RHINOLOGY

Nasal inflammation induced by a common cold: comparison between controls and patients with nasal polyposis under topical steroid therapy

Infiammazione nasale indotta dalla rinite virale: confronto tra pazienti controllo e pazienti trattati con corticosteroidi per poliposi nasale

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SUMMARY

The evolution of nasal inflammation during a common cold in patients with nasal polyposis under topical steroid treatment is not clearly defined in the literature. Objective of this study was to analyse nasal inflammation during a common cold in patients with nasal polyposis under topical steroid treatment in comparison with control subjects. Two groups of subjects (35 consecutive patients with nasal polyposis receiving medical treatment, and 17 control subjects without any symptoms of chronic rhino-sinusitis) were studied: 10 patients with nasal polyposis and 11 controls had a common cold during a one-year follow-up period. Nasal lavage was performed at baseline and during the common cold. Soluble inflammatory mediators and permeability markers were determined in the nasal lavage fluid, as well as total and differential counts of the cells present. At baseline, no significant difference between controls and patients was observed, except for eosinophils. Paired comparisons between baseline and cold in controls revealed that all measured parameters, except for eosinophils, increased in the second nasal lavage. In nasal polyposis patients, the total cell neutrophil counts tended to increase. However, most of the concentrations of soluble parameters did not vary significantly in the second lavage, except for interleukin-6. In conclusion nasal inflammation markers appear to be similar in patients with and without nasal polyposis during a common cold when nasal polyposis patients are under topical steroid treatment.

KEY WORDS: Nose • Common cold • Nasal polyposis • Therapy

RIASSUNTO

Durante la rinite virale comune, l'evoluzione dell'infiammazione nasale, per i pazienti sotto trattamento con corticosteroidi topici per poliposi nasale, non è chiaramente definita dalla letteratura. In questo studio abbiamo realizzato un'analisi comparativa dell'infiammazione nasale durante un episodio di rinite virale comune tra pazienti trattati con corticosteroidi topici per poliposi nasale e pazienti controllo. Due gruppi di pazienti (35 pazienti consecutivi con poliposi nasale trattata con terapia medica, e 17 pazienti controllo senza nessun segno di patologia rinosinusale cronica) sono stati analizzati. Complessivamente 10 pazienti con poliposi nasale e 11 controlli hanno presentato una rinite virale durante il periodo di sorveglianza di un anno. Un lavaggio nasale è stato eseguito all'inclusione ed un altro durante l'episodio di rinite. I mediatori solubili dell'infiammazione, gli indicatori di permeabilità sono stati analizzati nei lavaggi nasali come lo sono stati i conti cellulari totali e differenziali. A livello basale non si è evidenziata nessuna differenza statisticamente significativa tra i pazienti con poliposi nasale ed i controlli tranne per gli eosinofili. Il confronto tra valori basali e valori del secondo lavaggio hanno dimostrato per i controlli un aumento di tutti i parametri, eccetto degli eosinofili. Nella poliposi nasale il conto totale di neutrofili tendeva ad aumentare. Tuttavia nella poliposi nasale tutti gli altri parametri solubili, eccetto l'interleuchina-6, non hanno mostrato nessuna variazione significativa tra primo e secondo lavaggio. In conclusione gli indicatori di infiammazione nasale durante gli episodi di rinite virale sembrano simili per i pazienti con poliposi nasale trattata con corticosteroidi topici e i pazienti senza poliposi nasale.

