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Steroid-sensitive indices of airway inflammation in children with seasonal allergic rhinitis

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Previous studies involving adults have demonstrated that airway glucocorticosteroids inhibit plasma exudation and eosinophil activity in allergic rhinitis. This study explores the possibility that plasma exudation, exudative responsiveness, and the occurrence of eosinophil activity-related proteins are glucocorticosteroid-sensitive nasal mucosal indices in allergic children. Using a placebo-controlled, parallel-group design effects of nasal budesonide (64 μg per nasal cavity b.i.d) were determined in children with seasonal allergic rhinitis. Nasal lavage fluid levels of eotaxin, eosinophil cationic protein (ECP), and α_2-macroglobulin, indicating plasma exudation, were determined, the latter with and without challenge with topical histamine. Nasal lavage fluid levels of α_2-macroglobulin and ECP increased significantly during the pollen season, and the acute plasma exudation response to histamine was significantly greater during than outside the season. There was a trend towards a seasonal increase in nasal lavage fluid levels of eotaxin. Budesonide significantly inhibited the seasonal increase in α_2-macroglobulin as well as the exudative hyperresponsiveness to histamine. Any tendency of increases in mucosal output of eotaxin and ECP was abolished by the glucocorticosteroid treatment. We conclude that mucosal exudation of plasma, as a global sign of active inflammatory processes, is a glucocorticosteroid-sensitive facet of allergic rhinitis in children. Exudative hyperresponsiveness, potentially caused by several weeks of mucosal inflammation, emerges as a significant feature of allergic rhinitis in children, and its development is prevented by local treatment with a glucocorticosteroid drug. The seasonal increase in ECP and the trend for an increase in eotaxin were absent in the glucocorticosteroid-treated subjects.

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As an inflammatory disease of the nasal mucosa, allergic rhinitis is characterized by plasma exudation (1–3). Because this mechanism involves tissue distribution and airway luminal entry of adhesive, leukocyte-activating, growth- factor active, and other biologically active proteins, pharmacological inhibition of plasma exudation is a potentially important action (4). However, it is only in adults that drug (glucocorticosteroid) treatment has been demonstrated to inhibit plasma exudation (5, 6). In adult seasonal allergic rhinitis, it has further been demonstrated that the nasal mucosa exhibits abnormally great exudative responses to histamine challenges (7, 8). Whether the exudative hyperresponsiveness is also expressed in the nasal mucosa of allergic children and whether it is at all affected by glucocorticosteroid treatment are not known.

The eosinophil granulocyte, a potential effector cell in allergic airway diseases (9), may be of special importance in allergic rhinitis as in this disease the nasal mucosa is particularly rich in highly degranulated eosinophils (10). Glucocorticosteroid treatment inhibits the eosinophilia of allergic rhinitis in adults (11). This action may largely reflect reduced recruitment of eosinophils

to the nasal mucosa as elimination of the nasal tissue eosinophils, by either apoptosis or luminal entry, may not be affected by glucocorticosteroids *in vivo* (12–14). A few previous reports have suggested that glucocorticosteroids are anti-eosinophilic agents in children suffering from allergic rhinitis (15), but this possibility has not been extensively studied.

This study examines children with seasonal allergic rhinitis during as well as outside their active disease period. Inflammatory indices appearing on the nasal mucosal surface are examined by use of an efficient sampling technique that can be handled by the children themselves (16). Specifically, the present study examines the possibility that nasal mucosal outputs of plasma (α_2-macroglobulin), an eosinophil chemoattractant (eotaxin), and an eosinophil granule protein (eosinophil cationic protein; ECP) are glucocorticosteroid-sensitive indices in the allergic child. In addition, the possibility that seasonal hyperresponsiveness develops and that it is a glucocorticosteroid-sensitive disease variable is also explored in these patients.

Methods

Study design

Children with allergic rhinitis, receiving topical glucocorticosteroid treatment or placebo, were examined during a birch pollen season. Nasal lavages with saline were carried out once before and at two occasions during the season. In addition, nasal challenges and lavages with histamine were carried out once during and once after the pollen season. This particular design was chosen to avoid any effects of a histamine challenge carried out close to the pollen season on allergen-induced nasal mucosal outputs of eotaxin, ECP, and α_2-macroglobulin. The levels of eotaxin and ECP were determined in the saline lavages. Furthermore, the levels of α_2-macroglobulin were determined in nasal lavage fluids obtained after saline as well as the histamine exposure.

