Effects of topical corticosteroid on lymphoid cells in nasal lavage

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The aim of this study is to determine whether the budesonid nasal spray, which is a nonhalogenated glucocorticoid with higher local consequences, effects the lymphoid cells in nasal polyposis and whether the effects of budesonid can be observed in nasal lavages of patients with nasal polyposis. Twenty-two patients with nasal polyposis were investigated in this study. A control group of 25 healthy persons was also included. Each patient was given 400 yg of budesonid spray per day for a duration of four weeks. Effects of budesonid on lymphoid cells were evaluated by flow cytometric analysis of nasal lavage obtained from patients before and after the treatment. These values were compared statistically with those obtained from the control group. Following the treatment the ratios of T cells/Naturel killer cell, T helper cell/T suppressor cell and T cell/Active Tcell showed a significant increase whereas the ratio ofTcell/B cell did not change significantly. It can be concluded that budesonid has some effects on of activation and kinetics T lymphoid subsets in nasal mucosa and that these effects can be detected by flow cytometrical analyses of nasal lavages of patients. [Turk J Med Res 1997; 15(2):60-63]

Key Words: Topical corticosteroid, Nasal polyposis, Lymphoid cells

Nasal polyps are obstructing tissue formations containing oedema fluid, but with an intact surface epithelium similar to that of the human nose (1,2). Histologically, polyps are characterized by infiltration of inflammatory cells and proliferation of connective tissue in the stroma and tissue eosinophilia. Eosinophils are the most common inflammatory cells in about 80% of nasal polyps (3). Other inflammatory cells include macrophages, plasma cells, mast cells and lymphocytes (4). Nasal polyposis is, therefore an inflammatory disease.

T and B lymphocytes, which are regulatory and effector cells in complex process of inflammatory responses, are found in the nasal mucosa (5-7). HLA-DR$^+$ epithelial cells, which are also present in the nasal mucosa, may activate CD$^+_8$ T lymphocytes (8).

Nasal lavage has been shown to be a useful tool in the study of inflammatory cells and their mediators in diseases affecting the nose (9-14). Although mucosal infiltration by T cell was demonstrated in upper airways of patients with rhinitis, these cells could not be detected in nasal lavages in the previous studies (15,16).

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Stoop et al (7,17) demonstrated that, CD$^+_4$ cells are found significantly in higher amounts in healthy subjects than in patients with nasal polyps.

Budesonide is a non halogenated glucocorticosteroid with a higher ratio of local antiinflammatory activity over the systemic one (18). Glucocorticosteroids reduce the number of T lymphocytes that accumulate close to surface of the nasal mucosa of patients with allergic rhinitis and nasal polyps (9,19,23).

We studied the effects of budesonide on lymphoid cells in nasal lavage of patients with nasal polyposis.

MATERIALS AND METHODS

Patients: This study was carried out on 22 patients with nasal polyps ranging in age from 19 to 45 (with a mean age of 28 years).

Control Group: The control group was composed of 25 healthy subjects (7 women and 18 men, ranging in age from 18 to 34 years; with a mean age of 24). These subjects did not have any upper respiratory disease such as nasal allergy, nasal polyps, chronic sinusitis and common cold.

Drug Administration: Patients were given 400 u.g budesonide nasal spray once a day for a duration of 4 weeks. This treatment was well tolerated by the patients.

Nasal Lavage Technique: A nasal lavage technique, described in detail by Naclerio et al (11), was applied. With their heads bent back, the patients was given 5 ml

of isotonic saline solution in room temparature in each nasal cavity for 10 seconds. The lavage fluid was then collected in a plastic cup and was immediately chilled and centrifuged at 4°C at 500 g for 10 min. The sol phase was separated from cellular phase, and the supernatant was aliquoted for subsequent analyses.

Lymphocytes obtained before and after the treatment from nasal lavage of patients with nasal polyposis were detected with flow cytometric analysis.

Statistical Analysis

All the data are expressed as the mean valuelstandard deviation. The significance of the differences between the data was determined by Mann-Whitney-U tests. A p value of less than 0.05 was considered to be significant. A comparison was made between the result of flow cytometric analysis of lymphoid subsets in nasal lavage before and after the treatment. Statgraf version 5.0 statistical program was used in data analysis.

Flow cytometric analysis

Following steps were taken to obtain a total cell count using flow cytometric analyses:

1) Supernatant was taken out, then 20 ml of CD_3/CD_{19}, CD^+_4/CD^+_8, $CD_3/HLA\text{-}DR^+$, CD_3/CD_{16+56}, CD_{23} monoclonal antibody per tube and 200 ml of nasal secretion were added to each tube.
2) Tubes were incubated for 20 minutes at room temperature.
3) They were analysed by Coulter-Multi Q prep device.
4) Tubes were flow cytometrically analysed by Coulter Epics Elite ESP device and approximately 10.000 cells were counted per each tube.

