Effect of Inhaled Fluticasone on Lung Inflammation Administered During and After Guinea Pig Sensitization

Mohammad Hossein Boskabady*, Mohammad Javad Eslamizade*, Abas Tabatabaei*, Habib Nemati* and Fariba Mansouri*

*Department of Physiology and Pharmacological Research Centre of Medical Plants, Mashhad, Iran
*Department of Pathology, Imam Khomeini Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
*Department of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

ABSTRACT

Objective: The effect of an inhaled corticosteroid, fluticasone propionate (FP) on lung inflammation of sensitized guinea pig was examined.

Material and methods: Four groups of guinea pigs (n = 8) were sensitized (S) with ovalbumin (OA). Control group was given similar solutions without OA. One S group was treated with inhaled 250 μg inhaled FP twice/day during, other group after sensitization for 18 days and two groups were treated with placebo, one during, and the other after sensitization. One day after the last treatment, tracheal responses of all animal groups to methacholine and OA were examined. Total and differential white blood cell (WBC) counts of lung lavage and lung pathology were also examined.

Results: Tracheal responsiveness to both methacholine and OA and WBC of both placebo groups were significantly higher than those of control group (P < 0.01 for all cases). The lungs of placebo groups showed variable pathological changes (non significant to P < 0.001) compared to control group. Tracheal responsiveness in two treated groups with FP to both methacholine and OA were significantly decreased compared to placebo groups (P < 0.01 to P < 0.001). Treatment with FP leads to improvement in total (P < 0.001) and differential WBC counts (non significant to P < 0.001) as well as mucosal detachment (P < 0.001), but not other pathological changes.

Conclusions: These results showed a protective effect of FP on tracheal responsiveness and lung inflammation. In addition, this study showed that treatment with inhaled fluticasone propionate, during sensitization (development of inflammation and pathological changes) was more effective than after sensitization (establishment of inflammation and pathological changes).

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Efecto de la fluticasona inhalada sobre la inflamación pulmonar administrada durante y después de la sensibilización de cobayas

RESUMEN

Objetivo: Se examinó el efecto de un corticosteroidé inhalado, el propionato de fluticasona (PF), sobre la inflamación pulmonar de cobayas sensibilizados.

Material y métodos: Se sensibilizó con ovalbúmina (OA) a 4 grupos de cobayas (n = 8). El grupo de control recibió soluciones similares sin OA. Durante un período de 18 días, un grupo sensibilizado fue tratado con 250 μg de PF inhalado 2 veces al día durante la sensibilización; el otro grupo después de ella y los otros 2 grupos fueron tratados con placebo, uno durante la sensibilización y otro después de ella. A las 24 h del último tratamiento, se examinaron las respuestas traqueales de todos los grupos de animales a metacolina...
Introduction

Asthma is an inflammatory disease of the respiratory tract and this inflammation can cause hyperreactivity of the respiratory tract to many stimuli. A direct correlation has been identified between respiratory tract inflammation and hyperreactivity of the airways and severity of the disease. Many inflammatory cells participate in the pathogenic of this inflammation in asthma including eosinophils, mastocytes, macrophages and neutrophils. This inflammation of the respiratory tract causes extensive but variable obstruction of the air flow that is reversible spontaneously or with treatment. Eosinophilia of the respiratory tract is the main factor seen in the development of allergic inflammation of the respiratory tract.

Asthma is also characterised by a greater reactivity of the airways to different physiological and environmental stimuli, such as exercise, cold air, dust mites and animal hair. In this disease, one of the predominant anamopathological characteristics is the dehiscence of the layer of epithelium and its detachment into the bronchoalveolar fluid.Steroids are the basis of asthma treatment. However, different tests have demonstrated that they do not improve this condition or they can even cause even more deterioration of the epithelial cells of the airways. In some studies it has been shown that these drugs induce apoptosis of the epithelium of the respiratory tract and additional denuding. It produces detachment from the basal membrane to the pulmonary lavage fluid and, consequently, this effect is controversial.

In asthma, the epithelium of the airways is a target for inflammatory and physical stimuli. In anamopathological studies of patients with asthma, a frequent finding is epithelial lesions, even in cases with mild clinical symptoms. According to what has been demonstrated by endobronchial biopsies, epithelial lesions are seen in about half the patients with mild asthma and in almost all of the patients with persistent asthma. One of the characteristics of chronic remodelling of the respiratory airways and the distinctive mark of persistent, chronic asthma is the detachment of the epithelial cells and the denuding of the mucosa of the respiratory airways.

