Respiratory Medicine (2013) **107**, 1339–1345

Available online at www.sciencedirect.com

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

Vascular endothelial growth factor and cysteinyl leukotrienes in sputum supernatant of patients with asthma

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Received 30 April 2013; accepted 17 June 2013
Available online 8 July 2013

KEYWORDS
Asthma;
VEGF;
Cysteinyl leukotrienes;
Inflammation;
Angiogenesis;
Vascular remodeling

Summary
Background: Vascular endothelial growth factor (VEGF) is considered to be the most important angiogenic factor in asthma. Cysteinyl leukotrienes (Cyst-LTs) have been implicated in vascular permeability in asthma. Cyst-LTs receptor antagonists modulate vascular permeability by reducing VEGF expression.
Objective: We aimed to determine the levels of VEGF and Cyst-LTs in sputum supernatants of patients with asthma and to investigate possible associations within them and with airway vascular permeability (AVP) index. Possible confounding factors were also assessed.
Methods: One hundred twenty one patients with asthma (38 with severe refractory asthma, 41 smokers) and 30 healthy subjects (15 smokers) were studied. All subjects underwent lung function tests, and sputum induction for cell count identification and VEGF, Cyst-LTs, measurement in supernatants. AVP index was also assessed.
Results: Both VEGF & Cyst-LTs (pg/ml) levels were significantly elevated in patients with asthma compared to normal subjects (median, interquartile ranges 845 [487–1034] vs. 432 (327–654) and 209 [171–296] vs. 92 [75–114] respectively, $p < 0.001$ for both). Multivariate regression analysis in the whole group showed a significant association of Cyst-LTs levels in sputum supernatants with VEGF levels in sputum supernatants and AVP index. A similar positive

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0954-6111/$ - see front matter
http://dx.doi.org/10.1016/j.rmed.2013.06.014

association was observed between VEGF levels in sputum supernatants and AVP index. The presence of Severe asthma was a significant covariate for both associations.
Conclusion: Our results indicate that Cyst-LTs may modulate vascular permeability by up-regulating VEGF expression. The above effect seems to be affected by asthma severity.

Introduction

Angiogenesis is a complex multiphase process, potentially involving a great number of growth factors, cytokines, chemokines and numerous other mediators but the specific role of each molecule has not been clearly defined. During chronic inflammation, the vascular remodeling process is the consequence of a pro-angiogenetic action, in which many growth factors and inflammatory mediators are involved [1]. Airway remodeling in asthma also involves an increase in angiogenesis, a process most likely to be mediated by several angiogenic mediators including vascular endothelial growth factor (VEGF) [2].

VEGF is considered to be the most important angiogenetic factor, that induces vascular endothelial cell proliferation, tubule formation and increases microvascular permeability [3]. The latter is a common feature of vascular remodeling in asthma and is modulated by the release of different inflammatory mediators, cytokines, proteases and growth factors.

Cysteinyl leukotrienes (Cyst-LTs) are important molecules that promote both airway inflammation and remodeling [4]. Evidence suggests that Cyst-LTs play an important role in the airway remodeling observed in persistent asthma that includes increases of airway goblet cells, mucus, blood vessels, smooth muscle, myofibroblasts, and airway fibrosis. Cyst-LTs can transcriptionally activate VEGF production via cysLT1 receptors, indicating that Cyst-LTs may be important in the angiogenic process of airway remodeling [5]. Furthermore in vitro and in vivo studies support that the administration of a Cyst-LTs antagonist leads to alterations of VEGF levels [6,7].

In the present study, we aimed to determine the levels of both Cyst-LTs and VEGF in sputum supernatants of patients with asthma and to investigate possible associations with airway vascular permeability as assessed by airway vascular permeability (AVP) index. Furthermore, we wanted to determine whether significant confounding factors such as underlying severity, atopy and smoking significantly affect the above mediators and processes.

Materials & methods

Subjects

Patients were recruited from an open cohort of asthmatic patients who were followed up in the asthma clinics of the 1st and 2nd Respiratory Medicine University Departments in Athens for at least 2 years. The recruitment period was between June 2008 and September 2012. The diagnosis of asthma was established according to GINA guidelines [8]. The diagnosis of Severe Refractory asthma (SRA) was established according to ATS criteria [9]. Patients with symptoms of acute rhinitis, nasal congestion, nasal polyps, or a history of aspirin hypersensitivity were excluded since these conditions are related to eicosanoid inflammation [10–12]. Patients receiving leukotriene modifiers were also excluded. Subjects with any other respiratory disease or any concomitant malignant, heart, renal, liver or collagen disease were excluded. Patients with a respiratory tract infection or asthma exacerbation in the past 8 weeks prior to admission were also excluded. The study was approved by the ethics committees of both hospitals and all subjects provided an informed consent.

Induced sputum

Sputum was induced as previously described using all the modifications for safe measurements according to the underlying asthma severity [13,14]. Briefly, patients inhaled 3% saline at room temperature nebulized by an ultrasonic nebulizer (DeVilbiss Co., Heston, UK) at the maximal saline output (4 ml/min). The total period of sputum induction was 15 min. Subjects were encouraged to cough deeply at 3-min intervals until the 15-min induction time had been completed. Sputum was processed using selected plugs as previously described [15]. Dithiothreitol (DTT) was added in a volume equal to four times the weight of the sputum specimen and it was further diluted with phosphate buffered saline (PBS) in a volume equal to the sputum plus DTT. Total cell counts were performed on a hemacytometer using Trypan blue stain. Slides were prepared by using cytospin (Shandon, Runcorn, UK) and were stained with May-Grunwald and Giemsa for differential cell counts. Cell counting was performed by an observer blind to the clinical characteristics of the subjects. At least 500 inflammatory cells were counted in each sample. A sample was considered adequate when the patient was able to expectorate at least 2 ml of sputum and the slides contained $<$10% squamous cells on differential cell counting. Total cell count expressed as the number of cells $\times$ 10^6 and the percentage (%) of sputum inflammatory cells were used for our analysis. Sputum supernatants were kept at -70 °C for further measurement of Cyst-LTs, VEGF and albumin.

Lung function

Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC), were measured using Master Screen Body (Viasys Healthcare, Jaeger, Hoechberg, Germany) according to the American Thoracic Society guidelines [16].

Atopic status

A positive skin prick test to any of twenty common aeroallergens (including mites, grasses, trees, fungus, domestic animals) was used to confirm atopy.