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Sex difference in counts of α4 and α7 nicotinic acetylcholine receptors in the nasal polyps of adults with or without exposure to tobacco smoke

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Main Article

Dr K Jáuregui-Renaud takes responsibility for the integrity of the content of the paper

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Abstract

Objective. To assess counts of α4 and α7 nicotinic acetylcholine receptors in nasal polyps of adults with or without long-term exposure to cigarette tobacco smoke.
Methods. Twenty-two patients with and 22 patients without exposure to cigarette tobacco smoke participated in the study. After endoscopic polypectomy, the fragments of the nasal polyps were analysed by immunohistochemistry.
Results. Compared to patients with no exposure, patients with exposure showed higher counts of α4 and α7 nicotinic acetylcholine receptors (*t*-test, $p < 0.05$). However, in patients with no exposure, multivariate analysis showed gender dimorphism, with lower counts in males than in females, and no influence from other variables (analysis of covariance, $p > 0.05$).
Conclusion. Exposure to cigarette tobacco smoke may induce increased counts of α4 and α7 nicotinic acetylcholine receptors in nasal polyps of adults, with lower counts in males than females without exposure to tobacco smoke.

Introduction

Nasal polyposis is a chronic disease of the upper airway. It has been related to allergy, asthma, infection, cystic fibrosis and aspirin sensitivity.[1] However, irrespective of any specific cause, chronic persistent inflammation is recognised as a major factor.

Exposure to cigarette smoke is a risk factor for rhinosinusitis[2] and infection.[3] Tobacco smoke may decrease the effectiveness of mucociliary clearance, provoke cytological changes and disrupt the secretion of antimicrobial peptides.[4] Nicotine is an important constituent in cigarette smoke. Short-term exposure to nicotine increases intracellular calcium and cell migration in several cell types, while long-term exposure suppresses the immune and inflammatory responses.[5]

Nicotine from tobacco products may have direct effects on nasal mucosa cells through activation of nicotinic acetylcholine receptors ('nAChRs'). These can be expressed by a variety of cell types, including: lymphocytes, macrophages, dendritic cells, endothelial cells and epithelial cells.[6,7] In non-neuronal tissues, acetylcholine can be released into the extracellular space via organic cation transporters,[8] with autocrine and paracrine effects.[9] This non-neuronal system is thought to be involved in the regulation of cell functions such as cell-to-cell interaction, apoptosis and proliferation.[10]

The nicotinic acetylcholine receptor family consists of acetylcholine gated cation channels that form homologous or heterogeneous receptor subtypes.[11] In the human brain, the α4β2 nicotinic receptor is the most abundant subtype, with the greatest nicotine-induced up-regulation.[12] However, induced up-regulation of β2 nicotinic acetylcholine receptors by tobacco smoke seems to be different between males and females.[13]

The α7 nicotinic acetylcholine receptors subtype has been involved in several biological processes such as cell proliferation, apoptosis and angiogenesis in cancer.[14,15] In the airway epithelium, *in vitro* modulation of α7 nicotinic acetylcholine receptors controls the proliferation of human airway epithelial basal cells.[16]

Evidence suggests that direct toxic effects of inhaled nicotine on the respiratory tissues may interfere with the functioning of non-neuronal cholinergic networks by displacing from nicotinic acetylcholine receptor its natural ligand acetylcholine, which acts as a local hormone in a variety of non-neuronal locations.[17,18] Information on the distribution of nicotinic acetylcholine receptors in the nasal mucosa is limited. However, in specimens obtained from the inferior or middle turbinate of healthy subjects, and in patients with chronic sinusitis, inflammatory polyps and sinus allergy, there is evidence of transcripts for the α (1, 2, 3, 4, 6, 7) and β (2, 3, 4) subunit genes,[6] with a significantly lower

frequency of positive samples for β4 messenger RNA in diseased tissues as compared to normal tissues, and significant differences on β3 messenger RNA between genders.[6]

This study aimed to assess counts of α4 and α7 nicotinic acetylcholine receptors in nasal polyps from adult patients with and without long-term exposure to tobacco cigarette smoke.

Materials and methods

The study protocol was approved by the local committee of research and ethics, and informed consent was obtained from all participants. Forty-four patients with nasal polyps who underwent endoscopic polypectomy participated in the study. All were living within the same area of Mexico City. Inclusion in the study was considered consecutively when: nasal polyposis was diagnosed for the first time, and patients had no evidence of aspirin intolerance, cystic fibrosis, Churg–Strauss syndrome, fungal sinusitis, systemic infection, anatomical abnormality or pregnancy. In addition, none of the patients included had received immunotherapy, corticosteroids (nasal or systemic), cromolyn, anti-inflammatory treatment or anti-leukotrienes in the three months prior to participating in the study.

Exposure or no exposure to cigarette smoke was determined by means of the questionnaire designed for the National Addictions Survey of Mexico.[19] Regarding cigarette smoke exposure, patients were classified in terms of two groups (Table I). Group 1 comprised 22 patients exposed to cigarette smoke (mean (± standard deviation) age of 49.5 ± 11.4 years; 14 were males). They had been exposed to cigarette smoke during the previous 10–63 years (29.1 ± 12.8 years), but only 13 reported active smoking during the last 30 days. They were advised to avoid exposure during the week prior to polypectomy. Group 2 consisted of 22 patients who had not been exposed to cigarette smoke (aged 47.9 ± 13.2 years; 7 were males).

