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Liquid chromatography–mass spectrometry measurement of leukotrienes in asthma and other respiratory diseases☆

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ABSTRACT

Leukotrienes (LTs), including cysteinyl-LTs (LTC_4, LTD_4 and LTE_4) and LTB_4, are potent inflammatory lipid mediators which have been involved in the pathophysiology of respiratory diseases. LC–MS/MS techniques for measuring LT concentrations in sputum supernatants, serum, urine and exhaled breath condensate (EBC) have been developed. In asthmatic adults, reported LTB_4 and LTE_4 concentrations in sputum range from 79 to 7220 pg/ml and from 11.9 to 891 pg/ml, respectively. Data on sputum LT concentrations in healthy subjects are not available. In EBC, reported LTE_4 concentrations range from 38 to 126 pg/ml (95% CI) in adult asthma patients and from 34 to 48 pg/ml in healthy subjects. LTB_4 concentrations in EBC range from 175 to 315 pg/ml (interquartile range) in asthmatic children, and from 25 to 245 pg/ml in healthy children. Enabling an accurate quantitative assessment of LTs in biological fluids, LC–MS/MS techniques provide a valuable tool for exploring the pathophysiological role of LTs in respiratory disease and might be useful for assessing the effects of therapeutic intervention. This review presents the analytical aspects of the LC–MS/MS techniques for measuring LT concentrations in biological fluids and discusses their potential utility for the assessment of airway inflammation and monitoring of pharmacological treatment in patients with asthma phenotypes and other respiratory diseases.

1. Introduction

Leukotrienes (LTs) include LTB_4 and cysteinyl LTs (CysLTs) (LTC_4, LTD_4, and LTE_4). These potent lipid mediators derive from arachidonic acid through the 5-lipoxygenase (5-LO) activity [1–6] (Fig. 1). The pathway for the complete synthesis of CysLTs is expressed in several inflammatory cell types and becomes activated during atopic airway inflammation [3,5]. Although they lack the complete synthetic pathway, platelets and endothelial cells can produce CysLTs from the chemically reactive intermediate LTA_4 via mechanisms of intercellular transfer [5]. LTs have a pivotal pathophysiological role in asthma [1–4,7], as demonstrated by the efficacy of CysLT receptor antagonists [8], but their importance may vary among patients with asthma.

CysLTs induce pathophysiological changes that contribute to airway obstruction in patients with asthma [1–3]. LTC_4, LTD_4, and LTE_4 are the most potent endogenous bronchoconstrictors. They have similar contractile activity on human airway smooth muscle in vitro. This effect has been confirmed by bronchoprovocation studies in healthy control subjects [3], whereas asthma patients are hyper-responsive to inhalation of LTC_4, LTD_4, and LTE_4 [3]. CysLTs are elevated in adults and children with exercise-induced bronchoconstriction [9,10]. CysLTs increase lung microvascular permeability in experimental animals and increase mucus secretion in isolated human and animal airways [3]. Inhalation of CysLTs in asthmatic patients causes recruitment of eosinophils into airway mucosa and increases sputum eosinophil counts [11]. In addition to their local effects in the airways, CysLTs have several systemic effects that contribute to the inflammatory process that characterizes asthma [4,12]. The reduced Th2 cell-dependent inflammatory

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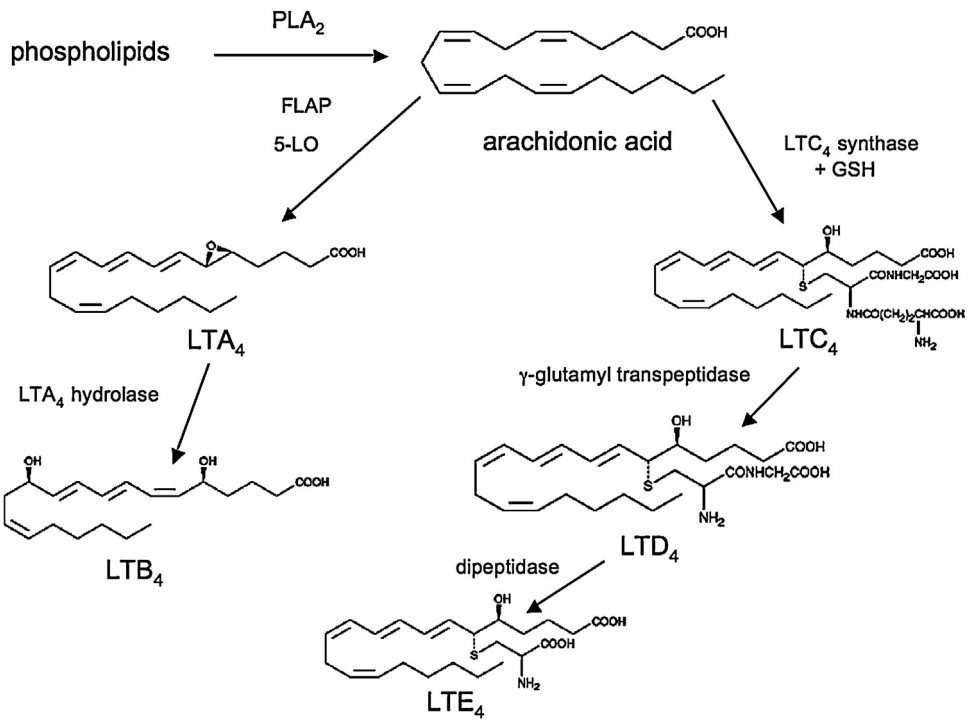

