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Quantitative analysis of nasal vascularization in allergic patients treated with mometasone furoate*

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SUMMARY

The purpose of this study was to compare vascularization of the nasal mucous membrane among non-allergic, non-treated allergic and allergic patients treated with mometasone furoate, by means of the stereology method in quantitative analysis. Three groups of patients (GP), each containing 10 patients were examined. The first group (GP 1) had a negative inhalatory allergen test while the second (GP 2) and third (GP 3) group both had positive results with the same test. GP 3 included allergic patients treated with mometasone furoate for 15 days before analysis, when a small piece of the nasal mucous membrane was taken from the frontal pole of the lower nasal shell. The specimens were examined immunohistochemically for expression of CD31 and VEGF-C. Vascular phase was determined by using length density (L_v). Differences in CD31 and VEGF-C expression were compared using one-way ANOVA and Tukey HSD post-hoc tests. CD31 expression in GP 1 had significantly lower values than in the GP 2 and GP 3 groups ($p < 0.001$). VEGF-C expression in GP 1 was significantly lower than in GP 2 ($p = 0.007$), but not in GP 3 ($p = 0.292$). We have shown that 15-day treatment with mometasone furoate results in a significant reduction of the density of vascular elements in allergic patients.

Key words: vascularization, nasal mucous membrane, allergy, mometasone furoate, stereology

INTRODUCTION

Angiogenesis plays an important role in diverse pathological mechanisms. It has been suggested that vascularization of the nasal mucous membrane in patients with allergies differs from that found in patients with healthy mucosa and that changes in the vascular network contribute to the pathogenesis of allergic rhinitis [(1)]. An increased number of blood vessels could be one of the factors influencing the overall increase in the surface of the blood vessel walls followed by increased permeability as well as transition of a greater number of eosinophiles and other inflammatory cells into the surrounding tissues. The greater the number of vessels and subsequently their wall permeability, the likelihood of more intensive inflammatory reaction increases. Thus, the question is whether local medicament therapy (mometasone furoate) of allergic patients could lead to a substantial change in nasal mucous membrane vascularization.

MATERIALS AND METHODS

Patients

For the purpose of this study, three groups of patients (GP), each consisting of 10 patients were tested. The patients were grouped according to the results of an allergy test to inhalatory allergens, including: grass, weed, tree pollen, feathers, herbal fibbers, fungi, fabric, animal hairs, domestic dust, dermatophagoides pteronyssimus and bacteria. The first group (GP 1) (patients without allergies; with a negative allergic reaction to all of the allergens) included four female and six male patients, age range from 23 to 47 years (average age 31.5 years). The second group (GP 2) (allergic patients; with a positive allergic reaction to one or more allergens) included three female and seven male patients, age range from 18 to 37 years (average age 27.5 years). The third group (GP 3) (allergic patients treated with mometasone furoate) consisted of five female and five male patients, age from 18 to 46 years (average age 31.9 years). Signed informed consent was obtained from all patients before the procedure. This study has been approved by the Ethics Committee of the School of Medicine, University of Rijeka.

Immunohistochemistry and vascular density analysis

A small piece of the nasal mucous membrane, size 5x5 mm, was excised from the lower nasal shell and used for the analy-

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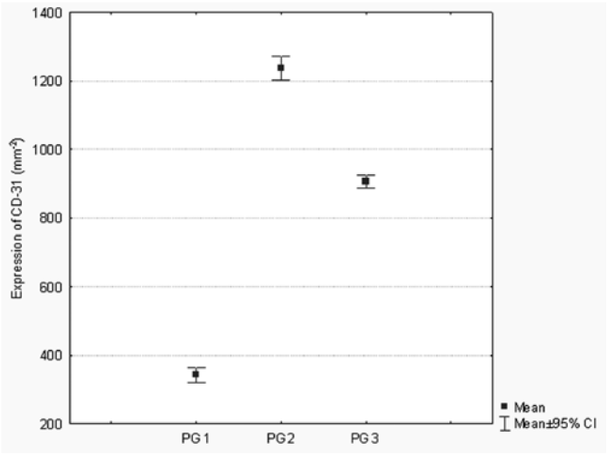

Figure 1. Expression of CD31 in GP 1 (patients without allergies), GP 2 (patients with allergies), and GP 3 (patients with allergies treated with mometasone furoate), ($p<0.001$*).
* Significant difference between all three groups

