Lipoxin formation in human nasal polyps and bronchial tissue

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Chopped human nasal polyps and bronchial tissue produced lipoxin A_4 and isomers of lipoxins A_4 and B_4, but not lipoxin B_4, after incubation with exogenous leukotriene A_4. In addition, these tissues transformed arachidonic acid to 15-hydroxyeicosatetraenoic acid. The capacity per gram of tissue to produce lipoxins and 15-hydroxyeicosatetraenoic acid was 3–5-times higher in the nasal polyps. Neither tissue produced detectable levels of lipoxins or leukotrienes after incubation with ionophore A23187 and arachidonic acid. Co-incubation of nasal polyps and polymorphonuclear granulocytes with ionophore A23187 led to the formation of lipoxins, including lipoxins A_4 and B_4. The results indicate the involvement of an epithelial 15-lipoxygenase in lipoxin formation in human airways.

Lipoxin; Lipoxygenase; Arachidonic acid; Nasal polyp; Bronchial tissue; Human

1. INTRODUCTION

Metabolism of arachidonic acid by 5-, 12- and 15-lipoxygenases leads to formation of the corresponding hydroperoxyeicosatetraenoic acids (HPETE), which are reduced to hydroxyeicosatetraenoic acids (HETE). Alternatively, 5-HPETE may be converted to an unstable epoxide intermediate, leukotriene (LT)A_4, which is further metabolized to the biologically active leukotrienes B_4, C_4, D_4 and E_4 (for review see [1]). Leukotriene A_4 is not only a key intermediate in the formation of leukotrienes, but is also converted to lipoxins. Thus, we have recently described lipoxin production from endogenous sources of arachidonic acid via platelet-dependent lipoxygenation at C-15 of granulocyte-derived LTA_4 [2]. This transformation was probably catalyzed by the platelet 12-lipoxygenase (12-LO) [3,4]. The lipoxins, including the biologically active lipoxin (LX)A_4 and LXB_4, are trihydroxylated arachidonic acid derivatives, containing a conjugated tetraene [1]. They were originally isolated and characterized after incubation of human leukocytes, possessing high 5-LO activity, with exogenous 15-H(P)ETE [1]. In accordance, the importance of interaction between the 5- and 15-lipoxygenases in lipoxin formation was postulated. However, the involvement of the latter enzyme in lipoxin formation from endogenous substrate was not convincingly demonstrated. Human nasal, tracheal and bronchial epithelium possesses high 15-LO activity, with 15-HETE as the major arachidonic acid metabolite [5–8]. Furthermore, 15-HETE has been detected in nasal and bronchoalveolar lavage fluid [9,10]. Therefore, it was of interest to investigate the capacity of tissues derived from the human respiratory tract to synthesize lipoxins from synthetic or granulocyte-derived LTA_4.

The present paper demonstrates that human nasal polyps and bronchial tissue convert exogenous LTA_4 to LXA_4 and lipoxin isomers. Furthermore, transcellular formation of lipoxins A_4 and B_4 from endogenous substrate in mixed incubations of granulocytes and nasal polyps is described.

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2. EXPERIMENTAL

2.1. *Materials*

Arachidonic acid was purchased from Nu-Chek Prep. Inc. (Elysian, MN, USA). Synthetic LTA_4 methyl ester was from Salford Ultrafine Chemicals (Manchester, UK) and was saponified as described [11]. Ionophore A23187 was obtained from Calbiochem-Behring (La Jolla, CA, USA), Dextran T-500 was from Pharmacia Fine Chemicals (Uppsala, Sweden) and Lymphoprep from Nyegaard & Co. (Oslo, Norway). Fatty acid free human serum albumin was from Sigma (St. Louis, MO, USA). Synthetic standard of 5-HETE was a kind gift from Dr T. Miyamoto (ONO Pharmaceutical, Osaka, Japan). Biosynthetic standards of 15- and 12-HETE were prepared from incubations of arachidonic acid with soybean lipoxygenase (Sigma) and human platelets [12], respectively. Synthetic lipoxin standards were kindly provided by Dr K.C. Nicolaou (Univ. of Penn., PA, USA).

2.2. *Tissue preparation*

Nasal polyp tissue was obtained from patients undergoing nasal polypectomies and bronchial tissue was dissected from macroscopically normal parts of human lungs derived from patients undergoing surgery due to lung carcinoma. The tissues were collected within 30 min after surgical removal, kept on ice, chopped with fine scissors and carefully rinsed several times in ice-cold phosphate-buffered saline (PBS (pH 7.4), 0.9 mM Ca^{2+}) until the buffer was macroscopically free of contaminating blood.