PAROLE CHIAVE: *Naso • Raffreddore comune • Poliposi nasale • Terapia*

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Introduction

Nasal polyposis (NP) is a chronic inflammatory disease of the paranasal sinus mucosa leading to the protrusion of oedematous polyps from the ethmoid complex into the nasal cavity [1]. Polyps are covered with respiratory ciliated pseudo stratified epithelium and oedematous stroma filled with inflammatory cells such as eosinophils, mast cells, and lymphocytes. Eosinophilia is the main characteristic of NP inflammation, although it is still unclear why these cells accumulate [2]. They are the main targets of cytokines such as interleukin (IL)-5, IL-3, RANTES, and eotaxin which are

chemoattractant, activate recruitment of mature eosinophils and prolong their survival [3 4]. Several other pro-inflammatory cytokines [GM-CSF, IL-6, IL-8, tumour necrosis factor-alpha (TNFα)] have been shown to be up-regulated in various nasal polyp tissues [3-5]. Inflammatory mediators involved in NP have been traditionally studied during sinus surgery [3 4] in nasal secretions [2]; some Authors have also used nasal lavage, a simple and non-invasive method for studying inflammatory mediators [6].

Nasal polyps can cause nasal blockage, anterior and posterior rhinorrhea, smell and taste disorders, sneezing, and facial pain, therefore their effect on daily life can be significant [7]. Topically applied corticosteroids have a favourable effect on symptoms, most probably by down-regulating the expression and production of previously blocked cytokines (IL-5) and reducing the number of eosinophils. The disease can be exacerbated by two known factors: one is the common cold, as observed with asthma, and the second the interruption of treatment. Other factors that could exacerbate nasal polyposis have not yet been clearly identified. Similarly, despite the large number of papers on NP published in the literature, nasal inflammation during these exacerbation periods has not been extensively studied until recently. The aim of this study is to compare nasal inflammation in patients with and without nasal polyposis during a common cold.

Materials and Methods

Patients and control group

This prospective study included two groups of patients. First, 35 consecutive patients with nasal polyposis (19 males, mean age: 52.5 years, range 30-80), non smokers, were included. All patients were examined and treated by the same physician for the duration of the study. The diagnosis of nasal polyposis was based on two criteria: (i) the presence of bilateral polyps in the nasal cavity on endoscopic examination, and (ii) the existence of bilateral opaque areas located in the ethmoidal sinuses on computed tomography (CT) scans (axial and coronal planes) performed without contrast medium. Five patients (14.3%) had typical hypersensitive reactions to aspirin or to non-steroidal anti-inflammatory drugs. Overall 14 patients (40%) had asthma, and 8 received inhaled corticoids and beta-2-mimetics at the time of the study. Patients with asthma did not receive montelukast sodium orally. All NP patients were treated with two different therapeutic measures [8]. First, lavage of the nasal cavities was carried out twice a day with a physiological solution. Then, 30 minutes after the lavage, patients received intra-nasal steroid spray (budesonide) at a daily dosage of 512 µg (128 µg twice a day in each nasal cavity). Second, 17 control subjects (4 males, mean age: 34.8 years, range 24-54), non smokers, without symptoms of chronic rhino-sinusitis were included.

Clinical score

Nasal function was checked on the basis of three criteria: nasal obstruction, anterior rhinorrhea, and anosmia. The severity of each symptom was evaluated according to a three-point scale: 0, no symptoms; 1, mild to moderate symptoms; and 2, severe symptoms [8]. With regard to the sense of smell analysis, anosmia was noted as grade 2, hyposmia as grade 1, and normal function as grade 0. A clinical global severity score (GSS) was obtained, ranging from 0 to 6, which represented the sum of the previous symptoms. The common cold was clinically defined by a two-point scale increase in GSS over 3 consecutive days.

Study design

A nasal lavage was performed in patients and controls at baseline (i.e., before the common cold). When the patients or controls returned with clinical symptoms of a common cold, a second nasal lavage was performed on the third day of the common cold. The study was approved by the local Ethics Committee, Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Paris Cochin (France). All subjects gave informed consent prior to the study.

Nasal lavage

Nasal lavage was performed as described elsewhere [9]. The nasal lavage fluid was centrifuged (500 g, 15 min). The supernatant was stored at -70 °C pending soluble inflammatory mediator assay. IL-5, IL-6, IL-8 levels were determined using commercially available kits (enzyme-linked immunosorbent assay (ELISA) kit, R&D Systems, Abingdon, UK) according to the manufacturer's instructions. Detection limits were respectively 3 ng/L, 0.7 ng/L, and 10 ng/L. Two permeability markers were also studied, albumin and urea: an immunonephelemetric method was used to as-

Table I. Inflammatory mediator levels in nasal lavage at baseline in the two study groups: controls and patients with nasal polyposis (NP patients).