Steroids may cause direct inhibitory effects on many of the cells that take part in the inflammation of the airways during this disease, including macrophages, T lymphocytes, eosinophils and epithelial cells of the airways. In cell cultures, this type of drug decreases the survival of eosinophils mediated by cytokines that stimulate apoptosis. This process could explain the decrease in the number of these white cells in the blood flow and airways of patients with asthma during treatment with this type of drug, especially the low density eosinophil fraction. Steroids do not inhibit the release of mediators of allergic reactions from mastocytes, but reduce the number of these cells in the airways. Besides their suppressor effects on inflammatory cells, these drugs also inhibit plasma exudation and mucosa secretion in inflamed airways. The lack of effect of inhaled steroids in pulmonary inflammation and airway reactivity has also been demonstrated.

In this study, we analysed the effect of an inhaled steroid, fluticasone propionate (FP), during and after sensitisation in guinea pigs, on anamopathological changes of the epithelium of the airways and their physiological properties.

Material and Methods

Sensitisation of the Animals and Groups of Animals

The sensitisation of the animals to OA was carried out using the method described by McCaig. To be brief, the guinea pigs were sensitized using 10 mg of OA (Sigma Chemical Ltd, United Kingdom) and 100 mg of Al(OH)3 dissolved in 1ml of saline administered by the intraperitoneal route. One week later, the animals received 1mg of OA and 100 mg of Al(OH)3 dissolved in 1ml of saline by the intraperitoneal route as a booster dose. From day 17, the animals were exposed to a nebulised solution of 4% AO during 18 ± 1 day, for 4min every day. The control group received similar solutions without OA. The aerosol was administered in a closed 30 ? 20 ? 20 cm chamber. The control animals were treated in a similar way, but saline was used in place of OA. The study was approved by the Research Committee of the Mashad University of Medical Sciences.

The study was carried out in 5 different groups of guinea pigs (n=8) according to the following (Table 1):

- Control group (group C): Received Al(OH)3 alone dissolved in 1ml normal saline and inhaled a water aerosol instead of OA.
- Treatment group A: Animals sensitised with OA and Al (OH)3, and, after a period of sensitisation with OA, treated with 250 μg of inhaled FP, 2 times a day (GlaxoSmithKline Research Triangle, NC) for 18 days (treatment A).
- Treatment group B: Animals sensitised with OA and Al(OH)3, and treated with 250 μg of inhaled 2 times a day, during the provocation period with OA, for 18 days (treatment B).
- Placebo group A: Animals sensitised with OA and Al(OH)3, and treated with inhaled placebo (compound made by GlaxoSmithKline Research Triangle, NC: CFC-free of HFA as4a; 1,1,1,2-tetrafluoroethane propellant), after the period of provocation with OA for 18 days (placebo A).
- Placebo group B: Animals sensitised with OA and Al(OH)3, and treated with inhaled placebo during the provocation period with OA for 18 days (placebo B).
Aerosol FP and placebo were administered with a container normally used with a spacer modified as previously described.\textsuperscript{14}

Tissue Samples

The guinea pigs were killed and the trachea was extracted. Each organ was cut into 10 rings (each one contained 2-3 cartilage rings). All the rings were opened in a direction contrary to the tracheal muscle and were sutured forming a tracheal chain.\textsuperscript{15}

Subsequently, the tissue was suspended in 20mL of an organ bath (Schuler type 809 organ bath, March-Hugstetten, Germany), that contained a Krebs-Henseliet solution formed by (mM): NaCl 120, NaHCO\textsubscript{3} 25, MgSO\textsubscript{4} 0.5, KH\textsubscript{2}PO\textsubscript{4} 1.2, KCl 4.72, CaCl\textsubscript{2} 2.5 and glucose 11. The Krebs solution was maintained at 37°C and was gassed with 95% O\textsubscript{2} and 5% CO\textsubscript{2}. The tissue was suspended under isotonic tension of 1g and it was allowed to reach equilibrium for at least one hour while it was washed with a Krebs solution every 15 min.

Assessment of the Tracheal Response to Metacoline

In each experiment the log of the cumulative concentration-response curves of the contraction of the tracheal chain induced by metacoline hydrochloride (Sigma Chemical Ltd, United Kingdom) was obtained. Every 3 minutes consecutive concentrations were added (including 10^{-7}–0^{-8}M), and the corresponding concentration was registered for each concentration at the end of the 3 minutes. The effect reached a plateau in all experiments. To obtain the curve, we represent graphically the percentage of contraction of tracheal smooth muscle due to each concentration of metacoline in proportion to the maximum concentration obtained from the final concentration compared to the log of metacoline concentration.

The effective concentration of metacoline that caused 50\% of the maximum response (EC\textsubscript{50}) was determined using the metacoline response curve for each experiment. Furthermore, the contractility of tracheal muscle obtained by 100 \mu M of metacoline was converted to grams, according to the calibration instructions of the electronic transducer.

Assessment of the Tracheal Response to Ovalbumin

The tracheal responses of all the animals to a solution of 0.1\% of OA was determined according to the following method: 0.5 mL of a 4\% OA solution were added to 10mL of the organ bath and the contraction of the tracheal chain was registered after 15min and, subsequently, this was expressed as a proportion (percentage) of the contraction obtained by 10 \mu M of metacoline.

