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Original Research Article

Reversal of IL-13-induced inflammation and Ca^{2+} sensitivity by resolvin and MAG-DHA in association with ASA in human bronchi

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ABSTRACT

The aim of this study was to investigate the effects of resolvin D1 (RvD1), as well as the combined treatment of docosahexaenoic acid monoglyceride (MAG-DHA) and acetylsalicylic acid (ASA), on the resolution of inflammation markers and Ca^{2+} sensitivity in IL-13-pretreated human bronchi (HB). Tension measurements performed with 300 nM RvD1 largely abolished (50%) the over-reactivity triggered by 10 ng/ml IL-13 pretreatment and reversed hyper Ca^{2+} sensitivity. Addition of 300 nM 17(S)-HpDoHE, the metabolic intermediate between DHA and RvD1, displayed similar effects. In the presence of 100 μM ASA (a COX inhibitor), the inhibitory effect of 1 μM MAG-DHA on muscarinic tone was further amplified, but not in the presence of Ibuprofen. Western blot analysis revealed that the combined treatment of MAG-DHA and ASA upregulated GPR-32 expression and downregulated cytosolic TNFα detection, hence preventing IκBα degradation and p65-NFκB phosphorylation. The Ca^{2+} sensitivity of HB was also quantified on β-escin permeabilized preparations. The presence of ASA potentiated the inhibitory effects of MAG-DHA in reducing the Ca^{2+} hypersensitivity triggered by IL-13 by decreasing the phosphorylation levels of the PKC-potentiated inhibitor protein-17 regulatory protein (CPI-17). In summary, MAG-DHA combined with ASA, as well as exogenously added RvD1, may represent valuable assets against critical AHR disorder.

1. Introduction

Inflammation and airway hyperresponsiveness (AHR) are hallmarks of asthma. AHR diseases have become increasingly prevalent, affecting more than 300 million people worldwide [8]. Current standard therapies, including corticosteroids and β-2 receptor agonists, effectively provide symptomatic control; however, none of the above treatment options are curative in patients with advanced severe asthma [7,30].

N-3 polyunsaturated fatty acids (n-3 PUFAs) are beneficial for human health and are found in fish oil under the form of eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and docosapentaenoic acid (DPA; 22: 5n-3) [3,22,34]. Several studies have shown that n-3 PUFAs display beneficial effects in a wide range of human diseases in which unresolved inflammation is suspected to be a key component of chronic-disease pathogenesis [10,12,38]. DHA, which is enriched in neuronal tissues [35], is converted into potent mediators, including resolvins and protectins, identified from inflammatory exudates [14,15,34]. Recent studies have demonstrated that DHA is a precursor to a potent family of bioactive docosanoid derivatives which include novel docosatrienes as well as the 17*S* epimer resolving series (17(S)-HpDoHE) in human blood cells and mouse brain [10,15]. 17(S)-HpDoHE is generally reduced to 17(S)-resolvin metabolites, which have been shown to inhibit both TNFα-induced interleukin-1β expression in human glioma cells and TNFα-induced leukocyte trafficking to the murine air pouch. In addition, the 17*S* series

Abbreviations: AHR, airway hyperresponsiveness; HB, human bronchi; BSM, bronchial smooth muscle; ASA, acetylsalicylic acid; MAG-EPA, eicosapentaenoic acid monoacylglyceride; PUFA, polyunsaturated fatty acid; TNFα, tumor necrosis factor alpha; RvD1, 17*S*,8*R*,17*S*-trihydroxy-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-docosahexaenoicacid; 17(S)-HpDoHE, 17(S)-hydroperoxy docosahexaenoic acid; VSM, vascular smooth muscle.

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of DHA metabolites is a potent regulator of peritonitis-activated leukocyte recruitment [14,15]. In light of the above, a new DHA sn1-monoacylglyceride – MAG-DHA – has been tested to assess its properties in various models of asthma [22,24], brain disease [32] and pulmonary hypertension [9,16,23]. This nontoxic compound is well absorbed by the gastrointestinal tract and its metabolites are found in lung tissues and blood circulation [24,36,37]. Furthermore, MAG-DHA metabolites have been shown to be vasodilating and pro-resolving compounds in an *in vitro* model of pulmonary hypertension [9,23]. Recent studies have further demonstrated that MAG-DHA and MAG-EPA reduce lung inflammation, leukocyte counts in bronchoalveolar lavages and mucus production in ovalbumin-sensitized guinea pigs [22]. In parallel, COX2 inhibition has been suggested to mediate the anti-inflammatory effects of n-3 PUFAs in pulmonary inflammatory diseases [22]. A key feature associated with the discovery of these novel DHA oxidative metabolites is the role of aspirin in the biosynthesis of these molecules [2,19]. Aspirin, the first chemically produced medication, is widely used for its analgesic and anti-inflammatory properties [27]. Inhaled aspirin protects against bronchoconstrictor challenges in asthma [1]. Aspirin downregulates the NFκB activity of activated B cells, leading to a lower production of proinflammatory cytokines and leukotrienes [2,17,27]. This discovery served as a basis for the development of nonsteroidal anti-inflammatory drugs (NSAIDs) which are widely used for the treatment of arthritis and other inflammatory conditions [1,36]. A new aspect of aspirin's mode of action (in the biosynthesis of oxidative lipid modulators) has been recognized with the identification of the second eicosanoid oxidative enzyme, COX2 [19]. Aspirin has indeed been shown to act on COX2 by acetylating its catalytic serine residue, which prevents prostaglandin formation [1,2,17]. Serhan and colleagues furthermore identified a pro-resolving pathway involving DHA by characterizing the chemical structures of aspirin-triggered DHA metabolites produced in murine systems and isolated human leukocytes [37–39].

