Journal of Chromatography B, 879 (2011) 2220–2228

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of cysteinyl leukotrienes in exhaled breath condensate: Method combining immunoseparation with LC–ESI-MS/MS

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ARTICLE INFO

Article history:
Received 10 November 2010
Accepted 6 June 2011
Available online 13 June 2011

Keywords:
Cysteinyl leukotriene
Exhaled breath condensate
Immunoseparation
Solid phase extraction
Lyophilization
LC–MS

ABSTRACT

A rapid and precise method for the identification and quantification of cysteinyl leukotrienes (leukotriene C_4, leukotriene D_4 and leukotriene E_4), essential markers of bronchial asthma, in exhaled breath condensate was developed. The protocol consists of immunoaffinity separation and a detection step, liquid chromatography combined with electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS). In particular, the selected reaction monitoring mode was used for its extremely high degree of selectivity and the stable-isotope-dilution assay for its high precision of quantification. The developed method was characterized with a high precision (≤7.7%, determined as RSD), an acceptable accuracy (90.4–93.7%, determined as recovery), a low limit of detection (≤2 pg/ml EBC) and a low limit of quantification (≤10 pg/ml EBC). It was compared to other simple, clinically appropriate combinations of pre-treatment methods (solid phase extraction and lyophilization) with LC/MS. Finally, the method (a combination of immunoaffinity separation with LC–MS) was successfully tested in a clinical study where a significant difference was found in the concentration levels of cysteinyl leukotrienes between patients with occupational bronchial asthma and healthy subjects.

1. Introduction

The analysis of exhaled breath condensate (EBC) is a relatively novel method with a good potential to become the preferred and completely non-invasive alternative to the currently practiced invasive (open lung biopsy [1–4], bronchoalveolar lavage [3–5]) and *semi*-invasive (method of induced sputum [6–8]) diagnostic methods for bronchial asthma. Early diagnosis of this life-threatening disease is essential to allow a physician to initiate an effective therapy and minimize harm to the patient. EBC is a liquid matrix that reflects the particular composition of the bronchoalveolar extracellular lung liquid, which replicates the conditions proceeding directly in the lungs and airways. The substances are contained in the exhaled breath in both gaseous and liquid phases (aerosol form). The aerosol particles and the substances present in the gaseous phase could be condensed by breathing over a condenser, which is readily available at specialized clinical facilities. In the obtained liquid, typically known as EBC, more than 1000 compounds have been identified so far, out of which a substantial number are considered to represent sensitive biomarkers of lung diseases (leukotriene B_4 – LTB_4, cysteinyl leukotrienes C_4, D_4 and E_4 – LTC_4, LTD_4, LTE_4, 8-isoprostane, malondialdehyde, 4-hydroxyhexenal, 4-hydroxynonenal, 8–hydroxyguanine, 8-hydroxyguanosine, 8-hydroxy-2′-deoxyguanosine, hydroxymethyluracil, *o*-tyrosine, nitrotyrosine and others) [9–11]. Based on the determination of their content in EBC, the type of ongoing pathological process, the severity of the disorder and the efficiency of a therapeutic procedure etc. can be assessed. In the case of bronchial asthma, cysteinyl leukotrienes (cys LTs) represent a specific group of biomarkers, whose concentration level is significantly elevated in airways and lungs as a result of an ongoing allergic reaction (e.g. aspirin-induced asthma) [12,13]. Leukotrienes are metabolites of arachidonic acid, which is present as phospholipid in cellular membranes [9,14,15]. The enzyme 5-lipooxygenase transforms arachidonic acid into an unstable epoxide, leukotriene A_4 (LTA_4), which can be further transformed by one of two possible enzymatic pathways (Fig. 1). During inflammation, the levels of LTB_4 are elevated by the action of LTA_4 hydrolase, while the second pathway is dominant during allergic reactions [16]. The first member of the family of cys LTs, LTC_4, is produced by LTC_4 synthetase. The next members of the series of cys LTs (LTD_4 and LTE_4) are formed by a gradual transformation occurring sequentially from $LTC_4 \rightarrow LTD_4 \rightarrow LTE_4$ by a consecutive action of the enzymes γ-glutamyltranspeptidase ($LTC_4 \rightarrow LTD_4$)

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doi:10.1016/j.jchromb.2011.06.004