All the experiments were carried out at random with a one hour rest period of the tracheal chains between every 2 experiments. Every 15 min the tissues were washed with a Krebs solution. In all the experiments the contractions were determined using an isotonic transducer (Harvard APP LTD, 50-6360 SINO 0210) and using a computer program with registers in a computer (model Acer # G781).

Pulmonary Lavage and WBC Count

At the same time as the tracheal chain was prepared, a cannula was placed in a bronchus and the other bronchus was closed with a forceps to prevent the manipulation of epithelial cells.

The lungs were washed with 5mL of saline 4 times (a total of 10mL). 1mL of bronchoalveolar lavage (BAL) was stained with Turk’s solution and a duplicate count was made in a haemocytometer (in a Burker chamber). Turk’s solution consisted of 1mL of glacial acetic acid, 1mL of a 1\% Gentian violet solution and 100mL of distilled water.

The remaining BAL was centrifuged at 2.500 \times g at 4°C for 10min. The supernatant was eliminated. A smear was prepared with the cells and stained with Wright-Giemsa. According to staining and morphological criteria, the analysis of the WBC count formula was carried out using a light microscope and counting 400 cells and calculating percentages. The WBC count was made with a Neubauer chamber using non-centrifuged BAL and a TORC stain.

Anatomopathological Assessment

After the BAL exams, the lungs and the residual trachea were extracted and introduced into a neutralised 10\% formaldehyde solution (37\%, Merck, Germany). After 7 days, the tissues were dried by passages through 70-100\% alcohol and washed by passages through xylol. Paraffin blocks of tissue were prepared and the samples were cut in 4 \mu m sections and stained with haematoxylin and eosin stain. The tissues were examined with a light microscope.

Statistical Analysis

To assess the efficacy of treatment in each group, the percentage of improvement was calculated in the following manner: Data obtained from the treatment group minus data obtained from the corresponding placebo group divided by data obtained in the same placebo group, multiplied by 100. In cases, the data obtained from the treatment group was greater than in the corresponding placebo group (i.e. [(treatment\textsubscript{A1}−placebo\textsubscript{A1})/placebo\textsubscript{A1}]100). In cases, treatment data was lower than in the corresponding placebo, the data obtained from the placebo group minus the data obtained from the corresponding treatment group divided by data obtained in the same treatment group, multiplied by 100 (i.e. [(placebo\textsubscript{A1}−treatment\textsubscript{A1})/treatment\textsubscript{A1}]100).

The data obtained for the tracheal response to metacoline (EC\textsubscript{50}) and OA, tracheal contractility, WBC count formula and anatomopathological score of the lung was documented as mean ± SEM (standard error of the mean). The normal distribution of the data was examined using the Kolmogorov and Smirnov test. The data of the placebo group and treated group were compared with the

<table>
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<th>Days 36-53</th>
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<tr>
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<td>IP Injection</td>
<td>IP Injection</td>
<td>Aerosol administration (4min/d)</td>
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</tr>
<tr>
<td>P\textsubscript{OA}</td>
<td>OA (10mg)</td>
<td>OA (1mg)</td>
<td>Placebo (2 times a day)</td>
<td>FP (250μg 2 times a day)</td>
</tr>
<tr>
<td>T\textsubscript{OA}</td>
<td>OA (10mg)</td>
<td>OA (1mg)</td>
<td>OA (4%)</td>
<td>FP (250μg 2 times a day)</td>
</tr>
<tr>
<td>P\textsubscript{FP}</td>
<td>OA (10mg)</td>
<td>OA (1mg)</td>
<td>OA (4%)</td>
<td>Placebo (2 times a day)</td>
</tr>
<tr>
<td>T\textsubscript{FP}</td>
<td>OA (10mg)</td>
<td>OA (1mg)</td>
<td>OA (4%)</td>
<td>Placebo (2 times a day)</td>
</tr>
</tbody>
</table>

IP: Intraperitoneal; OA: Ovalbumin; FP: Fluticasone propionate. Placebo and FP were administered from a container using a modified spacer.
control group using the t test for independent samples. The differences in the improvement of the different parameters were also examined using the t test for independent samples. Data comparison between the 2 treatment groups and the 2 placebo groups was carried out by means of a 2 way variance analysis (ANOVA) with a post hoc Bonferroni test. As level of significance a value was accepted of p < 0.05. All statistical analysis was performed using the Prism program.

Results

Histology

The anatomopathological changes in the lung of the sensitised groups were also included: Epithelial detachment, epithelial regeneration, mucus plug and inflammation were scored as follows: No pathological changes = 0; scattered changes = 0.5; local changes = 1; severe changes (in most lung regions) = 2.