	Controls n = 17	NP patients n = 35	p value
Total cells (10^3/ml)	16.8 (6.3-32.0)	14.9 (7.8-29.5)	NS
Epithelial cells (%)*	95.0 (61.0-100)	71 (38.5-84.5)	NS
Leukocytes (%)*	5 (0-39)	31 (15.6-61.5)	NS
Neutrophils (%)*	3.0 (0-39)	21.0 (2.0-55.0)	NS
Eosinophils (%)*	0	2.0 (0-13.0)	p < 0.001
Lymphocytes (%)*	0 (0-0.5)	0 (0-0.2)	NS
Urea (µmol/l)	560.0 (398.5-99.0)	530.0 (446.0-639.0)	NS
Albumin (mg/l)	16.6 (5.0-23.5)	19.2 (8.4-29.3)	NS
α1-antitrypsin (µg/l)	336.0 (157.0-534.0)	435.0 (261.0-702.0)	NS
IL-6 (ng/l)	1.5 (< DL-5.5)	5.9 (< DL-11.1)	NS
IL-8 (ng/l)	350.0 (64.0-565.0)	226.0 (79.0-398.0)	NS
IL-5 (ng/l)	< DL	< DL	NS

Results are expressed as median value (25th and 75th percentiles); DL: detection limit; NS: not significant; * Percentage is expressed against total cells

sess albumin and an enzymatic method for urea. The lower detection limits were 2 mg/L and 30 μmol/L, respectively. The anti-protease α1-antitrypsin (AAT) was also analysed (ELISA kit, R&D Systems). The pellet was sieved [9] and total cell counts were counted in Malassez cell. Differential cell counts of eosinophils, neutrophils, lymphocytes, and epithelial cells were performed on a cytospin stained with May-Grunwald Giemsa, and the percentage of each cell type was calculated for each sample.

Statistical analysis

Statistical analysis was performed using BMDP software. Data were presented as median values (25th and 75th percentiles). Kruskal-Wallis test was used to compare mediator distributions between controls and NP patients whereas Wilcoxon paired test when comparing exacerbated/cold situation to basic data, in each group. Data less than the detection limit were assigned a value of one-half the detection limit.

Results

Results at baseline

At baseline, mean GSS was respectively equal to zero and 1.6 ± 1.3 in controls and NP patients. During a common cold, this mean score increased to 3.3 ± 1.0 in controls, 3.9 ± 1.9 in NP patients. Only 10 out of the 35 NP patients and 11 of the 17 controls fulfilled the conditions (i.e., a common cold) and underwent two nasal lavages.

Considering the inflammatory mediator levels in nasal lavage fluid at baseline, total and specific cell (epithelial cells, leukocytes, neutrophils, eosinophils) counts in controls and NP patients are summarized in Table I. No significant difference between controls and NP patients was observed for either total cell counts per ml or specific cell counts per ml, except for eosinophils. Effectively, when the results are expressed as percentage, the eosinophil counts differed significantly between control subjects (0%) and NP patients [median value: 2% (0-13%)]. On the other hand, no significant difference was observed between controls and NP patients as far as concerns the concentration of soluble parameters (urea, albumin, AAT, IL-6, IL-8 and IL-5).

Results during common cold

According to paired comparisons of inflammatory mediators between baseline and cold (Table II) for the 11 controls subjects who underwent two lavages, all measured parameters, except for eosinophils, increased in the second nasal lavage: albumin ($p < 0.05$), AAT ($p < 0.01$), IL-6 ($p < 0.01$), and IL-8 ($p < 0.01$), total cell counts per ml ($p < 0.05$), leukocyte counts per ml ($p = 0.05$), neutrophil counts per ml ($p < 0.05$), although the cell percentages remained unchanged.

