analogs were constructed with specific modifications of the native structures that prolong the half-life of the compounds in blood and enhance both their bioavailability and bioactivity [32].

Considerable amounts of data tightly link LX actions and cytokine networks. In human enterocytes and leukocytes, LXA_4 and LXA_4 analogs inhibit the release of the proangiogenic cytokine IL-8 at the gene transcriptional level [33,34]. These reports are consistent with other findings showing LXA_4 reduction of IL-6 and IL-8 synthesis by human synovial fibroblasts [35]. IL-6 has been recently reported to induce angiogenic activity in a carcinoma cell line [36]. Conversely, LXA_4 and ATL synthetic analogs redirect the cytokine-chemokine axis in an in vivo model [37], stimulating the production of IL-4, a cytokine with anti-angiogenic properties [38]. Furthermore, the proteolytic activity necessary to digest the basement membrane, a crucial step in the angiogenic process, can be regulated by LX which, at nanomolar concentrations, prevented IL-1β-induced matria metalloproteinases (MMP) synthesis and induced a 2-fold increase of tissue inhibitor of metalloproteinase (TIMP)-1 protein levels [35]. Controlled local dissolution of the extracellular matrix is important not only for the coordinated movement of the EC, but also for the release of sequestered angiogenic molecules. Collectively, these data indicate that ATL could regulate EC responses in vitro and in vivo that are relevant for angiogenesis.

4. Lipoxins inhibit angiogenesis in vitro

The signaling pathways that control EC growth are dependent on the degree of cell-matrix adhesion; thus the angiogenic response requires conditions of increased cell-matrix adhesiveness. The adhesion of human umbilical vein endothelial cells (HUVEC) to the