Regarding this scoring, the anatomopathological changes in Placebo Group A, including mucosa detachment and mucus plug, were significantly greater than in the control group (p < 0.001 in both cases), but mucosa regeneration and inflammation were not significantly greater than in the control group. The anatomopathological changes in Placebo Group B, including mucosa detachment, mucosa regeneration, inflammation and mucus plug, were significantly greater than in the control group (p < 0.05–p < 0.001) (Fig. 1 and Tables 2 and 3).

Treatment with FP significantly improved mucosa detachment in treatment groups A and B in comparison with the corresponding placebo groups (p < 0.001). However, treatment with FP did not significantly improve the other anatomopathological changes (Fig. 1 and Tables 2 and 3).

Tracheal Response to Metacoline

In both placebo groups the metacoline concentration-response curves showed a left shift in comparison with the curves in the control group. However, the curves of both treated groups showed a right shift in comparison with the corresponding placebo groups (Fig. 2).

In the tracheal chains of placebo group A and of placebo group B the mean EC_{50} value was significantly lower than in the control group (p < 0.001 for both placebo groups) (Table 4). In the tracheal chains of both A and B treatment groups, the mean values of EC_{50} were significantly greater than the corresponding ones of the placebo groups (p < 0.001 in both cases). However, in the tracheal chains of both treatment groups the mean values of EC_{50} were significantly lower than in the control group (p < 0.001 in both cases) (Table 4).

According to the protocol followed in the treatment A group, tracheal reactivity to metacoline (EC_{50}) was not significantly worse than in the treatment B group protocol (Table 3).

Contractility

In placebo group A and placebo group B, the response to contractility of the tracheal chains with 100 μM of metacoline was significantly greater than in the control group (p < 0.001 for both placebo groups) (Table 4). In treatment groups A and B the responses to contractility improved significantly in comparison with the placebo groups (p < 0.001 in both cases) (Table 4). However, significant differences were detected in the responses to contractility in both treatment groups, A and B, and also between control groups (p < 0.05 y p < 0.01, respectively) (Table 4).

The absolute value of tracheal contractility to metacoline in placebo group B was significantly greater in comparison with placebo group A (p < 0.05) (Table 4). In the treatment A group protocol tracheal contractility response to metacoline at 100 μM was significantly worse than in the treatment B group protocol (Table 3).

Tracheal Response to Ovalbumin

The tracheal response to OA in the placebo groups A and B was significantly greater than in the control group (p < 0.01 for both placebo groups) (Table 4). In treatment groups A and B, tracheal response to OA was significantly worse than in the corresponding placebo groups (p < 0.001 and p < 0.01, respectively) (Table 4). However, tracheal responses in both treatment groups to OA continued to be significantly greater than in the control group (p < 0.01 for treatment group A and p < 0.001 for treatment group B) (Table 4).

The absolute value of tracheal response to OA in placebo group B was significantly greater in comparison with placebo group A (p < 0.001) (Table 4). The improvement in tracheal reactivity to OA in treatment protocol A was significantly worse than in treatment protocol B (p < 0.05) (Table 3).

WBC Count

The mean values of WBC count in BAL both for placebo groups A and B were significantly greater than for the control group (p < 0.001 for both placebo groups) (Table 5). In treatment groups A and B the total WBC count improved significantly compared to the
independent data. The comparison of the data between both treatment groups was done using a t test for
placebo group, multiplied by 100 (i.e. \((\text{treatmentA1} - \text{placeboA1}) / \text{placeboA1}\) × 100). The obtained from the corresponding placebo group divided by data obtained in the same
data was greater than in placebo – data obtained from the treatment group less data obtained from the
corresponding placebo groups. *p < 0.001, **p < 0.01, *p < 0.05.
Significant differences between data from each group treated with fluticasone propionate in comparison with the corresponding sensitised placebo group. *p < 0.001.
Significant difference between data from the 2 placebo groups. *p < 0.5, *p < 0.01.

Table 2
Mean values ± EEM of mucosa membrane detachment, mucosa membrane regeneration, inflammation and mucus plug in the control group (C) and groups that were treated (T) with fluticasone and placebo (P) after (A) and during (B) sensitization and significant differences in both placebo and treatment groups (n = 8, for each group)

<table>
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<tr>
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<th>C</th>
<th>P</th>
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<tr>
<td>Mucosa detachment</td>
<td>0.07 ± 0.07</td>
<td>1.57 ± 0.20</td>
<td>1.85 ± 0.14</td>
<td>0.28 ± 0.18</td>
</tr>
<tr>
<td>Mucosa regeneration</td>
<td>1.00 ± 0.3</td>
<td>1.34 ± 0.26</td>
<td>1.85 ± 0.14</td>
<td>0.85 ± 0.26</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0.42 ± 0.2</td>
<td>0.92 ± 0.07</td>
<td>1.71 ± 0.18</td>
<td>0.71 ± 0.18</td>
</tr>
<tr>
<td>Mucus plug</td>
<td>0.42 ± 0.2</td>
<td>2.14 ± 0.14</td>
<td>1.85 ± 0.14</td>
<td>1.57 ± 0.29</td>
</tr>
</tbody>
</table>