Prior to surgery, the Lund–Mackay scoring system for computed tomography was used to evaluate disease severity in the paranasal sinuses.[20] According to the opacity of the left or the right sinuses and the ipsilateral osteomeatal complex, unilateral scores (ranging from 0 (complete lucency) to 12 (complete opacity) and total bilateral scores (ranging from 0 to 24) were calculated.

Polypectomy was performed under general anaesthesia with orotracheal intubation.[21] The harvested biological material was analysed to confirm the diagnosis. The fragments of the nasal polyps were fixed in 10 per cent buffered formalin, embedded in paraffin, and stained with haematoxylin and eosin. The number of α4 and α7 receptors per square millimetre were assessed by immunohistochemistry using rabbit polyclonal antibodies (Anti-Nicotinic Acetylcholine Receptor alpha 4 antibody ab41172, at 1:25, and Anti-Nicotinic Acetylcholine Receptor alpha 7 antibody ab10096, at 1:25; Abcam, Cambridge, UK). All samples were analysed on slides, by 2 independent reviewers, using 10 randomly selected calibrated fields (with a Leica DM750 microscope (Leica Microsystems, Milton Keynes, UK), with magnification ×40).

After analysis with a Kolmogorov–Smirnov test, statistical analysis was performed according to data distribution using a *t*-test, Pearson's correlation coefficient and analysis of covariance; values of $p \leq 0.05$ were considered statistically significant.

TABLE I. CHARACTERISTICS OF 44 ADULTS WITH NASAL POLYPS, WITH AND WITHOUT EXPOSURE TO CIGARETTE TOBACCO SMOKE

Variables	Exposed*	Not exposed†	$p \leq 0.05$
Age (years)	49.5 ± 11.4	47.9 ± 13.2	–
Body mass index (kg/m^2)	26.6 ± 3.1	24.7 ± 3.9	–
Time of clinical evolution (years)	5.8 ± 5.1	8 ± 6	–
Lund–Mackay score			
– Bilateral	13 ± 4	14 ± 4	–
– Right	6 ± 2	6 ± 2	–
– Left	6 ± 2	7 ± 2	–

Data represent means ± standard deviations, unless indicated otherwise. *n = 22; †n = 22

Results

The general characteristics of the patients are described in Table I. The proportion of males was higher among patients exposed than those not exposed to cigarette tobacco smoke (*t*-test for proportions, $p = 0.03$). There was no difference between the groups in Lund–Mackay scores, for either unilateral or bilateral scores.

The frequency of allergy was similar in the two groups (exposed = 13 out of 22, and non-exposed = 15 out of 22),

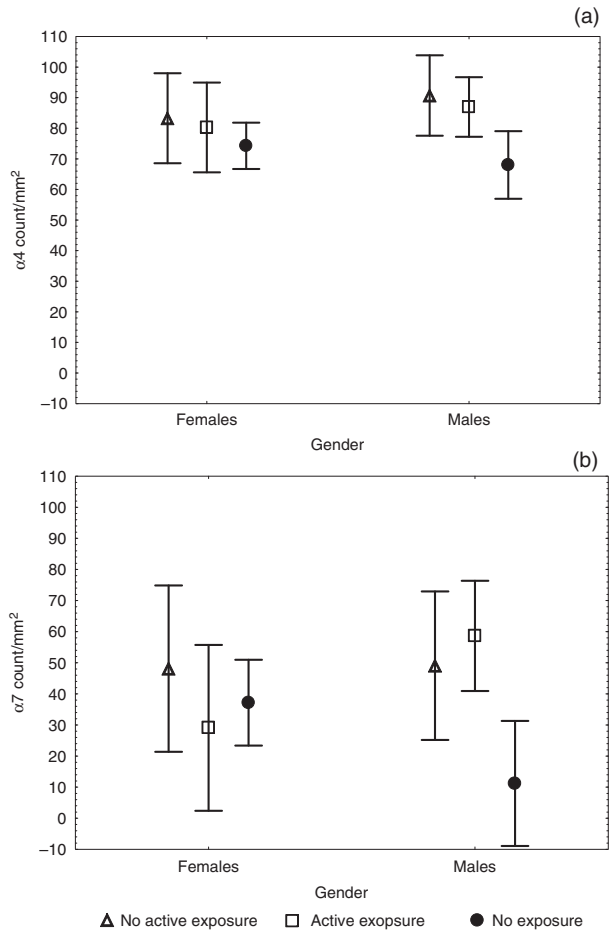

Fig. 1. Mean and 95 per cent confidence interval of the mean count per square millimetre of (a) α4 and (b) α7 nicotinic acetylcholine receptors in nasal polyps from adult patients (covariate mean age of 48.7 years) with or without (active or non-active) exposure to cigarette smoke.