Treatment

Topical glucocorticosteroid treatment (budesonide aqueous nasal spray, 64 μg per nasal cavity b.i.d) was given in a double-blind, placebo-controlled, randomized, and parallel group design. Nine patients received budesonide and nine placebo. The treatment started before the expected start of the pollen season and continued throughout the part of the study that was carried out during the pollen season. No other drugs were allowed except rescue medication: Loratadine tablets (Clarityn®, Schering-Plough, Brussels, Belgium) and cromoglycate eyedrops (Lomudal®, Aventis Pharma, Cheshire, England) in clinical doses. The patients were instructed to use rescue medication if more than moderate symptoms occurred.

Patients

Eighteen children (10 boys and 8 girls, 7–13 years old, mean age 10.6 years) participated in the study. The children had a history of birch pollen allergic rhinitis, which was verified by a positive skin-prick test. The children had no history of perennial nasal or bronchial disease, no history of recent respiratory tract infection, and no history of recent drug treatment. The study was approved by the local ethics committee and informed consent was obtained from the patients and their parents.

Symptom scores

The children scored nasal symptoms, i.e. rhinorrhea, blockage, and sneezes, as well as eye symptoms in a diary once daily during the pollen season. Score 0: no, 1: mild, 2: moderate, and 3: severe symptoms.

Nasal pool challenge and lavage technique

The nasal pool-device was used for saline lavages and for concomitant histamine challenge and lavage of the nasal mucosa (16). The nasal pool device is a compressible plastic container equipped with a nasal adapter. The adapter is inserted into a nostril and the sitting patient, leaning forward in a 60° flexed neck position, compresses the container. The nasal pool-fluid is then instilled in one of the nasal cavities and maintained in contact with a large and reproducible area of the mucosal surface for a determined period of time. When the pressure on the device is released the fluid returns into the container. In the present study, the volume of the nasal pool-fluid was 12 ml. The right nasal cavity was used for all lavages.

Isotonic saline and histamine lavages of the nasal mucosa

Nasal lavages with isotonic saline, each with a duration of 2 min, were carried out once before and at two occasions during the pollen season. Immediately after the second seasonal saline lavage, an additional 5-min saline lavage followed by a 5-min histamine (100 μg/ml) combined challenge and lavage was carried out. These three lavages were carried out with 5-min

intervals. A 2-min saline lavage followed by a 5-min saline lavage and a 5-min combined histamine (100 μg/ml) challenge and lavage was also carried several months after the pollen season had ended (December). These three lavages were carried out at 5-min intervals.

The lavages were carried out on the same days for all patients and at about the same time point of the day. The recovered lavage fluid was centrifuged (G = 105 ***g***, 10 min, 4°C) and aliquots were prepared from the supernatants and frozen (−20°C) for later analysis of eotaxin, ECP, and α_2-macroglobulin.

Analysis of eotaxin, ECP, and α_2-macroglobulin

The nasal lavage fluid levels of eotaxin were measured using a commercially available radioimmunoassay (Pharmacia-Diagnostics, Uppsala, Sweden). The detection level of the eotaxin assay was <5 pg/ml. ECP were measured by fluoroimmunoassay (Pharmacia-Diagnostics). The detection level of the ECP assay was <2 ng/ml. α_2-macroglobulin were determined using a radioimmunoassay sensitive to 7.8 ng/ml. Rabbit anti-human α_2-macroglobulin (Dakopatts, Copenhagen, Denmark) was used as antiserum and human serum (Behringwerke Diagnostica, Marburg, Germany) as standard. Human α_2-macroglobulin (Cappel-Organon Teknika, Turnhout, Belgium) was iodinated using the lactoperoxidase method (17). Tracer and standard (or sample) was mixed with antiserum before adding goat anti-rabbit antiserum (AstraZeneca, Lund, Sweden). The bound fraction was measured using a gamma counter. The intra- and inter-assay coefficients of variation were between 3.8–6.0% and 3.1–7.2%, respectively.

Statistics

Differences in lavage fluid levels of eotaxin, ECP, and α_2-macroglobulin were examined using Friedman's test. If statistical significance's emerged, further analyses were performed using Wilcoxon's signed rank test. Differences in nasal symptoms within each treatment group were similarly examined. Differences between budesonide and placebo treatment were examined using the Mann–Whitney *U*-test. p-values of less than 0.05 were considered significant. Data are presented as mean ± SEM.

