INTRODUCTION

Synthetic glucocorticosteroids belong to a group of compounds which are derivatives of adrenal cortex hormones (cortisone, cortisol, corticosterone). Due to their suggested and repeatedly proven strong anti-inflammatory, anti-allergic and immunosuppressive properties (also when compared to natural compounds) (1, 2), these compounds are currently used in nearly all medical fields and specialties. They form the base of therapy for inflammatory diseases, including, among others, the numerous diseases of the respiratory system, like the allergic, seasonal or perennial rhinitis and nonallergic rhinitis (3-6). In this case, the glucocorticosteroids, such as beclometasone, budesonide, fluticasone propionate or mometasone furoate are administered locally in the form of nasal aerosols, inhalation powders and water solutions (1, 7). Synthesizing glucocorticosteroids for localized treatment ensured strong, selective and simultaneously safe action, enabling high concentrations of the drug with a minimalized risk of side effects (with the appropriate application technique and proper dosage) even with long-term administration (7-9).

Modern treatment of rhinitis mainly concentrates on actions aiming to reduce the inflammation of nasal mucus. Numerous reports show that the research on topical steroid drugs mainly concentrate on the clinical symptoms related to the nose, such as itching, rhinorrhea, obstruction, burning sensations, etc. (7) and on the reactions of the immunological system (10-12). Cytological assessment is concentrated on exogenous cells which determine the type of the inflammation, such as eosinophils or neutrophils (13-15). Far less attention, however, is...
being paid to epithelial cells, which can determine the morphological effects of the ongoing inflammation process (16) or the administered treatment (17). It should also be stressed that, depending on their type, glucocorticosteroids can have a diversified influence on the cells of nasal mucous membrane (18). The available literature provides numerous conflicting information on this issue, which causes the mechanism of the anti-inflammatory action of glucocorticosteroids to remain partially unknown. Special attention should be also given to the fact that, despite proper administration, applying such drugs in a localized manner, nasally, together with orally administered drugs, might change the cytological image of the nasal mucous membrane (19).

The goal of the study was to cytologically assess the influence of fluticasone on the cells of nasal mucous membrane, with a special attention to the changes in the morphology of pseudostratified epithelium cells, performed in vivo in standard treatment conditions.

**MATERIALS AND METHODS**

**Patients**

The research was performed in cooperation with an allergist from the Allergy Clinic, Military Specialist Medical Clinic SP ZOZ in Kielce. The study was accepted by the Jan Kochanowski University in Kielce Bio-ethical Committee (No. 45/2011).

The study group was 40 patients (17 women and 23 men), aged 5 – 30 (median 12.4) with chronic rhinitis with suspected of allergy. The patients were qualified to participate in the study by a doctor based on long-term, clinically homogenous symptoms, such as runny or stuffy nose, nasal mucosa swelling, itching of nose. The control group were 10 healthy people (5 women and 5 men) aged 5 – 22 (median 14.20) who were showing no symptoms of rhinitis.