Anatomopathological changes in the lungs of the sensitised group included: epithelial detachment, epithelial regeneration, mucus plug and inflammation that were scored as follows: No pathological changes = 0; disperse changes = 0.5; local changes = 1; severe changes (in most regions of the lungs) = 2. Data from the placebo and treated groups were compared with the control group by means of a t test for independent data. Data comparison between the 2 treatment groups and the 2 placebo groups was carried out by means of a 2 way variance analysis (ANOVA) with a post hoc Bonferroni test.

Table 3
Differences in the improvement of tracheal response to metacoline and OA, total leukocyte count, and leukocyte formula in bronchoalveolar lavage fluid and anatomopathological changes in the 2 treatment groups A and B

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TA</th>
<th>TB</th>
<th>Value of p</th>
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</thead>
<tbody>
<tr>
<td>Mucosa detachment</td>
<td>85.71 ± 9.22</td>
<td>92.85 ± 7.14</td>
<td>NS</td>
</tr>
<tr>
<td>Mucosa regeneration</td>
<td>28.57 ± 18.44</td>
<td>57.14 ± 17.00</td>
<td>NS</td>
</tr>
<tr>
<td>Inflammation</td>
<td>42.85 ± 20.20</td>
<td>50.00 ± 10.91</td>
<td>NS</td>
</tr>
<tr>
<td>Mucus plug</td>
<td>25.71 ± 14.45</td>
<td>21.42 ± 10.10</td>
<td>NS</td>
</tr>
<tr>
<td>EC50</td>
<td>154.14 ± 21.83</td>
<td>288.33 ± 132.00</td>
<td>NS</td>
</tr>
<tr>
<td>OVA</td>
<td>36.71 ± 7.61</td>
<td>63.22 ± 9.28</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Contractility (reg)</td>
<td>103.33 ± 10.07</td>
<td>119.85 ± 24.04</td>
<td>NS</td>
</tr>
<tr>
<td>Total WBC count</td>
<td>32.87 ± 2.24</td>
<td>56.57 ± 3.44</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>49.28 ± 6.44</td>
<td>73.28 ± 5.32</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>150 ± 55</td>
<td>100 ± 56</td>
<td>NS</td>
</tr>
<tr>
<td>Monocytes %</td>
<td>93.14 ± 25.81</td>
<td>80.71 ± 45.41</td>
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<tr>
<td>Lymphocytes %</td>
<td>84.85 ± 10.48</td>
<td>24.28 ± 3.8</td>
<td>&lt; 0.001</td>
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</table>

NS: not significant.
All values are documented as mean ± standard error of the mean (SEM). The following percent improvements were obtained: in cases, treatment data was greater than in placebo – data obtained from the treatment group less data obtained from the corresponding placebo group divided by data obtained in the same placebo group, multiplied by 100 (i.e. \([\text{treatmentA1} - \text{placeboA1}] / \text{placeboA1}\) × 100). In cases, treatment data was greater than in placebo – data obtained from the treatment group less data obtained from the corresponding placebo group divided by data obtained in the same placebo group, multiplied by 100 (i.e. \([\text{treatmentA1} - \text{placeboA1}] / \text{placeboA1}\) × 100). The comparison of the data between both treatment groups was done using a t test for independent data.

WBC count in Bronchoalveolar Lavage Fluid

A significant decrease was identified in the percentage of neutrophils, lymphocytes and monocytes, and a significant increase in the percentage of eosinophils in BAL in both placebo groups in comparison with the control group (p < 0.005–p < 0.001) (Table 5). Treatment with FP significantly decreased the eosinophil percentage in both treatment groups compared to the eosinophil percentage in the placebo groups (p < 0.001 for treatment group A and p < 0.01 for treatment group B) (Table 5). In comparison with placebo group B there was a significant difference in the lymphocyte percentage (p < 0.001) in treatment B group (Table 5). However, significant differences continued to be detected in the eosinophil, neutrophil, lymphocyte and monocyte percentages in the treatment and control groups (p < 0.05–p < 0.001) (Table 5).

NS: not significant.
All values are documented as mean ± standard error of the mean (SEM). The following percent improvements were obtained: in cases, treatment data was greater than in placebo – data obtained from the treatment group less data obtained from the corresponding placebo group divided by data obtained in the same placebo group, multiplied by 100 (i.e. \([\text{treatmentA1} - \text{placeboA1}] / \text{placeboA1}\) × 100). In cases, treatment data was greater than in placebo – data obtained from the treatment group less data obtained from the corresponding placebo group divided by data obtained in the same placebo group, multiplied by 100 (i.e. \([\text{treatmentA1} - \text{placeboA1}] / \text{placeboA1}\) × 100). The comparison of the data between both treatment groups was done using a t test for independent data.

