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Circulating angiopoietin-1 and -2 in patients with stable and exacerbated asthma

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ABSTRACT

Background: Angiopoietin (Ang)-1 and -2 are involved in the pathogenesis of asthma and have been identified as markers of asthma severity.
Objective: To determine the relation between circulating angiopoietins and clinical variables of patients with asthma.
Methods: Fifty patients with bronchial asthma and 25 healthy controls were enrolled. Ang1 and Ang2 plasma levels were analyzed in patients with stable and exacerbated asthma.
Results: Plasma Ang1 levels were 28.4 ± 4.01 pg/mg in patients with bronchial asthma and 21.2 ± 5.21 pg/mg in healthy controls. Plasma Ang2 levels were 23.96 ± 1.38 pg/mg in patients with bronchial asthma compared with 36.8 ± 4.46 pg/mg in healthy controls ($P = .010$). The ratio of Ang2 to Ang1 was lower in patients with asthma than in control subjects. Plasma Ang1 concentrations were correlated with the ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC), and plasma Ang2 levels were correlated with FEV_1 percentage of predicted, FEV_1/FVC, and total immunoglobulin E values. The ratio of Ang2 to Ang1 was correlated with FEV_1 percentage of predicted and FEV_1/FVC. Although plasma Ang1 levels tended to be lower in the exacerbated state than in the stable state in patients with asthma, Ang2 levels were higher in the exacerbated state than in the stable state in patients with asthma ($P = .001$). Plasma Ang2 levels were correlated with initial eosinophil proportions and initial neutrophil proportions. Plasma Ang2 levels and the ratio of Ang2 to Ang1 were correlated with blood eosinophil proportions in the exacerbated state.
Conclusion: These results indicate that circulating angiopoietins could be a useful marker of asthma exacerbation.

Introduction

Asthma is characterized by chronic airway inflammation that is associated with structural changes, termed *airway remodeling*.[1] Increased angiogenesis is a well-documented feature of airway remodeling in asthma.[1] Angiogenesis can be initiated by endogenous angiogenic factors released from mesenchymal cells and/or inflammatory cells.[2]

Among these factors, angiopoietins regulate vascular homeostasis by the endothelial tyrosine kinase Tie receptor.[2] Angiopoietin-1 (Ang1) supports endothelial stabilization by Tie2 activation,[3–6] whereas angiopoietin-2 (Ang2) functions as a context-dependent Tie2 agonist and antagonist promoting pathologic angiogenesis, vascular permeability, and inflammation.[3,7,8] Ang2 levels, normally constrained during homeostasis, are increased in endothelial cells during vessel remodeling, particularly in tumor vasculature, and in diseases associated with increased vascular permeability and endothelial dysfunction, such as sepsis, acute lung injury, lung cancer, and chronic obstructive lung disease.[9–11] In addition to an increase in the Ang2/Ang1 ratio, decreased Tie2 levels have been reported in sepsis.[12–15] Serum Ang1 levels also have been reported to be higher in patients with asthma than in healthy subjects, and the serum Ang1/Ang2 ratio has been correlated with lung function, suggesting that angiopoietins might be useful markers for the diagnosis of

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asthma.[16] Therefore, this study analyzed circulating levels of Ang1 and Ang2 in healthy subjects and patients with asthma and examined changes in these levels in patients with stable vs exacerbated asthma as indicated by changes in lung function parameters. This report is based on a prior publication[17] in which the authors examined different measurements. In addition, the authors evaluated the possibility of a disease exacerbation marker for use in asthma monitoring.