Table II. Inflammatory mediator levels in nasal lavage at baseline and during common cold in controls and patients with nasal polyposis (NP patients).

	Controls n = 11			NP patients n = 10		
	Baseline	**Common cold**	**p value**	**Baseline**	**Exacerbation**	**p value**
Total cells (10^3/ml)	16.8 (0.7-31.1)	30.4 (10.4-80.5)	$p < 0.05$	12.6 (7.1-26.2)	31.3 (12.3-74.6)	$p = 0.07$
Epithelial cells (%)*	95.0 (54.5-98.5)	46 (18.5-86.5)	NS	78.5 (37.0-97.8)	40.5 (13-64.8)	$p = 0.07$
Leukocytes (%)*	5.0 (15-35.5)	54.0 (13.5-81.5)	NS	21.5 (2.3-63.0)	58.5 (24.0-86.3)	$p = 0.1$
Neutrophils (%)*	3.0 (0-35.5)	54.0 (13.5-80.8)	NS	11.0 (0-32.3)	34.0 (14.8-81.8)	$p = 0.1$
Eosinophils (%)*	0	0 (0-0.3)	NS	0 (0-16)	5 (0-24.5)	NS
Albumin (mg/l)	8.7 (5.1-27.2)	32.6 (24.9-88.8)	$p < 0.05$	16.8 (13.4-23.1)	16.0 (10.50-38.2)	NS
Urea (µmol/L)	532.0 (381.0-584.0)	643.0 (341.0-847.0)	NS	591.0 (526.0-667.8)	711.5 (548.8-940.3)	NS
α1-antitrypsin (µg/l)	252.0 (195.0-487.5)	976.0 (366.0-2757.0)	$p < 0.01$	480.5 (268.0-763.8)	762.0 (333.5-2881.8)	NS
IL-6 (ng/l)	1.5 (< DL-4.7)	79.3 (42.8-154.9)	$p < 0.01$	4.8 (0.5-11.1)	20.0 (8.1-51.0)	$p < 0.01$
IL-8 (ng/l)	448.0 (100.0-589.0)	786.0 (640.0-1346.0)	$p < 0.01$	185.5 (82.0-1225.0)	741.0 (122.0-1353.0)	NS
IL-5 (ng/l)	< DL	< DL	NS	9.5 (< DL-13.9)	< DL (< DL-2.3)	$p = 0.07$

Results are expressed by median values (25th and 75th percentiles); DL: detection limit; NS: not significant; *Percentage is expressed against total cells

In the 10 NP patients with a common cold during the follow-up period, total cell counts per ml ($p = 0.07$), percent leukocytes ($p = 0.1$) and percent neutrophils ($p = 0.1$) tended to increase during the common cold. Moreover, a decrease in the percentage of epithelial cells ($p = 0.07$) was observed (Table 2). The concentration of soluble parameters did not vary in the second lavage, except for the IL-6 concentration, which was almost four-fold higher ($p < 0.01$), and surprisingly, IL-5 levels, for which a non-significant decrease was observed.

Discussion

This prospective study was developed to determine the evolution of nasal inflammation during a common cold in patients with nasal polyposis in comparison to subjects without any symptom of chronic rhino-sinusitis. All patients with nasal polyposis received adequate medical treatment [8].

Nasal lavage is a convenient sampling method for studying nasal inflammation. Indeed, sampling can be performed at any time and whatever the physiopathological circumstances, in contrast to surgical biopsy, for example. We did not perform viral diagnosis or allergy testing since our purpose was not to establish the origin of exacerbation in NP patients but to evaluate the inflammatory status in the two groups.