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2.3. *Preparation of granulocytes*

Peripheral blood was collected using vacutainer tubes containing 7.5% (v/v) 77 mM EDTA. Suspensions of polymorphonuclear granulocytes (PMN; purity approx. 99%) were prepared by dextran sedimentation, hypotonic ammonium chloride lysis, Lymphoprep centrifugation [13] and resuspension in PBS.

2.4. *Incubation procedures*

Aliquots of chopped tissue (300 mg/ml PBS) were preincubated for 5 min at 37°C prior to a 30 min incubation without exogenous stimulus or with either leukotriene A_4 (10 μM) or arachidonic acid (75 μM) in the presence or absence of ionophore A23187 (5 μM). In other experiments polyp tissue was mixed with PMNs (15×10^6 PMN and 150 mg tissue (wet wt) per ml), preincubated for 5 min and incubated with ionophore A23187 (5 μM) for 30 min in the absence or presence of exogenous arachidonic acid (75 μM). In all experiments where lipoxins were analysed, human serum albumin (0.3 mg/ml) was added to the incubation medium [14]. Incubations were terminated by addition of 5 vols of ice-cold ethanol.

2.5. *Isolation, identification and quantitation of lipoxygenase products*

Precipitated material was removed by centrifugation and the supernatants were evaporated and dissolved in mobile HPLC phase. Analyses of HETEs and lipoxins were performed on a Nucleosil 120-3 C_{18} column (3 × 100 mm; Macherey-Nagel, FRG) eluted with methanol/water/acetic acid (72:28:0.01, v/v; 0.4 ml/min) and a Nova-Pak C_{18} column (3.9 × 100 mm; Waters Ass., Milford, MA, USA) eluted with acetonitrile/water/acetic acid (28:72:0.01, v/v; 1 ml/min), respectively. On-line product quantitation was performed using a computerized Diode Array Spectrophotometer (HP 8451A) or a variable wavelength ultraviolet detector (LDC Spectromonitor III) connected to an integrator (LDC/Milton Roy IC-10B). Products were identified using co-chromatography with synthetic or biosynthetic standards and ultraviolet spectroscopy.

3. RESULTS

3.1. *Formation of monohydroxyeicosatetraenoic acids*

Incubation of chopped nasal polyps without exogenous stimulus at 37°C for 30 min, led to production of 15-HETE, while no 12- or 5-HETE was formed. Addition of arachidonic acid (75 μM) markedly increased the 15-HETE synthesis and induced a minor 12-HETE production (Figs 1 and 2). Addition of calcium ionophore A23187 (5 μM) did not significantly alter the HETE formation. Detectable levels of 5-HETE could not be observed under any condition. Bronchial tissue converted exogenous arachidonic acid to 15-HETE, while 5- and 12-HETE was not formed. Comparison of the synthetic capacity showed that bronchial tissue produced 7.5 ± 5.3 nmol 15-HETE/g tissue (mean ± SD, $n = 4$) and polyp tissue 37.0 ± 18.4 nmol/g ($n = 3$) after incubation with arachidonic acid and ionophore A23187. Neither nasal polyps nor bronchial tissue produced detectable amounts of leukotrienes or lipoxins under these conditions.

3.2. *Metabolism of exogenous leukotriene A_4*

Chopped nasal polyps (Fig. 3A) and bronchial tissue converted exogenous LTA_4 (10 μM) to LXA_4, 6(S)-LXA_4 and the all-*trans*-isomers of LXA_4 and LXB_4,

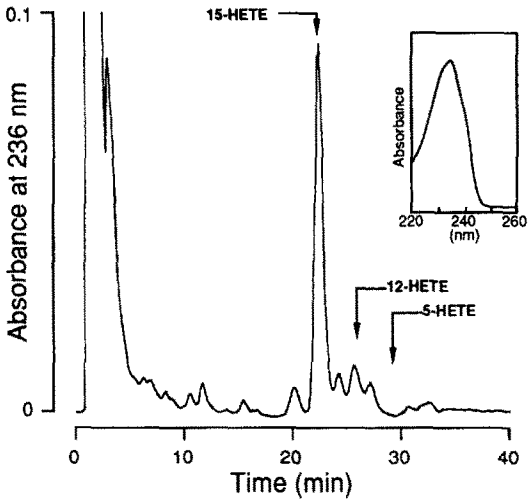

Fig. 1. RP-HPLC-chromatogram of HETEs formed by chopped human nasal polyps incubated with arachidonic acid (75 μM). Retention times of HETE standards are indicated. Inset: on-line ultraviolet spectrum of biosynthetic 15-HETE.

while LXB_4 was not formed (Table I). Addition of ionophore A23187 to the nasal polyps did not significantly alter the lipoxin production from exogenous LTA_4. Fig. 4 shows the ultraviolet spectrum of LXA_4 produced by bronchial tissue.