**Methods of smear preparation and assessment**

Two smears were taken from each patient with the use of a sterile cytology brush, from the surface of the nasal mucous membrane (exfoliative cytology), specifically from the inferior nasal concha (1 cm from its front edge). The cells on the brush were spread by hand on the slide with a single movement parallel to the slide edges. The obtained smears was immediately fixed in 96% ethanol and stained using the methods of Papanicolaou and Pappenheim in order to differentiate morphological changes (20, 21). Afterward the samples were analyzed in a blind test using the Nikon ECLIPSE 80i light microscope with a digital image analysis system Nikon Nis Elements in the magnification of ×400. Changes in the nasal mucous membrane of patients with rhinitis were analyzed based on a control image obtained in smears taken from healthy people. The results of the control group corresponded to the description of healthy nasal mucosa published by Tarchalska-Krynska (20). During the sample assessment, special attention was given to the morphological changes of epithelial cells. These concerned the morphological profile of the nucleus (change of size, karyorrhexis, non-nucleated form), cytoplasm (structure, changes of staining, vacuolation) and cell contour. In case of diagnostic doubt, cells were examined under 1000× magnification. In order to obtain the full picture, an assessment of the percentage of various types of cells in the smears was performed, according to the method of Tarchalska-Krynska (20). The inflammatory cells counted were neutrophils and eosinophils, and the epithelial cells counted were columnar cells, goblet cells and squamous cells. In the microscope field of view, all cell types (epithelial and inflammatory cells), as well as the cells with morphological changes, were counted together, to a total of 500 cells in the preparation. In order to obtain the most reliable results, the cells were counted in three repetitions (total counted: 1500 cells/preparation), and the final result was averaged. Determining cytograms for all patients enabled the emergence of 10 ill people (4 women and 6 men) aged 5 – 20 (median 11.7) with a similar cytological image, which became the basis for their final qualification to the fluticasone treatment group. In this group, there were 7 people with symptoms of perennial rhinitis, accompanied by typical nasal symptoms. Among the persons in 5 cases also demonstrated the profile of atopic diseases such as atopic dermatitis and asthma with symptoms from the conjunctiva (tearing, itching, burning) and the presence of allergen-specific immunoglobulin E for House Dust Mites D.P. (Dermatophagoides pteronyssinus) and D.F. (Dermatophagoides farinae). The remaining 3 people demonstrated characteristics of seasonal allergic rhinitis with typical nasal symptoms as well as the lack of extra-nasal symptoms and diseases associated and the presence of specific IgE to grass, birch, alder and hazel allergens.

The assessment of cytological image after fluticasone therapy was performed after 4 weeks of its use and compared to cytology taken from the same patients before treatment.

**Applied treatment**

Fluticasone propionate in the form of a nasal aerosol was locally applied nasally as two doses of 50 µg to each nostril once per day, in the total daily dose of 200 µg (for adults and children aged 12 or more), while children aged between 4 and 12 were given a single dose of 50 µg to each nostril once per day, in a total daily dose of 100 µg.

**Statistical analysis**

The statistical analysis of the results was performed using the nonparametric chi² test (STATISTICA 10.0, StatSoft Poland). Differences were considered statistically significant at P <0.05.

**RESULTS**

The assessment of the morphology of the nasal mucous membrane of patients with rhinitis symptoms before treatment was performed in reference to the smear from the mucous membrane of patients from the control group, which was picked as correct (Fig. 1a and 1b).

The analysis of smears from the nasal mucous membrane showed significant differences in the cytological image of patients with rhinitis before fluticasone treatment in comparison to the control group results. The statistically significant change was the presence of numerous cells of the stratified squamous epithelium. 37.24 acidophilic cells were present (χ²=35.45; P=0.0000) (Fig. 4). These were large polygonal cells, with translucent cytoplasm and a small pyknotic nucleus without a visible chromatin structure or with nuclear shadows visible in the center (Fig. 2d). Basophilic cells were present in a similar number: 30.80 (χ²=9.94; P=0.0016) (Fig. 4) and were characterized by a large round nucleus with a slightly granular structure (Figs. 2a, 2c and 2d). Some acidophilic cells were characterized by the presence of nuclei with signs of breakdown (Fig. 2d).

Another significant change was the disturbed ratio of columnar to goblet cells, in favor of the goblet cells, caused by a statistically significant decrease in the number of columnar
cells to 18.54 ($\chi^2=476.25; \ P=0.0000$) (Figs. 2a, 4 and 5). Furthermore, fairly frequently occurring free nuclei of these cells with very clear nucleoli could be observed (Fig. 2d).

The preparations also contained numerous bacteria, which were loosely distributed or adhered to the surface of squamous epithelial cells (Fig. 2c).
The dominant change was the statistically significant increase in the number of neutrophils to 333.34 ($\chi^2=289.92; P=0.0000$) (Figs. 2b and 4). These granulocytes showed the characteristics of poorly differentiated cytoplasm and of cell nuclei contours. A statistically significant number (10.84) among the observed inflammatory cells were also eosinophils ($\chi^2=11.12; P=0.0009$) (Figs. 2b and 4), while there were few basophils, lymphocytes and monocytes (Fig. 4).

No significant changes in the morphology of epithelial cells were shown, only the presence of 4.7 individual cells with cytoplasmic vacuolation (Figs. 2c, 2d and 8).