Naturally acquired viral rhinitis has been far less investigated than experimental viral challenge in asthmatic and healthy subjects. In our controls, an inflammatory process with a neutrophil influx, and an increase in cytokines IL-8 and IL-6 levels characterized cold. These findings had previously been reported by Fleming et al. [10] in an experimental study, in nasal lavage of healthy subjects during an acute cold. The IL-6 and IL-8 levels peaked on the second day after inoculation of rhinovirus 16 and returned toward baseline some weeks later. During a naturally acquired common cold in healthy subjects, Röseler et al. [11] described increased levels of IL-6 and IL-8 in nasal washes in symptomatic subjects compared to baseline values [11] in agreement with our results. With regard to exudation markers, as expected, a significant increase in albumin, urea, and AAT was also observed in our study during a common cold. AAT, also originating from serum, is involved in the protease-anti-protease balance by inhibiting neutrophil elastase released by triggered neutrophils on inflammatory site. Unlike albumin and urea, AAT levels had never been studied during a common cold.

In patients with medically treated nasal polyposis, at baseline, marker levels were not statistically different from those of controls, except for the presence of eosinophils, a hallmark of the disease. NP patients did not present more total cells in their nasal lavage than controls, but tended to have more leukocytes and fewer epithelial cells and increased eosinophils compared to percentage of neutrophil cells. Our results confirmed those of Jankowski et al. [2]. In histological studies, eosinophil cells infiltrate 80 to 90% of different polyp tissues [2]. Eosinophil cells are the targets of cytokines such as IL-5, which is chemotactic, activates mature eosinophils, and prolongs their survival. A number of studies have reported an increase in IL-5 levels, IL-5 immunoreactivity, IL-5mRNA expression, soluble IL-5 receptor α in polyp tissue compared to turbinate mucosa [3-5 12-14]. Immunohistochemical methods have shown that eosinophil cells might secrete IL-5 [3], which would create an autocrine effect on the inflammatory site. This increase in IL-5 levels was detected in untreated patients [3 15]. Indeed, Lee et al. [16] found a relation between eosinophil counts and IL-5 levels in nasal lavage, in NP patients who interrupted their treatment for four weeks. Corticosteroids mainly down regulate production of IL-5, and, consequently, reduce the number of eosinophil cells in bone marrow [17]. However, in our study, IL-5 levels were mostly undetectable, which is in agreement with the literature since the NP patients were under corticoid treatment [3 5], even though we observed persistence of eosinophil cells in nasal lavage fluid, without any relation to IL-5 levels. In our study, in which all NP patients were receiving local corticoid therapy at the time of baseline lavage, our results indicate that this therapy affects the levels of nasal inflammation markers as previously shown [5]. Indeed, so much so that these levels are no different from the levels of controls.

To our knowledge, our study is one of the first to deal with a panel of inflammatory markers to describe the global inflammatory process during a common cold in patients with nasal polyposis under topical steroid therapy. In the common cold, infectious agents induce a profound inflammatory response on the airway mucosa. This immune reaction leads to release of different inflammatory substances that are thought to play an important role in increasing the symptoms of nasal polyposis [18 19]. It has been shown that up to 80% of patients with a common cold have inflammation in paranasal sinuses [18]. During the common cold, nasal lavage fluid levels changed in NP patients, but with a pattern similar to controls with a cold, apart from eosinophilia and IL-5 that were always absent in control lavage. The nasal IL-6 level increased significantly, whereas albumin, urea and AAT increased only slightly. But the increase in IL-8 levels was not statistically significant, which might be due to the small sample size. At present, contrary to asthmatic or allergic rhinitis subjects, there are few data on nasal inflammation during a common cold in NP patients under medical treatment [20 21]. Keith et al. [20] observed increased albumin levels in NP patients compared to those without NP. In addition, studies have mainly focused on cytokines.

As shown by nasal score at baseline, NP patients under local steroid therapy have continuous nasal symptoms but the inflammatory profile is similar to that of controls apart from percent eosinophil, the latter being higher in the nasal lavage fluid of NP patients than in that of controls, in spite of corticotherapy. In the same way, during a common cold, the relative intensity of the inflammatory response seems to be similar in NP patients as compared to that in controls, presumably related to the effects of corticotherapy on cellular and soluble parameters.

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