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and the number of positive allergens during skin prick testing (AllerStand, Mexico City, Mexico) was also similar, ranging from 1 to 9 (median of 2). The most frequent allergen was dermatophagoides sp. (59 per cent in the exposed group *vs* 45 per cent in the non-exposed group; $p > 0.05$).

Bivariate analysis showed that both α4 and α7 nicotinic acetylcholine receptor counts were higher in the nasal polyps from adult patients exposed to tobacco smoke than in nasal polyps from patients with no exposure to cigarette smoke (student's *t*-test, $p < 0.05$). However, multivariate analysis showed that this difference was related to gender. Among patients with no exposure, males had lower counts than females (Figures 1 and 2), with no influence of age, body mass index or nasal allergy (analysis of covariance, $p > 0.05$). In addition, among those exposed to tobacco smoke, there was no influence of recent exposure (analysis of covariance, $p > 0.05$). The difference between males and females was more evident for the α7 nicotinic acetylcholine receptor count than for the α4 nicotinic acetylcholine receptor count (Figure 1b).

Discussion

The results of this study show that long-term exposure to cigarette smoke increases both α4 and α7 nicotinic acetylcholine receptor counts in nasal polyps. However, male patients with no exposure may show lower counts than female patients, independently of age, body mass index or nasal allergy.

The increase of nicotinic acetylcholine receptor counts related to tobacco exposure is in agreement with the effects of nicotine on inflammatory processes.[7,17] In human nasal epithelium, nicotine has anti-inflammatory effects via nicotinic acetylcholine receptors.[6,14] In macrophages, the main cholinergic receptor is the α7 subunit, which inhibits nuclear factor-κB signalling, thus inhibiting pro-inflammatory cytokine production.[14] Furthermore, nicotine suppresses inflammatory cytokines by heterologous α4β2 receptor activation.[22]

In those patients who were not exposed to cigarette smoke, the results showed lower counts of α4 and α7 nicotinic acetylcholine receptors in the nasal polyps of males compared to females. This finding is consistent with the already known gender dimorphism on cellular and humoral immune reactions,[23] in which differences in immune responses between sexes and reproductive phases are accompanied by variations in sex hormones.[24] Oestrogens and testosterone have an influence in antibody production: oestrogen increases immunoglobulin (Ig)-G and IgM production in both males and females,[25] directly and through a potentiating effect of interleukin (IL)-10 from monocytes. In contrast, testosterone decreases IgM and IgG production, both directly and indirectly, by reducing the production of IL-6 by monocytes.[26]

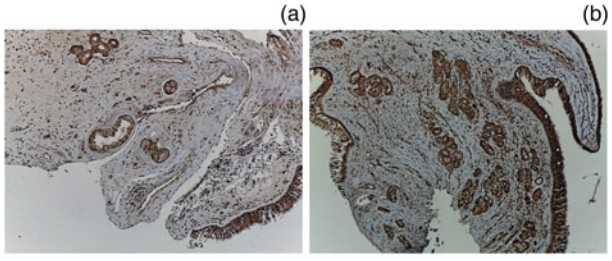

Fig. 2. Protein expression of α7 nicotinic acetylcholine receptors in a histological section of a nasal polyp from (a) a male and (b) a female without exposure to tobacco smoke. (Haematoxylin stain; ×10)

In the general population, the incidence and the prevalence of nasal polyps are higher in males than in females.[27] In addition, an increased parasite burden in males is hypothesised to be related to different pro-inflammatory (e.g. tumour necrosis factor α), type 1 T helper cell (e.g. interferon γ) and type 2 T helper cell (e.g. IgE) responses in males compared with females.[28] Further studies are needed to elucidate the mechanisms that contribute to sex hormone regulation of nicotinic acetylcholine receptor expression in nasal mucosa.

Gender dimorphism in the expression of nicotinic acetylcholine receptors may have to be considered, not only for the design and interpretation of studies on exposure to cigarette tobacco smoke, but also for the evaluation of cholinergic enhancers and nicotinic acetylcholine receptor agonists to treat conditions that can be modulated by inflammatory signalling.

- Toxic effects of nicotine on respiratory tissues may interfere with non-neuronal cholinergic networks
- Nicotine may have direct effects on nasal mucosa through nicotinic acetylcholine receptors
- Tobacco smoke may induce increased α4 and α7 nicotinic acetylcholine receptor counts in nasal polyps of adults
- Compared to females, males with no tobacco smoke exposure may have lower counts of α4 and α7 nicotinic acetylcholine receptors in nasal polyps

The main limitation of this study is its design. The cross-sectional design prevented us from discussing any causal relationship, and allowed us to identify just the most evident differences, without refuting other possible relationships among the variables. Although the number of women participating in the study was limited, and sex hormone cycles were not considered, the results showed a consistent difference between genders.

Conclusion

Exposure to cigarette tobacco smoke may induce increased counts of α4 and α7 nicotinic acetylcholine receptors in the nasal polyps of adult patients. Among those with no exposure to cigarette tobacco smoke, male patients may have lower counts of α4 and α7 nicotinic acetylcholine receptors than female patients. Further studies are needed to elucidate the clinical implications of this gender dimorphism.

Competing interests. None declared

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