RESULTS

Flow cytometric analyses of lymphocyte subsets in nasal lavage of patients at the times before treatment and healthy persons are shown in Table 1.

We calculated the ratios of T cell to Natural killer cell ($CD_3/CD_{16}.56$). T helper cell to T suppressor cell (CD^+4/CD^+8), T cell active to T cell ($CD_3/HLA\text{-}DR^+$) in nasal lavage from patients with nasal polyps before and after their treatment (Table 2). The difference between all these ratios were statistically significant ($p<0.05$) except for that between T cell/B cell which had a p value of >0.05 implying an insignificant difference between the values obtained before and after the treatment. There was no significant difference between the immunological parameters obtained from the patient following their treatment and those from the control group ($P>0.05$) (Table 3).

The eosinophil ratio in nasal lavage was found to be 65.8% but showed a significant decrease after the corticosteroid treatment and dropped to 8.4% (Figure 1).

Table 1. Lymphocyte subset ratios of nasal lavage of patients with nasal polyps at the times before treatment and healthy persons.*

	No	T cell/natural killer cell ($CD_3/CD_{16}.5_6$)	T helper cell/T suppressor cell ($CDVCD^+_8$)	T cell/B cell ($CD3/CD_{19}$)	T cell/active T cell (CD_3/HLA-DFO
Before treatment	22	3.9210.96	0.92±0.42	4.07±1.12	3.08±0.82
Healthy subjects	25	5.12±1.34	2.05+0.89	3.75±1.25	5.89+1.55

*p<0.05

Table 2. Immunological parameters of nasal lavage of patients before and after their treatment.

	No	T cell/natural killer cell ($CD_3/CD_{16},56$)	T helper cell/T suppressor cell ($CDVCD^+_8$)	T cell/B cell (CD_3/CD_{19})	T cell/active T cell (CD_3/HLA-DFO
Before treatment	22	3.92±0.96	0.92±0.42	4.07±1.12	3.08±0.82
Healthy subjects	22	4.92±0.94	, 1.98±0.40	4.36±1.02	4.45±0.92
p value		p<0.05	p<0.05	p>0.05	p<0.05

Table 3. Lymphocyte subset ratios of nasal lavage from the patients their treatment and those from the control group.

	No	T cell/natural killer cell (CD_3/CD_{16156})	T helper cell/T suppressor cell ($CDVCD^+_8$)	T cell/B cell (CD_3/CD_{19})	T cell/active T cell ($CD_3/HLA\text{-}DR^+$)
After treatment	22	4.92±0.94	1.98±0.40	4.36±1.02	4.4510.92
Healthy subjects	25	5.12±1.34	2.05+0.89	3.75±1.25	5.8911.55
p value		p>0.05	p>0.05	p>0.05	p>0.05

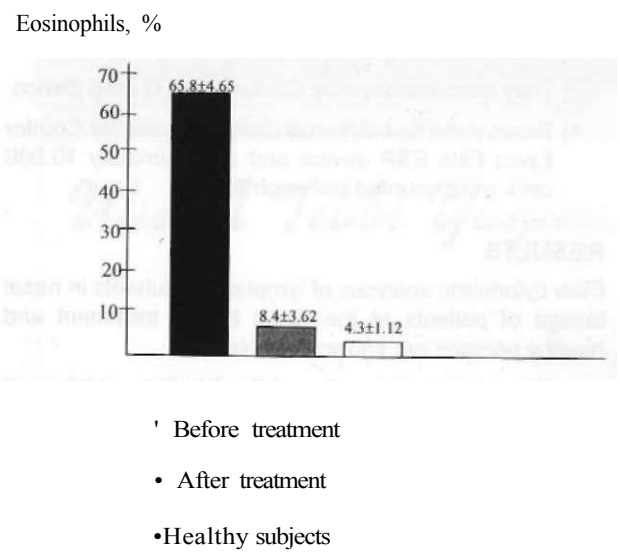

Figure 1. The eosinophil ratio in nasal lavage before and after treatment.

DICUSSION

Stoop et al (20) demonstrated that immunological and inflammatory processes play a prominent role in the development of nasal polyps. Chronic inflammatory response is characterized by an increase in the number of monocytes and lymphocytes (21). The role of eosinophils in continuing the inflammatory response through the release of highly toxic mediators is now well established. Other cells, such as neutrophils, platelets, monocytes and macrophages may also contribute to chronic inflammation, although their effects have yet to be defined.