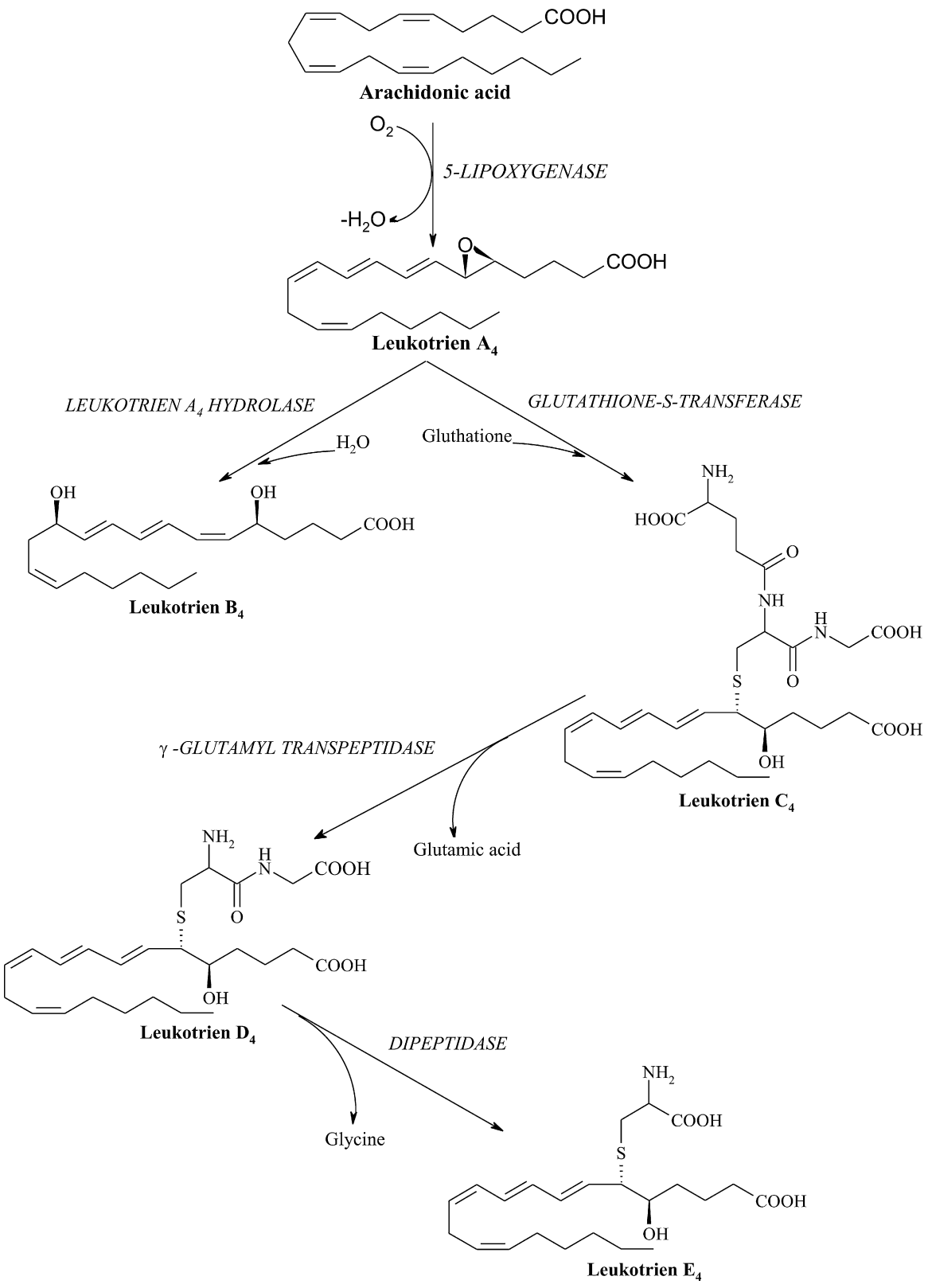

Fig. 1. Scheme of biosynthesis of leukotrienes *in vivo*.

and dipeptidase ($LTD_4 \rightarrow LTE_4$) [15,17]. The produced cys LTs interact with cys LTs receptors that are mainly found on the cells of smooth muscles, eosinophils and various other cells in the organism. Binding of cys LTs to cys LTs-1 receptors, localized in lungs and airways, cause bronchial and bronchiolar constriction and hyperaemia followed by tissue oedema and an excessive secretion of viscous mucus resulting in repeated episodic states of expiratory dyspnoea (breathlessness).

Several publications describe the determination of cys LTs in different body fluids (blood plasma [18,19], urine [20–22] and