Whereas the analysis of smears taken from the same patients after fluticasone therapy showed the presence of few bacteria, as well as a statistically significant reduction of the number of inflammatory cells such as neutrophils, to 167.17 ($\chi^2=110.22; P=0.0000$) and eosinophils to 1.10 ($\chi^2=8.43; P=0.0037$) (Fig. 6).

**Fig. 3.** The cytology of the nasal epithelium of patients with rhinitis after fluticasone treatment under a light microscope (a) Stained with the Papanicolaou method: 1-columnar cells of the pseudostratified epithelium, 2-columnar cells with cytoplasm vacuolation, 3-goblet cells, 4-acidophilic cells of the stratified squamous epithelium. Magnification ×400. (b) Stained with the Papanicolaou method: 1- columnar cells with a perinuclear halo, 2-acidophilic cells of the stratified squamous epithelium. 3- neutrophil. Magnification ×400. (c) Stained with the Papanicolaou method: 1-columnar cells of the pseudostratified epithelium, 2-goblet cells. 3-cells of the stratified squamous epithelium. Magnification ×400. (d) Stained with the Papanicolaou method: 1-cell of the stratified squamous epithelium with numerous vacuoles in the cytoplasm, 2-cell of the squamous epithelium without changes, 3-neutrophils. Magnification ×400. (e) Stained with the Pappenheim method: 1-cell of the squamous epithelium with a large vacuole with content, 2-neutrophil with signs of breakdown. Magnification ×400.
The studied granulocytes were showing characteristics of serious breakdown (Figs. 3d, 3e).

At the same time, changes in the ratio of cells of pseudostratified epithelial cells were observed, in the form of a statistically significant increase in the number of columnar cells to 214.70 ($\chi^2=217.16; P=0.0000$) (Figs. 3c, 6 and 7) and the equally statistically significant decrease in the number of goblet cells to 27.96 ($\chi^2=18.44; P=0.0000$) (Figs. 3c, 6 and 7).

A statistically significant increase in the number of basophilic cells of the stratified squamous epithelium was also shown, up to 60.60 ($\chi^2=10.77; P=0.0010$) (Fig. 6), with a statistically insignificant decrease of the number of acidophilic cells to 28.17 ($\chi^2=1.33; P=0.2483$) (Fig. 6). No significant changes were noted in relation to the number of basophils, lymphocytes and monocytes (Fig. 6).

Special attention was however drawn by the vacuolation changes in the form of one large vacuole or numerous small vacuoles in the cytoplasm of both columnar cells (Fig. 3a), and squamous epithelial cells (Fig. 3d and 3e). A significant increase in the number of vacuolated cells can be seen, up to 40.03, while the smears of patients before treatment only 4.70 were shown ($\chi^2=28.50; P=0.0000$) (Fig. 8). Among the cells with vacuolation changes, revealed the presence of forms with a perinuclear halo (Fig. 3b).

**DISCUSSION**

Progress in diagnosing and treating allergic diseases is based on the improved understanding of the mechanisms of these diseases, the development of new drugs, as well as on the improvement and broadening of indications regarding drugs which are already in use (22). The pathomechanism of inflammatory rhinitis is related to the inflow and activation of cells - mastocytes, basophils, eosinophils, T lymphocytes and neutrophils in the area of the inflammatory process (23, 24), and

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**Fig. 4.** Distribution of epithelial and inflammatory cells in smears of the nasal mucous membrane of patients before fluticasone treatment; *** P<0.001; ** P<0.01 compared to control (chi² test). Abbreviations: cc, columnar cells of pseudostratified epithelium; gc, goblet cells; ae, acidophilic cells of the squamous stratified epithelium; be, basophilic cells of the squamous stratified epithelium; N, neutrophils; E, eosinophils; B, basophils; L, lymphocytes; M, monocytes.

**Fig. 5.** Distribution of columnar cells (cc) and goblet cells (gc) in smears of the nasal mucous membrane of patients before fluticasone treatment; *** P<0.001 compared to control (chi² test).
its understanding allows us to correctly and justifiably use specific drugs and therapeutic methods (25).