WBC Count Formula in Bronchoalveolar Lavage Fluid

A significant decrease was identified in the percentage of neutrophils, lymphocytes and monocytes, and a significant increase in the percentage of eosinophils in BAL in both placebo groups in comparison with the control group (p < 0.005–p < 0.001) (Table 5). Treatment with FP significantly decreased the eosinophil percentage in both treatment groups compared to the eosinophil percentage in the placebo groups (p < 0.001 for treatment group A and p < 0.01 for treatment group B) (Table 5). In comparison with placebo group B there was a significant difference in the lymphocyte percentage (p < 0.001) in treatment B group (Table 5). However, significant differences continued to be detected in the eosinophil, neutrophil, lymphocyte and monocyte percentages in the treatment and control groups (p < 0.05–p < 0.001) (Table 5).

Figure 2. Log of the cumulative concentration-response curves of metacoline induced contraction in the isolated trachea in the control group (C) and groups that were treated (T) with fluticasone and placebo (P) after (A) and during (B) sensitization and (n = 8, for each group). In both placebo groups the metacoline concentration-response curves showed a left shift in comparison with the curves in the control group. However, the curves of both treated groups showed a right shift in comparison with the corresponding placebo groups. 1) Trachea contractile response (% of maximum effect). 2) Metacoline concentration (log M).

The absolute value of the eosinophil percentage in placebo group B was significantly greater compared to placebo group A (p < 0.001). The absolute value of monocyte percentage in placebo group A was...
Tracheal reactivity to metacoline was determined using the concentration of metacoline that caused 50% of the maximum response (EC50). Significant differences between data from each group treated with fluticasone propionate in comparison with the corresponding sensitised placebo group. *p < 0.001.

Significant difference between data from the 2 treated groups. **p < 0.001. **p < 0.001.

Table 4

Mean values ± standard error of the mean (SEM) of mucosa detachment, mucosa regeneration, inflammation and mucus plug in the control (C) and groups that were treated (T) with fluticasone and placebo (P) after (A) and during (B) sensitisation and significant differences in both placebo and treatment groups (n = 8, for each group).

<table>
<thead>
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<th>P_B</th>
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<td>CE50 (μmol)</td>
<td>4.85 ± 0.35</td>
<td>0.87 ± 0.12</td>
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<td>2.1 ± 0.19***</td>
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<td>OA (%)</td>
<td>21.98 ± 2.73</td>
<td>57.7 ± 1.39</td>
<td>97.85 ± 6.44***</td>
<td>36.14 ± 2.63***</td>
<td>70.7 ± 3.9****</td>
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<tr>
<td>Contractility (g)</td>
<td>0.11 ± 0.03</td>
<td>0.38 ± 0.04***</td>
<td>0.48 ± 0.02***</td>
<td>0.20 ± 0.01*</td>
<td>0.23 ± 0.02***</td>
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</table>

Tracheal reactivity to metacoline was determined using the concentration of metacoline that caused 50% of the maximum response (EC50). Tracheal reactivity to OA was determined by means of the percentage of contraction seen with a 0.1% OA solution compared with 10μM of metacoline. The contractility of tracheal muscle was determined by means of 10μM of metacoline. The data of the placebo group and treated group were compared with the control group using the t test for independent samples. Data comparison between the 2 treatment groups and the 2 placebo groups was carried out by means of a 2 way variance analysis (ANOVA) with a post hoc Bonferroni test. p < 0.05. **p < 0.001. ***p < 0.001.

Significant differences between data from each group treated with fluticasone propionate in comparison with the corresponding sensitised placebo group. *p < 0.001.

Significant difference between data from the 2 placebo groups. **p < 0.001. **p < 0.001.

Significant difference between data from the 2 treated groups. **p < 0.001.

Table 5

Mean values ± SEM of the total count (# of WBC in 1mL) and WBC count formula (percentage of each type of cell) in bronchoalveolar lavage fluid in the control group (C) and groups that were treated (T) with fluticasone and placebo (P) after (A) and during (B) sensitisation and significant differences in both placebo and treatment groups (n = 8, for each group).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>P</th>
<th>P_B</th>
<th>T_A</th>
<th>T_B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC count</td>
<td>378 ± 34.32</td>
<td>2.228 ± 60.6***</td>
<td>2.964 ± 168.93***</td>
<td>700 ± 40.82***</td>
<td>1.642 ± 73.54*****</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>8.85 ± 1.43</td>
<td>36 ± 2.65 ***</td>
<td>54.71 ± 3.7***</td>
<td>16.85 ± 1.43***</td>
<td>40.28 ± 3.69*****</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>5.14 ± 0.63</td>
<td>18.5 ± 0.26 ***</td>
<td>0.71 ± 0.28***</td>
<td>2.71 ± 0.68***</td>
<td>2.00 ± 0.65***</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>60.14 ± 3.65</td>
<td>44 ± 5.23***</td>
<td>36.7 ± 4.07***</td>
<td>36.71 ± 3.8***</td>
<td>9.42 ± 1.71***</td>
</tr>
<tr>
<td>Monocytes</td>
<td>7.57 ± 0.65</td>
<td>5.42 ± 0.65*</td>
<td>2.14 ± 0.67***</td>
<td>3.85 ± 0.5**</td>
<td>1.42 ± 0.61***</td>
</tr>
</tbody>
</table>