During chronic inflammation, the respiratory mucosa may become damaged by oedema, generated by the mediator induced increase in vasculer permeability. This vasculer leakiness allows more inflammatory permeability and inflammatory agent into tissues of the mucosa. More CD^+_4 cells are found in healthy subjects than in patients with nasal polyps (7,17). In our study, significantly more CD^+_4 cells were found in patients after the treatment than before.

Thus, T helper cell to T suppressor cell ratio in patients with nasal polyps (before the treatment) was significantly lower than that in healthy subjects ($p<0.05$). Following 4 weeks treatment with budesonid, a significant increase in this ratio was observed in the nasal lavages of patients.

HLA-DR+ cells possibly play a role in uptaking and presentation of antigens. $HLA\text{-}DR^+$ epithelial cells may also activate CD^+_4 cells as demonstrated by Mayer and Shlien (8). This could suppress the chronic inflammatory reaction in the polyps and middle turbinates of he patients. In our study, T cell to HLA-DR+ ratio in patients before the treatment was significantly lower than the ratio after the treatment ($p<0.05$).

Budesonide has a high relative binding affinity for glucocorticosteroid receptor in vivo (22). Glucocorticosteroids reduce the number of T lymphocytes that accumulate close to surface of the nasal mucosa in patients with allergic rhinitis and nasal polyps (9,19,23). The number of antigen processing Langerhans cells is also known to decrease in response to glucocorticosteroid treatment (23). There is also good evidence that glucocorticosteroids affect T lymphocyte kinetics, activation and lymphokine production, although direct evidence for the role of these cells in inflammation of the nasal and bronchial mucosa, has yet to be obtained (24).

Late nasal responses are accompanied by T cell recruitment and activation, local tissue eosinophilia and cytokine mRNA expression for cytokines. Treatment with topical corticosteroids is effective in inhibiting both early and late nasal responses. One mechanism by which this may occur may be the supression of IL-4 mRNA expression (by either T lymphocytes and/or mast cells) with consequent disruption of local IgE and inhibition of tissue eosinophilia (25).

Eosinophils activated by allergen provacation increase in the epitel in nasal mucosa and lamina propria. Following a corticosteroidal treatment the eosinophilia in the epithelium in nasal mucosa show a higher decrease compared to those in lamina propria (26). This decrease is also reflected in nasal lavage (27). In this study the eosinophil ratio in nasal lavage was found to be 65.8% but showed a significant decrease after the corticosteroid treatment and dropped to 8.4%.

Candidiasis during treatment with steroids is related to the total dose, and is usually associated with poor oral-hygiene (28%). We periodically examined the patients at 10 day intervals to see whether they develop candidiasis and found no cases.

The similiarity between the immunological parameters obtained from the patient following their treatment and those from the control group is important as it shows the efficiency of the treatment.

In summary, T and B lymphocytes play predominant role in the development of nasal polyposis. It is put forward in this study that the ratios of T cell to Natural killer cell ($CD_3/CD\text{-})_{6}.5_{6}$), T helper to T suppressor (CD^+_4/CD^+_8), T cell to active T cell ($CD_3/HLA\text{-}DR^+$) in nasal lavage of patients are normally lower than those in healthy persons. However, following treatment with budesonide, a significant increase occured in these ratios in nasal lavage.

Topikal kortikosteroidlerin nazal lavaj sıvısındaki lenfoid hücreler üzerine etkisi

Bu çalışmada, lokal etkili halojensiz glukokortikoid olan budesonid nazal spreyin nazal polipozda lenfoid hücreler üzerindeki etkisi ve bu etkinin nazal lavajda belirlenmesi a-

maçlanmıştır. Nazal polipozlu 22 hasta çalışmaya alınmış, 25 kişilik sağlıklı kontrol grubu kullanılmıştır. Her hastaya 4 hafta süreyle günde 400)jg budesonid sprey uygulanmıştır. Budesonid'in lenfoid hücreler üzerindeki etkisi, nazal lavaj sıvısında tedavi öncesi ve sonrası flow sitometrik inceleme ile değerlendirilmiştir. Bu değerler kontrol grubundan elde edilen değerler ile karşılaştırılmıştır. Tedaviyi takiben T hücre/Natural killer hücre>T helper/T suppressor hücre ve T hücresi/Aktif T hücresi oranları belirgin artış gösterirken T hücre/B hücre oranında belirgin bir değişiklik olmamıştır. Sonuç olarak Budesonid'in nazal mukozada T hücre alt tiplerinin aktivasyonu ve kinetiği üzerinde bazı etkileri olduğu ve bu etkilerin hastaların nazal lavajlarının flow sitometrik incelemesi ile belirlenebileceği düşünülmüştür.
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