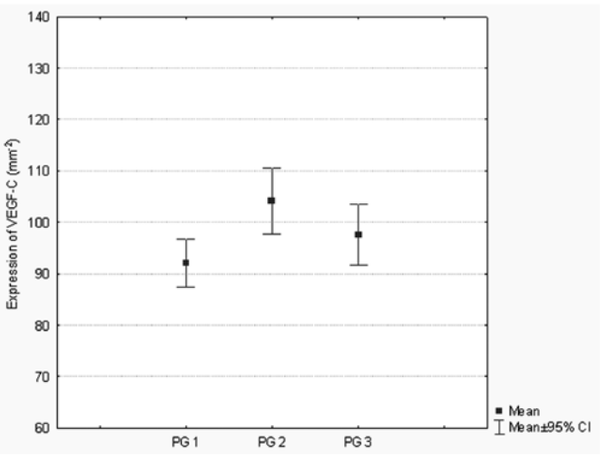

Figure 3. Expression of VEGF-C in GP 1 (patients without allergies), GP 2 (patients with allergies), and GP 3 (patients with allergies treated with mometasone furoate); ($p=0.010$*).
* Significant difference GP 1 vs. GP 2

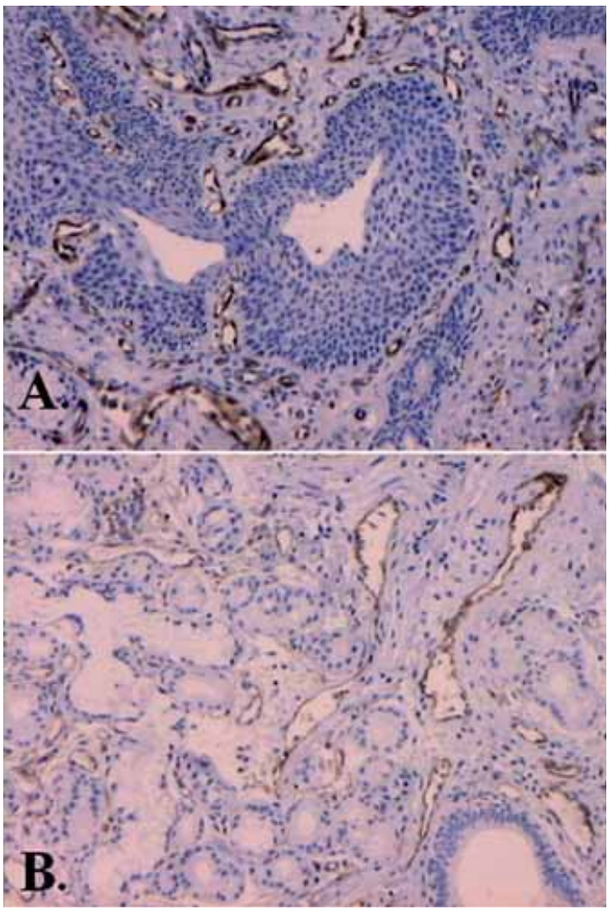

Figure 2. Immunohistochemical staining for CD31. A) In patients with allergy there was a mucous oedema and high number of CD31 positive vascular spaces. B) In patients treated with mometasone furoate there was a significant reduction of epithelial oedema and CD31 positivity. Magnification x200.