Methods

Subjects

Clinical data were collected from 50 patients with asthma who were registered in an asthma cohort of the Genome Research Center for Allergy and Respiratory Diseases in Korea. The biospecimens and data used for this study were provided by the biobank of Soonchunhyang University Bucheon Hospital (Bucheon, Korea), a member of the Korea Biobank Network. Asthma diagnoses were based on Global Initiative for Asthma (GINA) guidelines.[18] This study used the same sample as that examined by Moon et al,[17] although several different measurements and analyses are presented in the present report. All subjects had a clinical diagnosis of asthma that was supported by at least 1 of the following criteria: (1) variability in maximum diurnal peak expiratory flow greater than 20% over the course of 14 days; (2) an increase in the patient's forced expiratory volume in 1 second (FEV_1) greater than 15% after inhalation of 200 to 400 μg of albuterol; or (3) a positive methacholine provocation concentration less than 10 mg/mL that caused a decrease in FEV_1 of 20%. All subjects underwent standardized assessments, which included analyses of complete blood cell count with differential counts, immunoglobulin E (IgE) measurement, chest posteroanterior radiography, allergy skin prick tests, and spirometry. All data were collected at the time of diagnosis, before the administration of asthma medication, and during the period of exacerbation. Patients with asthma from the hospital cohort also were matched to healthy subjects according to age, sex, and body mass index. These healthy control subjects were recruited from among the spouses of patients or members of the general population and were included based on the following criteria: (1) negative responses on a screening questionnaire for respiratory symptoms and other allergic diseases; (2) FEV_1 values greater than 80% of predicted values; (3) provocation methacholine concentration less than 10 mg/mL that caused a decrease in FEV_1 of 20%; and (4) normal findings on simple chest radiographs. Of subjects who had been regularly followed for at least 2 years, 50 patients were diagnosed based on GINA guidelines.[18] Asthma exacerbation was defined according to GINA guidelines as episodes of a progressive increase in shortness of breath, cough, wheezing, or chest tightness, or some combination of these symptoms, accompanied by decreases in expiratory airflow and use of systemic corticosteroids (tablets, suspension, or injection), or an increase from a stable maintenance dose, for at least 3 days, and a hospitalization or emergency department visit because of asthma requiring systemic corticosteroids. Exclusion criteria included respiratory infection, chronic obstructive pulmonary disease, vocal cord dysfunction, obstructive sleep apnea, Churg-Strauss syndrome, cardiac dysfunction, allergic bronchopulmonary aspergillosis, or poor adherence to treatment. The local research ethics committee of the Soonchunhyang University Hospital research board approved the study protocol.

Spirometry

Spirometry was performed before and after bronchodilator use.[19] Baseline forced vital capacity (FVC) and FEV_1 measurements were obtained in the absence of bronchodilator use (within 8 hours). Basal and post-bronchodilator FEV_1 and FVC values were measured. A Vmax Series 2130 Autobox Spirometer (Sensor Medics, San Diego, California) was used and a calibration check was performed every morning at 8 AM.

Skin Prick Tests

Skin prick tests were performed using 55 common inhalant allergens, including dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cat fur, dog fur, cockroaches, grass, tree, pollens, ragweed, and *Aspergillus* species (Bencard Co, Brentford, United Kingdom).[20] Atopy was defined as having a wheal reaction from the allergen equal to or larger than the histamine wheal (1 mg/mL) or at least 3 mm in diameter. Total IgE was measured using the UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden).

Mediator Assays

Angiopoietin-1 and Ang2 were assessed in plasma samples using enzyme-linked immunosorbent assays according to the manufacturer's instructions. Ang1 and Ang2 levels were checked during asthma control and before and during exacerbation. The minimum detectable limits of Ang1 and Ang2 were 1.36 to 10.3 and 1.20 to 21.3 pg/mL, respectively (R&D Systems, Minneapolis, Minnesota). Blood was drawn to evaluate serum albumin levels using laser nephelometry. All values were expressed in picograms per milliliter. For Ang1 and Ang2, the intra- and interassay variabilities were 3.2% and 5.8%, respectively.

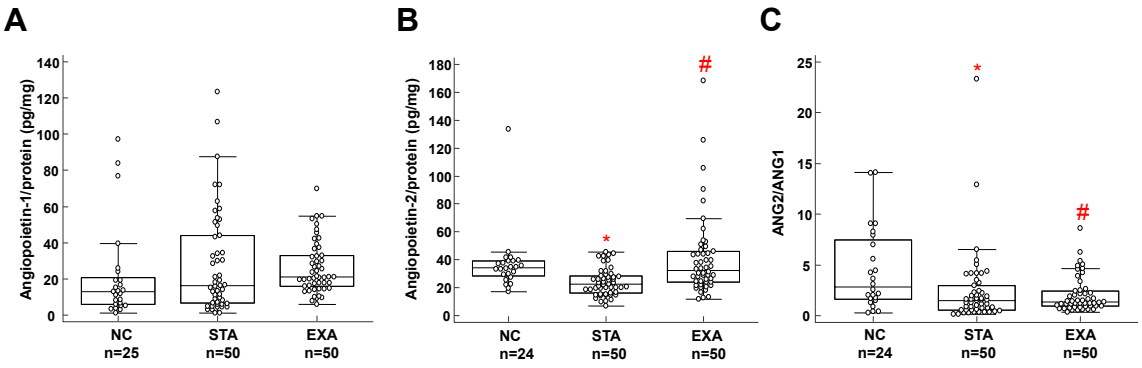

Figure 1. Circulating (*A*) angiopoietin-1 (Ang1) and (*B*) and angiopoietin-2 (Ang2) plasma levels and (*C*) Ang2/Ang1 ratio in patients with exacerbated asthma (EXC), patients with controlled asthma (STA), and healthy control subjects (NC). $^{*}P < .01$ compared with healthy control subjects. $^{\#}P < .01$ compared with patients with controlled asthma.