The cytological assessment of the smear from the surface of the mucous membrane is, unlike the biopsy, a completely noninvasive method, which can often be used in routine ambulatory diagnostic. It can be performed in every situation, in every patient of every age, and can be repeated numerous times in the same person (26). The safety and lack of age limits are confirmed by cytological studies in infants (27, 28). Since the picture obtained in cytological studies is a reflection of the processes taking place in the nasal mucous membrane, studies of this type provide many new and significant information about the type of the inflammatory reaction, as well allowing the monitoring of the effectiveness of the implemented treatment (24, 29 – 31). It should also be noted that the smear technique obtains the highest total number of inflammatory cells (approximately 3 – 4 times higher than in lavages and scrapings) and epithelial cells (in this case almost 70%, while in lavages 42%), which was proven in studies comparing all three methods of obtaining material for cytology (31, 32).

Without glucocorticosteroid drugs it would certainly be difficult to imagine modern therapy of these diseases, the pathogenesis of which is associated with the activation of various inflammatory cells (33). Besides nasal mucosa diseases

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**Fig. 6.** Distribution of epithelial and inflammatory cells in smears of the nasal mucous membrane of patients after fluticasone treatment; *** P<0.001, ** P<0.01 compared to before treatment (chi² test).

**Fig. 7.** Distribution of columnar cells (cc) and goblet cells (gc) in smears of the nasal mucous membrane of patients before and after fluticasone treatment; *** P<0.001, compared to before treatment (chi² test).
they are commonly used in the treatment of asthma, Crohn's Disease or infant meconium aspiration syndrome (MAS), where they, among other effects, significantly limit the influx of inflammatory cells (34-36).

It should be remembered that these drugs control numerous physiological processes in various organs and tissues and on many levels, from the proliferation and differentiation of cells, to the regulation of their metabolic activity and programmed death (37, 38). The cellular reaction to glucocorticosteroids is very diverse and shows great variability in its specificity and sensitivity (39).

Glucocorticosteroids, independent on the form of administration, can change the cytological image of the mucous membrane; it should however be noted that these changes can be beneficial or undesirable (19).

The cytological studies performed on a group of patients with rhinitis symptoms before their treatment, in comparison to the control smears, clearly show a disturbance in the biocenosis of the nasal mucous membrane. The presence of numerous bacteria has been shown (Fig. 2c), as well as of granulocytes with the predominance of neutrophils (Figs. 2b and 4). With the assumption that a cytogram described as correct should contain single neutrophils (13, 20, 25), the presented change suggests a pathological state. Furthermore, the observed granulocytes display the characteristics of poorly differentiated cytoplasm, as well as contours of nuclei, which suggests a chronic state. In acute inflammations granulocytes, existing as young forms, have clear and intensively staining nuclei and well differentiated cytoplasm (40).

Changes in the content of the cellular population of the nasal mucous membrane are also a confirmation of the pathological state. A disturbance in the ratio of columnar cells to goblet cells has been noted, to the benefit of the goblet cells (Figs. 2a, 4, and 5), while in proper cytograms the columnar cells should outnumber goblet cells by a ratio of 5 (25, 41, 42). The mechanism contributing to the increase of goblet cells is their over-proliferation, known as hyperplasia, which often accompanies inflammation in the respiratory epithelium (43, 44). According to some authors, the hyperplasia of goblet cells can accompany inflammation in which the dominant cell is the neutrophil, like in the chronic inflammation of nasal mucous membrane and rhinosinusitis with the exclusion of nasal polyps (45). It should also be stressed that the studied material showed the presence of free nuclei of columnar cells (Fig. 2d), which was a result of intensive cytolytic processes, and is also an example of changes commonly associated with a disturbed biocenosis (40, 46).

The presence of very numerous cells of the squamous stratified epithelium has also been confirmed (Figs. 2a, 2c, 2d and 4). The prevalence of these cells, in different stages of development, with the assumption that the material was properly obtained, suggests a pathological state (41). Since the analyzed smears showed a prevalence of cells originating from the deeper strata of the epithelium (basophilic with a large round nucleus with a mildly granular structure), it suggests a damage to the upper layers of the epithelium (47). The breakdown of the nucleus (karyorrhexis) observed in the basophilic cells is also, together with an enlargement and a dissolution (karyolysis) of this structure, a common occurrence in inflammation (40).