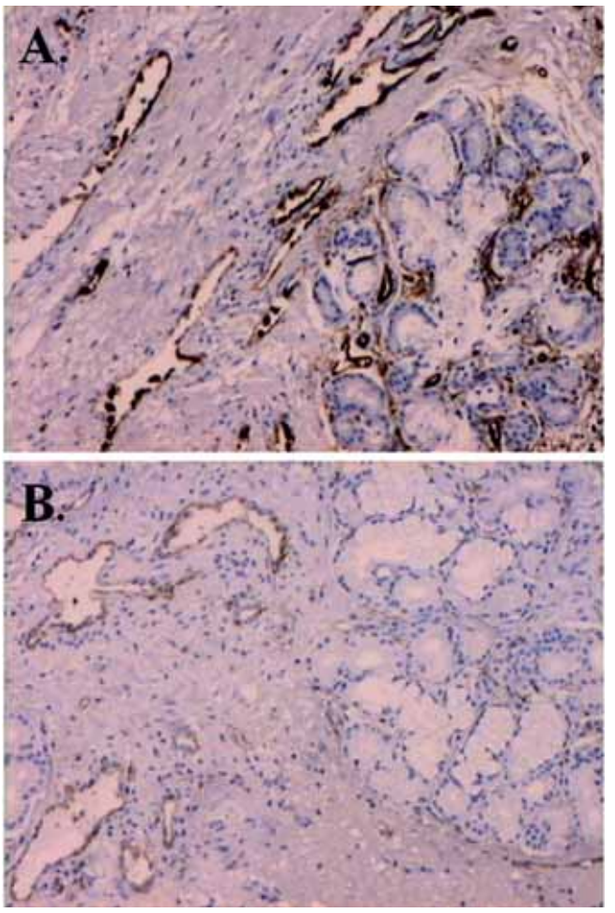

Figure 4. Immunohistochemical staining for VEGF-C. A) In patients with allergy there was a mucous oedema and high number of VEGF-C positive lymph capillaries. B) In patients treated with mometasone furoate there was a significant reduction of epithelial oedema and VEGF-C positivity. Magnification x200.

sis. After excision the mucous membrane was formalin fixed, paraffin embedded and 4 μm thick sections were cut. All sections were examined immunohistochemically for the expression of CD31 (1:50, clone JC 70A, DAKO A/S, Glostrup, DK) and VEGF-C (1:100, clone F 8/86, DAKO) [2-5].

The vascular phase of the nasal mucous membrane was determined by means of the stereological method using the length density variable. Length density is a relative stereological variable that gives us the length of a certain curve in a unit of volume. The property of length density has an exponent of -2 ($cm^1/cm^3 = cm^{-2}$ - our research made use of mm^{-2}). The trace of a one-dimensional curve in a plane is a dimensionless value called a transection point, marked Q. Its density in the test plane is referred to as density transection (Q_A). The formula employed to calculate the length density was $L_{vf} = 2 \times Q_f / A_t$); where Q_f in the formula stands for the number of transections, and At for the test plane surface, which in our case equals 0.013 mm^2. During the study approximately 100 measurements per specimen were taken and mean values were calculated for each sample.

Statistical analysis

Results are presented as the mean ± standard deviation. We used one-way ANOVA and Tukey HSD as a post-hoc test to compare differences in CD31 and VEGF-C expression between all three groups. All statistical values were considered significant at the p level of 0.05. Statistical analyses of data were performed by Statistica for Windows, release 6.1 (Stasoft, INC., Tulsa, OK, USA).

RESULTS

Values of CD31 expression in all GPs are shown in Figure 1. Histologically there was a significant decrease of microvasculature (blood capillaries) in allergic patients following mometasone therapy (Figure 2A and B). Statistical analysis of data showed that differences in CD31 values were significant at the level $p < 0.001$ in all comparisons. We detected a significant increase in CD31 expression in the GP 2 group (allergic non-treated patients) when compared to the other two GP's. Also, there was a significantly higher CD31 expression in GP 3 (treated allergic patients) compared to the non-allergic patients (GP 1).

Values of VEGF-C expression in all GPs are shown in Figure 3. Histologically there was a significant decrease in the number of lymph capillaries in allergic patients following mometasone therapy (Figure 4A and B). There was a significant increase in VEGF-C expression between GP 1 and GP 2 ($p = 0.010$). VEGF-C expression was increased in GP 3 compared to GP 1 although the difference did not reach statistical significance ($p = 0.292$).

DISCUSSION

Treatment of the group of allergic patients with mometasone furoate [6], which has a corticosteroid effect, 15 days before the surgical procedure, confirmed our assumption that the drug, besides its major effect in the late phase of inflammation (inhibition of interleukin release and other cytokines together with inhibition of the synthesis of leukotriens, which reduce vascular permeability) also reduces the number of blood vessels in the nasal mucous membrane. Mometasone furoate is powerful synthetic corticosteroid with a wide range of use as an anti-inflammatory agent for reducing seasonal and permanent allergic rhinitis [7-9]. Its glucocorticoid anti-inflammatory effect has minimal system activity and *in vitro* experiments have shown inhibition of pro-inflammatory Th2 cytokines [6]. It is known that mometasone furoate has an effect on nasal glucocorticoide receptors and inhibits leukotrien synthesis. Inhibition of leukotriens synthesis causes reduced permeability of the blood vessels [10, 11]. The intensity of the allergic reaction is, therefore, proportional to the permeability of the blood vessels. Thus, it is logical to assume that along with the permeability of the blood vessels for the intensity of the allergic reaction, the density of the blood vessels is of equal importance. Obtained results indicate that after a 15-day treatment with mometasone furoate the nasal mucous membrane of allergic patients showed significant reduction of the density of vascular elements. These effects are considered to be additional effects of the drug in the treatment of allergic diseases involving the upper respiratory system mucous membrane.

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