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Effects of academic exam stress on nasal leukotriene B4 and vascular endothelial growth factor in asthma and health

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ABSTRACT

Objective: To examine the effect of final exam stress on the concentrations of leukotriene B4 (LTB4) and vascular endothelial growth factor (VEGF) in the upper airways among healthy and asthmatic individuals.
Method: Nasal samples were collected from 12 individuals with asthma and 23 healthy controls early and late in a final exam period, and during a low-stress period in the semester. We determined LTB4 and VEGF concentrations using Enzyme-Linked Immunoassays.
Results: Mixed effects analysis of variance models showed that asthmatic participants with allergies in contrast to healthy individuals experienced a decrease in nasal LTB4 during the final exam period as compared to mid-semester (low stress period). There were no significant changes in nasal VEGF across the observation period. Changes in nasal LTB4 and VEGF were not associated with salivary cortisol, exhaled nitric oxide, or spirometric lung function.
Conclusions: Our results suggest that nasal LTB4 concentrations change in periods of psychological stress for asthmatic individuals with allergies.

1. Introduction

Psychosocial factors can cause an alteration of many inflammatory physiological processes that exacerbate infectious and inflammatory symptoms (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Trueba & Ritz, 2013). Inflammatory mediators and cytokines, such as leukotriene B4 (LTB4) and vascular endothelial growth factor (VEGF), are important macromolecules supporting innate immune function (Suzuki, Chow, & Downey, 2008) and are involved in allergic inflammation (Minai-Fleminger & Levi-Schaffer, 2009). Both VEGF (Puxeddu, Ribatti, Crivellato, & Levi-Schaffer, 2005) and LTB4 act as chemoattractants for leukocytes (Ohnishi, Miyahara, & Gelfand, 2008).

Leukotrienes are a group of proinflammatory lipid mediators produced by many different cells of the innate immune system, including granulocytes, mast cells and macrophages. LTB4, especially, is elevated in several inflammatory disorders such as allergic rhinitis, rheumatoid arthritis, chronic obstructive pulmonary disease, atopic dermatitis, allergic conjunctivitis and asthma, and is known to cause bronchoconstriction in most individuals but more so among asthmatics (Bisgaard, Ford-Hutchinson, Charleson, & Taudorf, 1985). LTB4 is a significant mediator in allergic diseases including persistent asthma (Gladue et al., 1996; Ohnishi et al., 2008). In asthma specifically, LTB4 was found to be elevated in bronchoalveolar lavage, plasma and sputum (Ohnishi et al., 2008). LTB4 is related to asthma exacerbations and the development of airway hyperresponsiveness (Mitsunobu et al., 2000). Corticosteroid treatment has been found to reduce LTB4 and this was related to increases in forced expiratory volume in the 1st second (FEV_1; Ohnishi et al., 2008).

Research examining the connection between LTB4 and psychological factors is scarce, but it has been proposed that prostaglandins are elevated in individuals with major depressive disorder (MDD), and prostaglandins share a precursor with leukotrienes (Van Lieshout, Bienenstock, & MacQueen, 2009). Others have implied that depression is associated with lower LTB4 levels (Maes, Christophe, Delanghe, Altamura, Neels, & Meltzer, 1999). The U.S. Food and Drug Administration in 2008 issued a warning on the use of medications that modify leukotrienes linking

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it to suicide, but there is little evidence for this link (Philip et al., 2009) and more recent studies found opposite associations in children, adolescents, and young adults (Schumock, Stayner, Valuck, Joo, Gibbons, & Lee, 2012). There is evidence that stress hormones such as glucocorticoids (Peers & Flower, 1990) and epinephrine (Gongadze & Kezeli, 2002) may modulate LTB4, but this has not been tested in vivo. Thus there is a need for more research on the relationship of leukotrienes with psychological factors.

VEGF has several roles in normal organismic functioning, including endothelial cell proliferation and induction of blood vessel growth (Barnes, 2008). VEGF is thought to also have an important role in mounting a healthy immune response by increasing the recruitment of immune cells and facilitating their infiltration of infection sites (Puxeddu et al., 2005). In asthma, VEGF has been found to induce the growth of blood vessels, but also to increase vascular leakage (Barnes, 2008). Inflammation causes cells to produce VEGF, which fosters angiogenesis and perpetuates inflammation by facilitating the movement of inflammatory and immune cells to tissues (Puxeddu et al., 2005). VEGF is produced by many different cell types such as macrophages, neutrophils, epithelial cells, fibroblasts, and smooth muscle cells (Puxeddu et al., 2005).

VEGF may have an important role in allergic conditions, as it is involved in Th2 cytokine production, and the ensuing airway pathophysiology such as airway remodeling and hyperreactivity (Lee et al., 2004; Thatayatikom & Liu, 2005). Some evidence suggested that allergen exposure may lead to increased nasal VEGF secretion, which may be related to elevations in eosinophil inflammation in the nasal mucosa of individuals with allergies (Choi et al., 2009). Lee et al. (2004) suggested that VEGF regulation might provide a therapeutic avenue for allergic disorders.