The obtained changes, especially the neutrophilic reaction, indicate a chronic infectious rhinitis, in which neutrophils can often be the only cells in the smear (48).

A comparison of the cytological image of patients with rhinitis before treatment and the same patients after treatment with fluticasone enabled the demonstration of significant differences in epithelial reaction and number of inflammatory cells.

The consequence of the drug activity was the decrease of inflammation of nasal mucosa, confirmed mainly through the reduction of the number of neutrophils (Fig. 6), which can be related to results of similar cytological studies with the use of another glucocorticoid-mometasone in allergic rhinitis (19).

The observed decrease of goblet cells, with a simultaneous, also statistically significant increase of the number of columnar cells (the proper ratio of columnar to goblet cells) shows a gradual regeneration of the nasal mucous membrane (Figs. 3c, 6 and 7). The confirmation of the results obtained in relation to goblet cells were the cytological studies of Lin et al. (18) on glucocorticosteroids used in people with rhinitis and the long-standing research of Mygind (49) and Sorensen (50) on the activity of budesonide based on the assessment of nasal mucous membrane segments under an electron microscope. Whereas the stimulating effect of glucocorticosteroids on columnar cells was shown in the research on mometasone performed by Tarchalska-Krynska (19) and Meltzer (51). Another confirmation of the beneficial effect of topical glucocorticoid therapy on the proper proportion of columnar to goblet cells comes from the results of our own cytological studies on the use of budesonide (52, 53).

In comparison to the state before treatment, fluticasone therapy did not influence the general number of squamous stratified epithelial cells (Fig. 6), because an increase in basophilic cells was obtained, together with a simultaneous decrease in the number of acidophilic cells, which suggests a low effectiveness of the drug in relation to cells of this type, which had also been confirmed in other studies (20).

However, special attention has been drawn by the increase of vacuolated epithelial cells (Fig. 8), mainly columnar. Some cells were characterized by the presence of a single vacuole (Fig. 3e) or numerous small vacuoles in the cytoplasm (Figs. 3a and 3d),
others were characterized by perinuclear vacuolation, a clear nuclear halo (Fig. 3b). The intensified vacuolation of the cell cytoplasm might suggest an induction of autophagic processes. According to numerous reports, autophagy plays a significant role in numerous aspects of inborn and adaptive immunity, including, among others, the activation of the immunological system, survival of infected cells (54) or the elimination of intracellular pathogens, including bacteria and viruses (55, 56). It should be stressed that according to numerous literature data, autophagy can be associated not only with macrophages, but also with cells not belonging to the immunological system (57). Whereas the so-called perinuclear halo effect, according to the available literature, appears because of karyoplasm shrinkage and can be the result of reversible cytoskeleton damage (58). In our previous research we had confirmed the effectiveness of topical glucocorticosteroid therapy in the treatment of nasal mucosa diseases, however we have also revealed changes which require further study. As described in professional literature (59), the noninvasive study of nasal nitric oxide (NO) levels can be an excellent supplementation of the cytological studies both in the assessment of the inflammatory reaction of the nasal mucous membrane and the reaction to anti-inflammatory treatment. As shown in professional literature (60), the study design should also include the fact that the very use of hypertonic saline solution in nasal aerosol form can have a significant effect on the nasal NO levels.

In summary, the obtained results of the cytological study of nasal mucosa shows that therapy with fluticasone propionate used in the dose of 100 µg to 200 µg in patients with diagnosed chronic infectious reaction of nasal mucosa caused a significant decrease of inflammation visible in the cytological image in the form of reduction of bacteria and the accompanying nasal neutrophilia. Simultaneously, no harmful action of the applied glucocorticosteroid was observed, which was confirmed by the lack of significant changes in the number of squamous epithelial cells with a clearly increased number of columnar cells. Whereas the observed escalation in vacuolation of these cells, with a simultaneous lack of apoptosis characteristics, doesn't show the characteristics of degenerative changes, but might suggest an additional mechanism of glucocorticosteroids in nasal mucosa inflammation.

Conflict of interests: None declared.

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