The data from the placebo groups and treated groups were compared with the control group using the t test for independent samples. Data comparison between the 2 treatment groups and the 2 placebo groups was carried out by means of a 2 way variance analysis (ANOVA) with a post hoc Bonferroni test.

Significant difference between data from the control group and the other groups: *p < 0.05. **p < 0.01, ***p < 0.001.

Significant difference between data from each group treated with fluticasone propionate in comparison with the corresponding sensitised placebo group. *p < 0.001.

Significant difference between data from the 2 placebo groups. **p < 0.001, **p < 0.01, **p < 0.001.

Significant difference between data from the 2 treated groups. **p < 0.001.

The main anatomopathological characteristic of asthmatic patients is inflammation of the airways and all prophylactic medication administered for treatment of this disease tries to reduce this condition.

Furthermore, the increase of mucus secretion by the goblet cells of the airway epithelium and the increase of serum concentrations of IgE and IgG1 are associated with airway inflammation and asthma. Kopf (1993), Cohn (1998) and Zhu (1999) showed the involvement of IL-4 and IL-13 in the induction of changes in IgE isotope in B lymphocytes and an increase in airway goblet-cell mucus secretion. As a result, in this study, the reduction of lymphocytes could be caused by the increase in the number of B lymphocytes. In experimental models of asthma studied in greater detail, the lymphocytes are recruited in the airways and activated as a response to the inhalation of specific allergens.

In this research, FP administrated during and after induction of inflammation in the airways caused by allergen provocation was effective in the prevention of inflammation of the airways in both treatment protocols. In previous studies FP was also administered during induction of an inflammatory response, but Vanacker administered it during and after the induction of inflammation.

In our study, the effects of the administration of an inhaled steroid, FP, was examined using 2 treatment protocols to determine tracheal reactivity to metacoline and OA, WBC and WBC count formula in BAL fluid and anatomopathological changes of the airways in sensitised guinea pigs.

The results demonstrated a greater tracheal response to metacoline and OA, an increase in the response to contractility, an increase in WBC count and eosinophil percentage, but a decrease in neutrophils, lymphocytes and monocytes in the BAL of sensitised animals compared to the control group. The histological assessment of lung tissue also showed an increase in mucosa detachment, mucosa regeneration, inflammation of the airways and mucus plug in sensitised animals. These values were similar to the results described in previous studies. Furthermore, El-Mezayen showed that the inflammatory response is characterised by an increase in the number of inflammatory cells and concentration of cytokines Th2 in BAL fluid, eosinophilia of the airways and goblet cell hyperplasia. Furthermore, these observations are compatible with some studies previously carried out in murine models with atopic asthma, which revealed that eosinophils, lymphocytes, neutrophils and monocytes invaded lung tissue after sensitisation.

significantly greater in comparison with placebo group B (p < 0.01) (Table 5).

The mean values of improvement of eosinophil percentage in BAL in the treatment A protocol was significantly greater than in the treatment B protocol (p < 0.05) (Table 3). In protocol treatment A group the improvement in mean values of lymphocyte percentage in BAL was significantly greater than for protocol treatment B group (p < 0.001) (Table 3).

Discussion

In this study, the effects of the administration of an inhaled steroid, FP, was examined using 2 treatment protocols to determine tracheal reactivity to metacoline and OA, WBC and WBC count formula in BAL fluid and anatomopathological changes of the airways in sensitised guinea pigs.
where 0.1mg of FP did not have quantifiable effects on inflammatory or structural changes, such as goblet cell hyperplasia and thickening of the wall of the airways. The increase of eosinophilic inflammation in BAL fluid and in the mucosa of the airways, as well as the increase in the deposit of fibronectin induced by the allergen, was inhibited by FP treatment with doses of 1 mg and more. The inhibition of goblet cell hyperplasia and airway wall thickening required 10 mg of inhaled FP. With this, systemic effects were also observed. However, the differences in percentages of improvement of anatomopathological changes between both treatment protocols do not significantly support the fact that improvement of these changes in treated sensitised animals is due exclusively to the FP effect. Some studies have also shown that steroids cause apoptosis in the airway epithelium, additional denuding and, therefore, detachment from the basal membrane up the pulmonary lavage fluid.