Results

The regional birch pollen counts demonstrated a mild pollen season. Accordingly, there were only small differences in symptom scores (Fig. 1): In

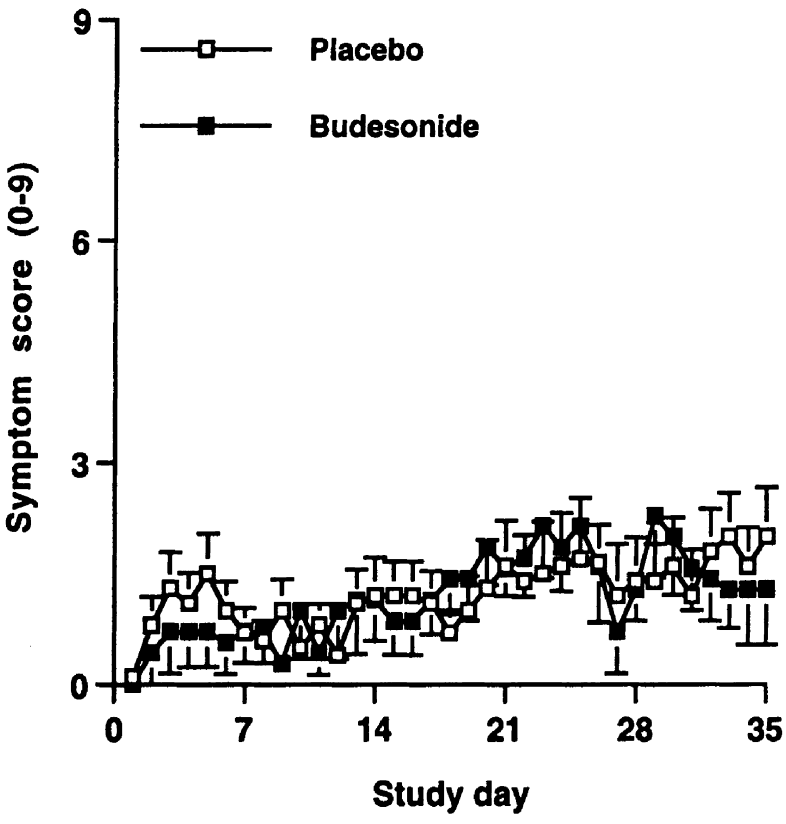

Fig. 1. Nasal symptom scores (mean ± SEM) during the study period. The scores demonstrated mild symptoms. Yet, in patients receiving placebo, the nasal symptom scores were significantly increased on study days 3–6, 9, 11, 15, 21, 29 and 32–35 (p-values < 0.05).

patients receiving placebo, nasal symptom scores increased very mildly but significantly on 13 of the study days (p-values < 0.05, cf. preseason levels). In patients receiving budesonide, nasal symptom scores increased significantly on 11 of the study days (p-values < 0.05, cf. preseason levels). Focusing on the cumulative seasonal symptoms, budesonide failed to reduce symptoms of allergic rhinitis. Loratadine tablets and cromoglycate eyedrops were used very infrequently during the study period.

In patients receiving placebo, nasal lavage fluid levels of eotaxin increased during the pollen season, but this increase failed to reach statistical significance (Fig. 2). At the observations during the pollen season, the levels of eotaxin were lower in patients receiving budesonide (cf. placebo), but this effect failed to reach statistical significance.

In patients receiving placebo, nasal lavage fluid levels of ECP increased during the pollen season, and this increase reached statistical significance at the first as well as the second seasonal observation ($p < 0.05$, cf. before the pollen season) (Fig. 3). This observation was not seen in patients receiving budesonide. At the observations during the pollen season, the levels of ECP were lower in patients receiving budesonide (cf. placebo), but this effect failed to reach statistical significance.

In patients receiving placebo, nasal lavage fluid levels of α_2-macroglobulin increased during the pollen season, and this increase reached statistical significance at the first ($p < 0.01$) as well as the second ($p < 0.05$) seasonal observation cf. before the pollen season) (Fig. 4). This effect was not seen in patients receiving budesonide. At both points of observation during the pollen season, the levels of α_2-macroglobulin were attenuated in patients receiving budesonide ($p < 0.05$, cf. placebo).