Psychosocial factors have been found to be associated to changes in several immune and inflammatory parameters including VEGF. Individuals experiencing prolonged work-related stress show VEGF increases in serum compared to non-stressed individuals (Åsberg et al., 2009). Social support has been found to have an inverse association with serum and tumor cell VEGF concentrations in patients with cancer (Lutgendorf et al., 2008, 2002). Similarly, perceived loneliness has been found to have a positive association with serum VEGF concentrations in patients with colorectal cancer (Nausheen et al., 2010). Regarding airway VEGF, we have previously found that VEGF collected from exhaled breath condensate was elevated in response to academic examination stress (Trueba, Rosenfield, Oberdörster, Vogel, & Ritz, 2013).

The study of inflammation in the nasal passages in asthma is of particular interest given a high comorbidity of asthma with allergic rhinitis (Marple, 2010; Ryan, 2008). Asthma is more severe with comorbid rhinitis, and exacerbations of sinonasal inflammation are associated with asthma exacerbations. Nasal lavage is a minimally invasive method to study inflammation in the nasal cavity (Roponen, Seuri, Nevalainen, Randell, & Hirvonen, 2003). Allergic cytokines (Noah, Henderson, Henry, Peden, & Devlin, 1995), including LTB4 (Shaw, Fitzharris, Cromwell, Wardlaw, & Kay, 1985) and VEGF (Lee et al., 2009) can be measured from nasal samples. Using this technique we sought to examine the association between academic stress and concentrations of nasal LTB4 and VEGF in healthy individuals and asthmatics. If their susceptibility to stress could be demonstrated, further exploration of these parameters as early precipitants of asthma symptom worsening or exacerbation would be justified. To our knowledge this is the first study to examine changes in LTB4 during real-life psychological stress and the effects of psychological stress on nasal VEGF in an ecologically valid behavioral setting.

We designed our academic final exam paradigm to capture prolonged effects of stress measuring repeatedly over a period of 5–7 days. Several studies have used academic final exam stress to test the effects of prolonged stress on immune parameters (e.g., Bosch, de Geus, Ring, & Amerongen, 2002; Kang & Fox, 2001; Trueba, Rosenfield et al., 2013). We hypothesized that final exam stress increases LTB4 and VEGF concentrations in nasal samples and more so in individuals with asthma and allergies. We also aimed to study the associations of nasal LTB4 and VEGF with other psychological and physiological markers of the stress response, including negative affect, salivary cortisol, and exhaled nitric oxide as a measure of central airway inflammation in asthma (Barnes et al., 2010). In addition, we statistically controlled for potential confounds such as the effects of season and corticosteroid medication use by including these as covariates in our analyses.

2. Methods

2.1. Participants

Undergraduate psychology students from a university in the Southwestern US were recruited to participate in this study. In order to participate in the study, all students had to be able to perform the physiological measurements, including nasal sampling and spirometric lung function tests, and had to have a minimum of three final examinations scheduled. Inclusion criteria for the asthma group were a diagnosis of asthma and nasal allergies or allergic rhinitis. For the healthy controls, prior allergic rhinitis diagnosis was an exclusion criterion. Report of other medical conditions (angina, myocardial infarction, congestive heart failure, transient ischemic attacks, or cerebrovascular accidents) was an exclusion criterion for all participants.

In addition, students who had been taking antibiotics or oral corticosteroids, or who had received injected corticosteroids in the past 6 weeks were also excluded. In addition, none of our participants were using any topical corticosteroids. The local Institutional Review Board approved the study and all participants provided written informed consent.

2.2. Sample preparation and immune measurements

Nasal fluid sampling was performed using Intranasal Mucosal Atomization Device™ (LMA MAD Nasal™ syringe; Teleflex Medical Inc, Morrisville, NC), with which participants instilled 3 mL saline solution (0.9% sodium chloride; Teleflex Medical Inc HUD20039 ADDIPAK Unit Dose Solutions, Morrisville, NC) into each nostril. The liquid was then dispensed into a kidney dish from where it was collected into small storage tubes (Roponen et al., 2003). All collected samples were immediately stored at −80 °C. Before performing the ELISA, samples were concentrated two-fold by evaporating water using an Eppendorf VacuFuge attached to a Fisher Scientific Maxima Dry vacuum pump. Enzyme Immunoassay kits (Enzo Life Science, Plymouth Meeting, PA) were used to determine the amounts of LTB4 and VEGF. All samples were analyzed in duplicate. The detection limits of the kits were 5.6 pg/mL for LTB4 and 14.0 pg/mL for VEGF. The intra- and inter-assay coefficients of variation were <10%.

To ensure that the increased NaCl concentration that resulted from concentrating the nasal samples did not interfere with the immune assays, control ELISA assays were performed using the standards for LTB4 provided by the assay kit. Saline was added to the samples at the same concentration that would be present after VacuFuge treatment of the nasal samples.

Saliva samples were collected with cotton swabs (Salivettes; Sarstedt, Inc., Newton, NC). Participants placed the swabs in their mouth for 2 min and then transferred them into individual plastic capsules. Samples were frozen at −80 °C until they were analyzed. Following centrifuging, salivary cortisol concentrations were