The prophylactic effect of FP on the tracheal reactivity of sensitised animals could be due to its suppressor effect on airway inflammation. This conclusion is supported by the anti-inflammatory effect of all steroids, especially FP, on airway reactivity, inflammatory cells in BAL fluid and pulmonary anatomopathological changes in sensitised guinea pigs. However, in some studies, FP did not mitigate bronchial hyperreactivity induced by allergens, in spite of inhibiting eosinophils and T lymphocytes in the airways and goblet cell hyperplasia. In the BAL fluid of both groups treated with FP total WBC count and eosinophil and neutrophil percentages improved in comparison with what was observed in untreated sensitised animals. Steroids have proved to inhibit neutrophil apoptosis in a manner that is concentration dependent. Furthermore, steroids slightly increase the inhibiting effect of the granulocyte-macrophage colony stimulating factor on neutrophil apoptosis. The data found in this study suggests that FP extends the survival of human neutrophils inhibiting apoptosis at clinically relevant concentrations by means of an effect on the glucocorticoid receptor. However, treatment with FP leads to an additional reduction of monocyte and lymphocyte percentages in BAL fluid in both treatment groups. T lymphocytes play an essential role in airway inflammation in asthma. Their accumulation at the site of inflammation is related to an increase of their recruitment from peripheral blood and their long survival rate. Cell apoptosis is one of the mechanisms involved in lymphocyte T homeostasis control that caused the deletion of autoreactive T lymphocytes. In vitro studies it has been shown that FP could induce lymphocyte apoptosis in culture. It has also been shown that steroids reduce monocyte recruitment in the airways.

The changes in observed eosinophils in sensitised animals treated with FP or placebo are supported by studies that show an increase of eosinophils in BAL fluid in sensitised guinea pigs and a reduction of the eosinophil percentage in BAL fluid and lung dendritic cells in sensitised animals treated with FP in comparison with sensitised untreated groups.

All the parameters in placebo group B (sensitised animals treated with inhaled placebo during sensitisation, SP) were greater than in placebo group A (sensitised animals treated with inhaled placebo after sensitisation, PA), which shows a greater PB sensitisation in comparison with PA, that may be due to an allergen-free period. Currently available data suggest that the prevention of allergens, including avoiding inhaled allergens, food allergens, and smoking, would only attenuate asthma symptoms, that is, bronchial exacerbations, wheezing and hyperreactivity. Similarly, in the study carried out by Vanacker, the total WBC count and the WBC count formula in BAL fluid returned to control values both in animals treated with FP and in the placebo group after interruption of exposure to OA for 2 weeks. However, in Vanacker’s study, the animals exposed to OA maintained a greater reactivity of their airways to carbachol in comparison with the control group at the end of the 2-week allergen-free interval. The data obtained in this study also showed that, although all parameters, including tracheal reactivity, improved in the placebo group with an allergen-free period (P), they continued to be significantly different from the control group.

Improvements in percentages of all parameters except total WBC and eosinophil counts and mucosa detachment in the treatment group B (sensitised animals treated with inhaled FP during sensitisation, TB) were greater than in treatment group A (sensitised animals treated with inhaled FP after sensitisation, TA), including mucosa regeneration, inflammation, neutrophils, monocytes and lymphocytes. The reason for improvement of most parameters in treatment group B is probably the administration of FP during sensitisation. These findings highlight the importance of administering antiinflammatory treatment as soon as possible in asthmatic patients. This data is coherent with studies that show that early treatment of airway inflammation could modify the prognosis of asthma by preventing permanent loss of pulmonary function and even induce a remission in some patients. However, improvements in total WBC count, percentage of eosinophils and mucosa detachment in group TA was greater than in group TB. These findings suggest that inhaled FP could mainly affect airway inflammation (reduction of WBC and eosinophil counts and mucosa detachment) more than tracheal reactivity. The reason for the lower degree of improvement of this data in group TB is probably due to the greater change in this group.

To conclude, the results of this study show a protective effect of FL on tracheal reactivity, lung inflammation and mucosa detachment in sensitised animals. Furthermore, this study showed that treatment with an inhaled steroid, FP, during sensitisation (development of inflammation and anatomopathological changes) was more effective than after sensitisation (establishment of inflammation and anatomopathological changes). Consequently, steroid treatment must be initiated as soon as possible after the development of respiratory tract inflammation in asthmatic patients. Our findings also indicate the importance of achieving an allergen-free environment in the treatment of this disease. As a result, it is necessary to carry out an in depth investigation of the effect of an allergen-free environment and early antiinflammatory treatment in asthmatic patients as well as considering the treatment for this common disease. It would also be interesting to examine the effect of the administration of FP during sensitisation and to determine the different parameters after a certain period of time to study an allergen-free environment with greater precision. A study with a longer treatment period could also show a more substantial therapeutic effect on the different parameters.

Acknowledgements

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References