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ORIGINAL ARTICLE

Comparison of inflammatory cytokine release from nasal epithelial cells of non-atopic non-rhinitic, allergic rhinitic and polyp subjects and effects of diesel exhaust particles in vitro

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Abstract
Background: Although studies have reported an association between air pollutants and increased allergic airway diseases, such as allergic rhinitis and nasal polyposis, the underlying mechanisms are not fully understood. A limited number of studies have suggested that diesel exhaust particles (DEP) play a role in atopy and the pathogenesis of allergic upper airway diseases. The aim of this study was to investigate the effect of DEP on inflammatory cytokine release, and mRNA expression of transcription factors such as JNK and NF-β in primary nasal epithelial cells (NECs), in vitro.
Methods: NECs from non-atopic, non-rhinitic subjects (controls) and patients with allergic rhinitis and nasal polyps were cultured and incubated with 0–100 μg/ml DEP for 24 h. ELISA and RT-PCR were used to assess the release of IL-8, GM-CSF, and RANTES, and mRNA expression for JNK and NF-κB, respectively.

Abbreviations: DEP, diesel exhaust particles; Ig-E, immunoglobulin-E; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; BECs, bronchial epithelial cells; NECs, nasal epithelial cells; NF-κB, nuclear factor kappa B; AP, activator protein; MAPK, mitogen-activated protein kinase; RANTES, regulated on activation, normal T cell expressed and secreted; JNK, c-Jun N-terminal kinase; RT-PCR, real-time polymerase chain reaction; SF, serum free; CO_2, carbon dioxide; sICAM-1, soluble intercellular adhesion molecule-1.

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Results: Compared to control cells, NECs from subjects with atopic polyps released significantly greater amounts of IL-8 (median = 887 vs. 176.6 pg/μg cellular protein; $p < 0.0001$) and RANTES (median = 0.191 vs. 0.02 pg/μg cellular protein; $p < 0.001$). While 50 μg/ml DEP induced release of RANTES in NECs from patients with allergic rhinitis, 100 μg/ml DEP decreased IL-8 levels in NECs from both control and allergic rhinitic subjects. DEP did not affect mRNA expression for JNK and NF-κB from NECs of subjects with polyps.
Conclusions: NECs from subjects with various pathologies may respond differently to DEP.

Introduction

The number of diesel engine vehicles has increased owing to their decreased cost and high efficiency, and diesel exhaust particles (DEP) have become a serious health concern worldwide.[1] DEP play an important role in the exacerbation of allergic airway diseases.[2,3] Animal studies have shown that exposure to DEP results in increased production of specific immunoglobulin (Ig)-E, interleukin (IL)-4 and -5, and granulocyte-macrophage colony-stimulating factor (GM-CSF).[4,5] Human bronchial epithelial cells (BECs) have been reported to release IL-8 and GM-CSF following DEP exposure.[6] A limited number of studies with nasal epithelial cells (NECs) obtained from nasal explants of un-characterised patients reported that DEP increased the release of IL-8 and GM-CSF with inducing eosinophil degranulation.[7–9] The potential triggering mechanisms that cause the release of these inflammatory cytokines after DEP exposure are unclear.

Studies report that DEP activate oxidative stress pathways, such as nuclear factor (NF)-κB and activator protein (AP)-1.[10–13] Additionally, the mitogen-activated protein kinase (MAPK) pathway has been shown to be involved in the DEP-induced inflammatory effects in macrophages and BECs.[2,6,14,15] Since the nasal cavity is often the first site of exposure to noxious agents, we are interested in determining how DEP affect this area, especially in those with the common conditions of having allergic rhinitis or nasal polyposis. Therefore, we investigated the effects of DEP on the release of IL-8, GM-CSF, regulated on activation, normal T cell expressed and secreted (RANTES), and the expression of mRNA for c-Jun N-terminal kinase (JNK) and NF-κB in primary NEC cultures from well-characterised non-atopic, non-rhinitic subjects (control) in comparison to NEC from patients with allergic rhinitis or nasal polyposis.

Material and methods

Study subjects

Eighteen volunteers with a mean age of 34.15 ± 10.1 years participated in the study. Of these, six were control, five had allergic rhinitis, and seven had nasal polyposis. Table E1 (online depository) presents the study subject characteristics. Atopy was defined as the presence of one or more positive skin prick test reaction to 10 common aeroallergens (ALK, Denmark and Allergopharma, Germany), which included *Dermatophagoides pteronyssinus*, mixed grass pollens, tree mix, composites, weed pollens, cat, dog, *Alternaria alternata*, *Aspergillus* mixture, and *Blattella germanica*.

Symptomatic patients were included in the study if they had a confirmed history of allergic rhinitis by skin prick test (wheal diameter >3 mm) and reported at least one of the following naso-ocular symptoms: stuffy, itchy, and runny nose; sneezing; teary and itchy eyes. Patients that received immunotherapy in the last year and patients having acute upper respiratory tract infections or taking antibiotics within one month were excluded. Study subjects were asked to stop their nasal or oral steroids and antihistamines at least two weeks before the study. A baseline questionnaire was used to collect data on demographic details, clinical features (predominant symptoms, age of onset, and duration of disease), accompanying disorders (perennial rhinitis, asthma, and other disorders), smoking status, and familial atopy history. Six patients were former smokers. Five patients with nasal polyposis received nasal steroids regularly until the study. Of three patients with asthma, two were taking inhaled steroids. The Local Ethics Committee of Gaziantep University approved the study (Protocol No: 07/2011-31), and informed consent was obtained from all participants.

Reagents

All reagents were of tissue culture grade and purchased from Sigma Chemical Company (Interlab, Turkey) unless stated otherwise.

Nasal tissue

Nasal explants were obtained from subjects who underwent septoplasty, concha resection, polypectomy or endoscopic sinus surgery. Biopsies were taken from the inferior turbinate of the patients with allergic rhinitis and controls. Polyps overflowing from the middle meatus were completely resected during endoscopic sinus surgery. Each explant was processed for tissue culture within 30 min of resection.

Primary nasal epithelial cell cultures

An explant cell culture technique was used to culture the primary NECs.[6,16,17] Each specimen was observed with a dissecting microscope, and the epithelium was dissected from the underlying tissue, and processed for culture as fully described elsewhere.[6,16,17] The purity and identity of

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