Fig. 1. Biosynthetic pathway of leukotrienes. FLAP, five-lipoxygense-activating protein; GSH, glutathione; 5-LO, 5-lipoxygenase; LT, leukotriene; PLA_2, phoshpolipase A_2.

response in LTC_4 synthase null mice demonstrates a central role for CysLTs in airway inflammation induced by allergen challenge [13]. Due to their biological effects, CysLTs might be actively involved in airway remodelling which includes eosinophilic inflammation, mucus gland hyperplasia and mucus hypersecretion, collagen deposition beneath the epithelial layer and in the lung interstitium at sites of leucocyte infiltration, and airway smooth muscle cell hyperplasia [14].

LTB_4 may contribute to airway narrowing by producing local oedema and increasing mucus secretion, although it has no bronchoconstrictor effect in healthy subjects and patients with asthma [2,3]. Due to its potent chemoattractant activity for neutrophils, LTB_4 might be functionally involved in the neutrophilic phenotype of asthma that characterizes patients with severe asthma [15] or asthma exacerbations. LTA_4 hydrolase inhibition attenuates allergic airway inflammation and airway hyper-responsiveness (AHR) in a mast cell-dependent murine model of allergic airway inflammation [16]. Persistently elevated plasma LTB_4 concentrations in children 1 month after an asthma exacerbation [17], and elevated LTB_4 concentrations in exhaled breath condensate (EBC) in adults with mild asthma [18] and children with mild-to-moderate persistent asthma [19], could indicate a pathophysiological role of LTB_4 in persistent asthma of lesser severity [20]. However, the pathophysiological role of LTB_4 in asthma is not completely defined and requires further studies.

In this review, we present the analytical aspects of the LC–MS/MS techniques for measuring LT concentrations in biological fluids and discuss their potential utility for the assessment of airway inflammation and monitoring of pharmacological treatment in patients with asthma phenotypes and other respiratory diseases.

2. Mass spectrometry measurement of LTs in biological fluids: analytical aspects

MS techniques for measuring LTs concentrations in sputum supernatants, EBC, bronchoalveolar lavage (BAL) fluid, serum, plasma, and urine in healthy subjects and patients with respiratory disease, including asthma, have been developed. Unlike measurement of LT concentrations in serum, plasma and urine which reflects systemic LT production, measurement of LTs in sputum supernatants, EBC, and BAL likely reflects LT production within the respiratory system and is, therefore, more suitable for assessing airway inflammation. However, BAL is invasive and not indicated in patients with asthma who are at higher risk for bronchospasm during this procedure due to their AHR.

2.1. Sputum supernatants

A sensitive and specific solid-phase extraction (SPE) ultra high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method for simultaneous measurement of LTB_4 and CysLTs in sputum supernatants has been developed and validated [21]. Stable-isotope dilution method was used for measuring sputum LTB_4 and LTE_4 concentrations, but it is not known whether deuterated internal standards were also used for measuring LTC_4 and LTD_4, making the results related to these LTs less convincing. However, this limitation applies to similar studies aiming at quantifying LT concentrations in biological studies by LC–MS/MS as discussed further on in the text. After ultracentrifugation, samples were stabilized by protease inhibitors, spiked with stable-isotope labelled internal standards, and subjected to SPE and UHPLC analysis on Acquity UHPLC (Waters, USA) with a reversed-phase C-18 column (BEH C18, 2.1 mm × 50 mm, 1.7 μm particle size, Waters, USA) [21]. Column oven temperature was 55 °C. Sample injection volume was 10 μl. Mobile phase A consisted of water with 0.1% formic acid. Following 0.5 min equilibration phase with 18% mobile phase B (acetonitrile with 0.1% formic acid), prostaglandin E_2 was eluted by 4.5 min isocratic elution with 27% phase B; LTB_4 and CysLTs were eluted by a 35–36% gradient of mobile phase B in the next 4.5 min [21].

LT concentrations were measured with a triple quadrupole mass spectrometer (API 5000, Applied Biosystem/MDS SCIEX, Foster City, USA) coupled with an electrospray ionization (ESI) source