Histamine produced significant plasma exudation, i.e. increased nasal lavage fluid levels of α_2-macroglobulin ($p < 0.001$) (Fig. 5). In patients receiving placebo, the exudative responsiveness to histamine was significantly increased during the pollen season ($p < 0.05$, cf. histamine challenges

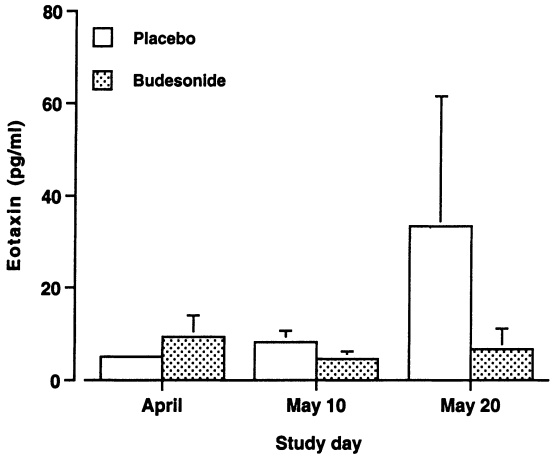

Fig. 2. Levels of eotaxin in nasal saline lavages obtained before (April) and at two occasions (May 10 and 20) during the birch pollen season. In patients receiving placebo, eotaxin levels tended to be increased by the seasonal allergen exposure, but this effect failed to reach statistical significance. Budesonide prevented the seasonal increase in eotaxin levels, but again this effect failed to reach statistical significance.

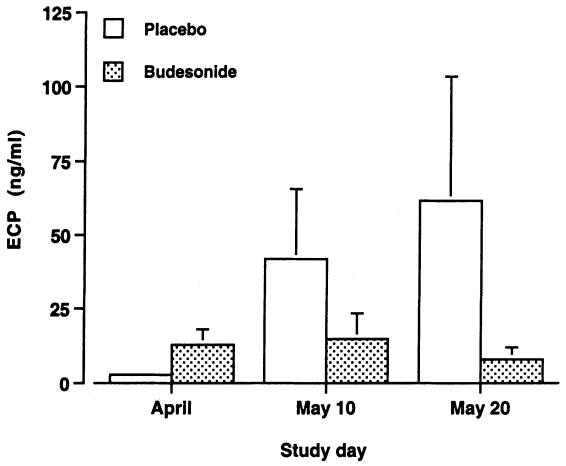

Fig. 3. Levels of ECP in nasal saline lavages obtained before (April) and at two occasions (May 10 and 20) during the birch pollen season. In patients receiving placebo, ECP levels were significantly increased at seasonal allergen exposure (significance levels are given elsewhere). Budesonide reduced the seasonally increased ECP levels, but this effect failed to reach statistical significance.

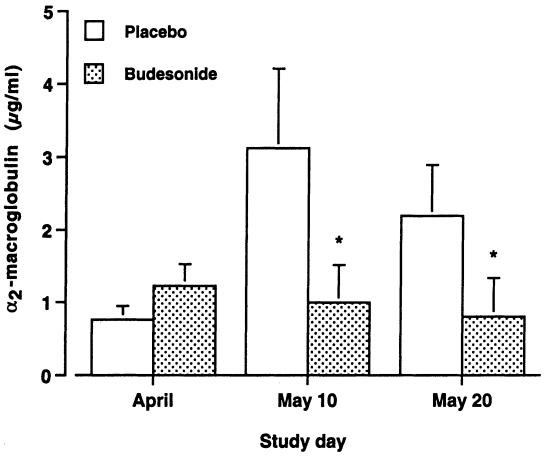

Fig. 4. Levels of α_2-macroglobulin in nasal saline lavages obtained before (April) and at two occasions (May 10 and 20) during the birch pollen season. In patients receiving placebo, α_2-macroglobulin levels were significantly increased by the seasonal allergen exposure (significance levels are given elsewhere). Budesonide attenuated the seasonal increase in α_2-macroglobulin levels (*$p < 0.05$).

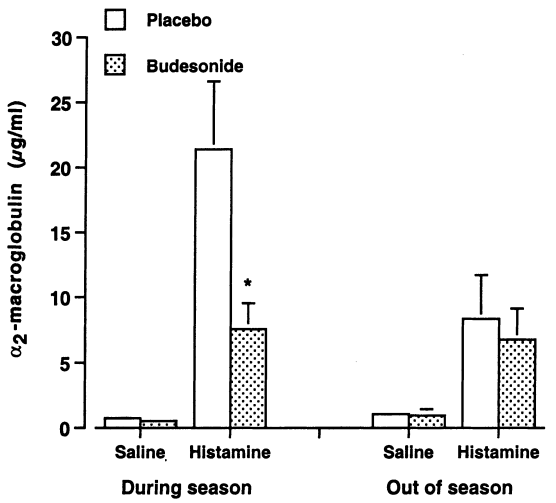

Fig. 5. Levels of α_2-macroglobulin in saline and histamine lavages obtained during (May 20) and well after (December) the birch pollen season. In patients receiving placebo, a significant exudative hyperresponsiveness developed during the pollen season (significance levels are given elsewhere). Budesonide attenuated the seasonal exudative hyperresponsiveness (*$p < 0.05$).

carried out well after the pollen season). Budesonide attenuated the seasonal exudative hyperresponsiveness ($p < 0.05$, cf. placebo).