3.3. *Ionophore A23187-induced formation of lipoxins in co-incubations of human nasal polyps and granulocytes*

Granulocytes were mixed with chopped nasal polyps and the suspension was stimulated with ionophore A23187 for 30 min at 37°C. In these incubations both LXA_4 and LXB_4 were formed from endogenous substrate (Table I). The production of lipoxins increased about 3-fold in the presence of exogenous arachidonic acid (Table I, Fig. 3B). Granulocytes alone produced undetectable or trace amounts of lipoxins.

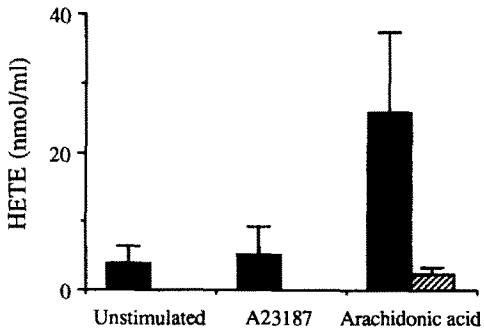

Fig. 2. Formation of 15-HETE (filled bars) and 12-HETE (hatched bars) in suspensions of chopped human nasal polyps without stimulus or in the presence of ionophore A23187 (5 μM) or arachidonic acid (75 μM). Each value represents the mean ± SD of five to six duplicate experiments.

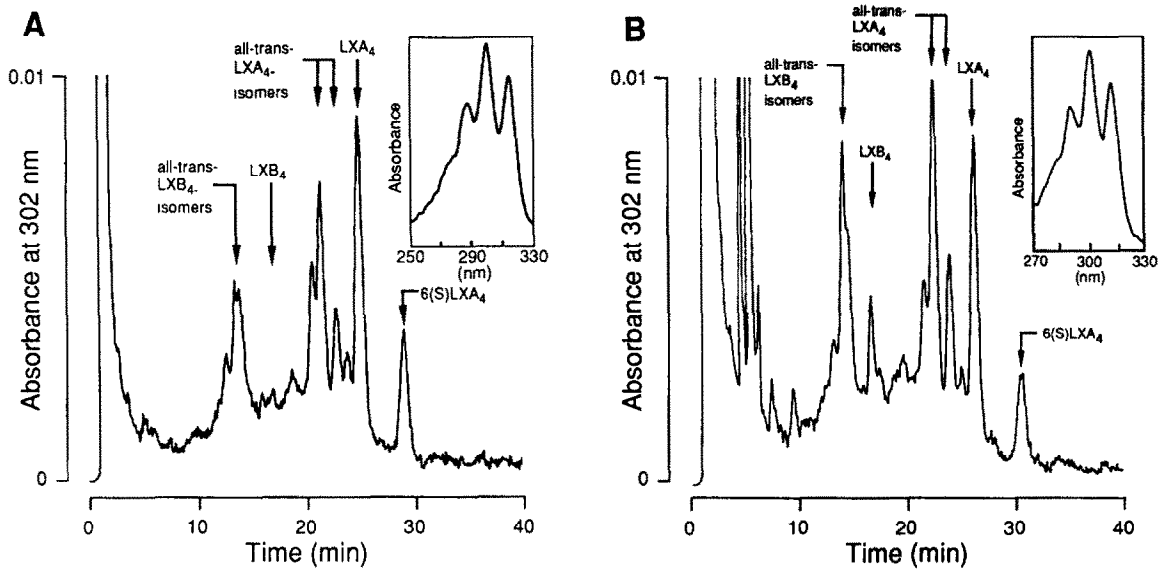

Fig. 3. RP HPLC-chromatograms of lipoxins formed in (A) suspensions of chopped nasal polyps incubated with LTA_4 (10 μM) or (B) mixed suspensions of chopped nasal polyps and granulocytes incubated with ionophore A23187 (5 μM) and arachidonic acid (75 μM). Retention times of authentic lipoxin standards are indicated. Inset: on-line ultraviolet spectra of biosynthetic (A) LXA_4 and (B) LXB_4.

4. DISCUSSION

In the present report, we demonstrate that human nasal polyps possess considerable 15-lipoxygenase activity and a capacity to transform authentic or granulocyte-derived LTA_4 to lipoxins. Similar results were obtained using human bronchial tissue, although the capacity of this tissue to convert exogenous LTA_4 to lipoxins was markedly lower and paralleled by a less pronounced 15-LO activity. This is in agreement with a reported decrease in immuno-reactive lipoxygenase along the human airways [15].