DHA also has the capacity to reduce airway overreactivity in TNFα-pretreated human bronchi *in vitro* [24]. Both MAG-DHA and MAG-EPA decrease plasma levels of inflammatory cytokines and angiogenesis factors in healthy adults [2]. The exact mechanism by which n-3 PUFAs reduce inflammation markers and concomitant AHR is still largely unknown. Moreover, n-3 PUFA-derived mediators, including E-series resolvins (such as resolvin E1 from EPA) and D-series resolvins and protectins from DHA, appear to exert potent anti-inflammatory actions both *in vitro* and *in vivo* by interaction with pro-resolving receptors such as CMKLR1 (also called ChemR23 and GPR-32 receptors, respectively) [17,36,38].

Aspirin-triggered resolvins and related omega-3 derivatives were first identified in murine exudates [18]. Aspirin-triggered lipoxin A4 (AT-LXA4) decreases COX2 expression levels in LPS-treated mice [13] and attenuates the NFκB activation pathway in BV-2 microglia cells [44]. While EPA and DHA do not modulate platelet aggregation, the combination of EPA or DHA with aspirin has been shown to synergistically reduce this aggregation [2].

IL-13 is a Th2 immunomodulating cytokine that plays a central role in the initiation of AHR [4,7,44]. IL-13 exhibits stimulatory activity in multiple cell types – including mast cells, eosinophils, pulmonary epithelial cells, and bronchial smooth-muscle (BSM) cells – by acting on IL-13 receptors [28,33]. *In vitro*, IL-13 has been shown to induce BSM contraction in agonist-induced Ca^{2+} sensitization *via* an upregulation of the RhoA/Rho-kinase signaling pathway [4,21,29]. The signal transducers and activators of transcription (STATs), including STAT6, are latent cytoplasmic proteins that undergo tyrosine phosphorylation by Janus kinases (JAKs) after IL-13 stimulation [28,40,44]. Once phosphorylated, STAT6 is translocated to the nucleus, where it regulates gene expression [42]. Mutational studies have revealed that STAT6 and NFκB are required for the upregulation of RhoA induced by IL-13 and TNFα in human BSM cells [4,6]. Other reports have shown that inhibition of STAT-6 prevents IL-13-induced Rho activation [29,41]. The PKC/CPI-17 pathway is also an important regulator of intestinal and bronchial smooth-muscle contraction under normal conditions whereby changes in PKC signaling can contribute to motility dysfunction under proinflammatory conditions [12,24,25]. Alternatively, CPI-17 phosphorylation results in an inhibition of myosin light-chain phosphatase activity (MLCP) which, in turn, maintains steady-state tension in BSM [11]. In contrast, CPI-17 dephosphorylation is associated with BSM relaxation [26]. PKC/CPI-17 signaling may also have a significant role in the hypercontractile phenotype associated with enhanced Th2 cytokine (IL-13, IL-4) production [7,8,11].

The present study was aimed at evaluating the effects of resolvin D1 and of MAG-DHA, alone or in combination with aspirin (ASA), on inflammatory markers and Ca^{2+} sensitivity induced by IL-13 in human bronchi *in vitro*. The role and implication of IL-13-mediated NFκB and CPI-17 phosphorylation was also tested in n-3 PUFA-treated HB. Herein, we report the first evidence that MAG-DHA in combination with ASA induces cumulative inhibitory effects on airway inflammation and mechanical tension. These effects are likely related to an upregulation of GPR-32 expression and ultimately lead to a reduction in both inflammation and Ca^{2+} sensitivity.

2. Materials and methods

2.1. Tissue preparation and organ culture of human bronchial rings

The study was approved by the Ethics Committee of the Université de Sherbrooke (Protocol No. CRC 05-088-S1-R6). Human lung tissues were obtained from 15 patients undergoing surgery for lung carcinoma. Following lobectomy and transport in sterile physiological saline solution, lung samples, distant from the malignant lesion, were dissected by the pathologist. Tissue sample dissection and culture were performed as previously described [24]. Bronchial rings were treated for 48 h (every 24 h for 2 days, unless specified otherwise) in the absence (control) or the presence of 10 ng/ml IL-13, either alone or in the presence of 100 μM acetylsalicylic acid (ASA), 1 μM MAG-DHA, 1 μM MAG-DHA + 100 μM ASA, 300 nM 17(S)-HpDoHE or 300 nM RvD1 prior to pharmacological challenge to assess their mechanical properties. For complementary experiments, explants were also treated for 48 h with either 10 ng/ml IL-13, IL-13 + 1 μM MAG-DHA, IL-13 + 1 μM MAG-DHA + 1 μg/ml anti-GPR-32, IL-13 + 300 nM RvD1 and IL-13 + 300 nM RvD1 + 1 μg/ml anti-GPR-32.

To further assess the putative involvement of 1 μM MAG-DHA combination with various concentrations of aspirin (ASA) or Ibuprofen (another NSAIDs), a set of complementary experiments were performed on untreated HB (control) or treated HB with 10 ng/ml IL-13, IL-13 + 1 μM MAG-DHA, Il-13 + 1 μM MAG-DHA various concentrations of these compounds. All culture plates were maintained in a humidified incubator at 37 °C under 5% CO_2 and culture medium were changed every 24 h.

2.2. Mechanical-tension measurements

The mechanical effects induced by pharmacological agents and eicosanoids were performed using an isolated organ-bath system (Radnoti Glass Technology, Monrovia, CA), as previously described [25]. Passive and active tensions were assessed with Grass FT03 transducer systems coupled to Polyview software (Grass-Astro-Med, West Warwick, RI) to allow data acquisition and analysis.