Discussion

This study demonstrates that treatment with a nasal glucocorticosteroid inhibits plasma exudation and exudative hyperresponsiveness, and may reduce eosinophil activity, in children who suffer from seasonal allergic rhinitis. These data indicate that allergic rhinitis, irrespective of age, is characterized by glucocorticosteroid-sensitive inflammatory processes.

The nasal pool-device, as confirmed in this study, is readily handled by 7 year olds. We have previously demonstrated that children using this device regularly manage to recover over 85% of the lavage fluids (16), which is far better than obtained with other methods of nasal lavage employed in children (16). Because only 18 children were recruited and a parallel-group design was used the power of the present study was low. Yet, the present methodology may have contributed to data consistency and, hence, to the detection of differences between small groups of patients in this study. We could thus demonstrate statistically significant increases in nasal lavage fluid levels of ECP and α_2-macroglobulin as well as development of exudative hyperresponsiveness during the season. Furthermore, significant inhibition of the exudative indices was produced by glucocorticosteroid treatment in this study. It is also possible that this treatment, in agreement with previous observations (11, 18, 19), would have reduced nasal lavage fluid levels of ECP and eotaxin more clearly than in this study had a greater number of patients been included.

Given the low power and the mild seasonal allergen exposure, the exudative and anti-exudative effects that turned out significant in this study may be considered quite characteristic features of allergic rhinitis in children. In challenge experiments involving adults as well as children, we have previously demonstrated that luminal entry of plasma extends to threshold inflammatory stimulation, that it occurs in airways with a maintained epithelial integrity, that it is largely a non-sieved process and, hence, that lavage fluid levels of the large plasma protein α_2-macroglobulin is a useful index of this response (20). Plasma exudation may therefore be viewed as a global measure of the overall inflammatory process, especially reflecting the extent to which the airway mucosal tissue itself is affected by the inflammation (20). The present data indicate that the exudative response is well developed in children. This feature is important in creating a bioreactive proteinaceous milieu for *in vivo* inflammatory processes. For example, as a consequence of its non-sieved nature, the kinin and coagulation systems (20) as well as the complement proteins will also be exuded (21, 22).

As shown by the saline lavage levels in this study, and as corroborated by the present efficacy of glucocorticosteroids, the exudative response provides a means of monitoring disease intensity and treatment effects in the allergic child. Because luminal entry of plasma, in contrast to luminal entry of cells, directly reflects the magnitude of subepithelial events, plasma exudation indices may provide more useful quantitative data on disease activity than the study of inflammatory cells (20). Interestingly, Benson et al. (23), in a study involving 60 schoolchildren with allergic rhinitis, could demonstrate glucocorticosteroid-sensitive increases in both eosinophil numbers and in interleukin-5 levels during seasonal allergic rhinitis. While the eosinophil count seems well established as a gross index of allergic airway disease, the interleukin-5 levels may too frequently be below detection limit to be of regular use (23). Thus, if the present promising findings regarding consistency of plasma exudation are confirmed in larger groups of children with allergic rhinitis, α_2-macroglobulin may become of great importance as an index for monitoring of this disease. In addition, the present study demonstrated that the exudative response to histamine challenge was greater

during than outside the pollen season. Hence, during several weeks of allergic inflammation there is development of an exudative hyperresponsiveness in children similar to what has been observed in adults (7). Moreover, the present hyperresponsiveness was inhibited by glucocorticosteroid treatment. Further studies are warranted to examine whether this latter original observation in allergic children carries over to adult patients with seasonal allergic rhinitis.

In conclusion, the present data indicate that mucosal exudation of plasma and the exudative hyperresponsiveness are both characteristic features and glucocorticosteroid-sensitive indices of seasonal allergic rhinitis in children. Inhibition of plasma exudation and reducing hyperresponsiveness likely reflect clinically important anti-inflammatory efficacy of drug treatment. Hence, monitoring of exudative properties of the nasal mucosa is potentially of value for assessment of treatment effects in children suffering from allergic rhinitis.

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