No detectable amounts of 5-HETE or leukotrienes were observed after incubation of nasal polyps or bronchial tissue with arachidonic acid and ionophore A23187, excluding the presence of significant amounts of contaminating leukocytes in the tissue preparations.

Table I

Formation of lipoxins in human nasal polyps and bronchial tissue

Incubation conditions	Lipoxin A_4 (pmol/g tissue)	Lipoxin B_4 (pmol/g tissue)
Nasal polyps + LTA_4 (10 μM)	336 ± 108	n.d.
Nasal polyps + LTA_4 + A23187 (5 μM)	305 ± 113	n.d.
Nasal polyps + PMN + A23187	100 ± 30	70 ± 24
Nasal polyps + PMN + A23187 + AA (75 μM)	364 ± 131	151 ± 32
Bronchial tissue + LTA_4 + A23187	84 ± 96	n.d.

Aliquots of 300 mg tissue/ml (wet weight) or 15 × 10^6 PMN and 150 mg tissue/ml were incubated at 37°C for 30 min in the presence of various stimuli as indicated. Products were identified and quantified as described in section 2. Data are presented as mean ± SD of 5–6 duplicate experiments. AA, arachidonic acid; n.d., not detected

Small amounts of 12-HETE were formed after incubation of nasal polyps with exogenous arachidonic acid. However, although this may suggest a minor platelet contamination, platelets were probably not responsible for the conversion of LTA_4 to lipoxins in suspensions of chopped nasal polyps, since this conversion was unaffected by ionophore A23187. In contrast, formation of lipoxins by human platelets is markedly stimulated by addition of the ionophore [2]. Neither did the ionophore stimulate 15-HETE formation from endogenous or exogenous arachidonic acid by the polyps. The results suggest the involvement of an epithelial 15-LO (possibly also possessing minor 12-LO activity) in the production of lipoxins by airway tissue.

Transcellular lipoxin formation in mixed granulocyte/nasal polyp incubations probably involves at least two pathways. First, LTA_4, synthesized by the granulocyte 5-LO, may be released and further converted to the putative tetraene intermediate 5(6)-epoxy-15-hydroxyeicosatetraenoic acid [16] by the

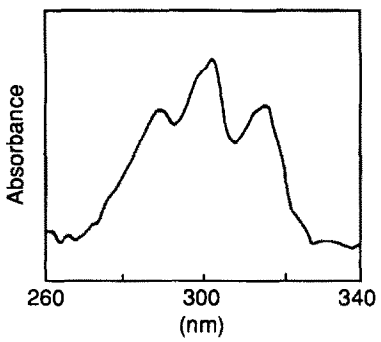

Fig. 4. Ultraviolet spectrum of LXA_4 formed by human bronchial tissue after incubation with exogenous LTA_4 (10 μM) and ionophore A23187 (5 μM).

15-LO in the polyp tissue. Subsequently, this intermediate may be hydrolysed to the trihydroxylated lipoxins. This hypothesis is supported by the finding that nasal polyps transformed synthetic LTA_4 to lipoxins. Second, 15-H(P)ETE, synthesized by the polyp tissue, may be further converted to the tetraene-epoxide intermediate by the granulocyte 5-LO [1]. The finding that nasal polyps produced 15-HETE and that the formation of this compound was markedly increased in the presence of exogenous arachidonic acid, which was also true for the lipoxin formation in mixed granulocyte/nasal polyp incubations, supports the proposed second route of formation.

Lipoxin B_4 formation was observed exclusively in the presence of granulocytes. These findings are in agreement with the results obtained with incubations of mixed platelets-granulocytes [2,4], indicating the involvement of a granulocyte-dependent epoxide hydrolase activity in LXB_4 formation.

We have previously reported production of lipoxins via platelet-dependent lipoxygenation at C-15 of granulocyte-derived LTA_4 [2]. The present results further indicate a role for LTA_4 as a key intermediate in lipoxin synthesis. In addition, the importance of 15-LO in the formation of lipoxins in human respiratory tissue is demonstrated. During pathophysiological conditions, epithelial cells or other extravascular cells possessing 15-lipoxygenase activity, may synthesize lipoxins in cooperation with activated leukocytes. Since LXA_4, although being a weak inflammatory agonist [1], has been reported to be a more powerful antagonist of LTB_4- and LTD_4-induced biological effects [17–19], transcellular lipoxin formation may be of importance for regulation of inflammatory reactions in the airways.

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