The effect of fluticasone on the airway inflammatory response to organic dust

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The effect of fluticasone on the airway inflammatory response to organic dust. A. Ek, L. Palmberg, K. Larsson. ©ERS Journals Ltd 2004.
ABSTRACT: Exposure to organic dust in a swine house causes acute airway inflammation and increased bronchial responsiveness to methacholine in healthy subjects. The aim of this study was to investigate whether an inhaled glucocorticoid, fluticasone propionate, alters the acute airway responses induced by exposure in a swine barn.

In 15 healthy subjects, analysis of nasal lavage fluids, serum samples and bronchial methacholine responsiveness were performed before and after exposure to organic dust in a swine house for 3 h. Seven subjects received fluticasone propionate (500 μg *b.i.d.* by inhalation and 100 μg intranasally once daily) and eight subjects received placebo during the 2 weeks prior to exposure.

Post-exposure plasma interleukin (IL)-6 levels and body temperature were significantly lower in the fluticasone group than in the placebo group. Intranasally administered fluticasone propionate significantly attenuated the plasma protein (assessed as albumin concentrations) leakage and IL-8 and tumour necrosis factor-α response induced by exposure. Fluticasone propionate inhalation exerted no influence on the increased bronchial responsiveness to methacholine induced by exposure.

In conclusion, glucocorticoid treatment attenuated the inflammatory response to inhaled organic dust without influencing the increased bronchial responsiveness to methacholine.
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Exposure in a swine confinement building for 3 h causes increased airway responsiveness and a massive influx of inflammatory cells into the airways in healthy subjects [1–3]. In bronchoalveolar lavage (BAL) fluid, a 75-fold increase in neutrophil numbers, more than doubling of alveolar macrophage numbers and a three-fold increase in lymphocyte numbers have been found following exposure [1, 2, 4]. In addition, the concentrations of pro-inflammatory cytokines (tumour necrosis factor (TNF)-α and interleukin (IL)-6 and IL-8) are elevated in nasal fluid and BAL fluid following exposure to swine dust [2, 4, 5]. Inhalation of swine dust also causes leakage of plasma proteins into the airways, as assessed by increased levels of α$_2$-macroglobulin, transferrin and albumin in BAL fluid [6]. Exposure in swine barns also induces systemic effects, such as increased body temperature and increased plasma levels of acute-phase proteins and IL-6 [1].

Glucocorticoids yield anti-inflammatory effects by modulating gene expression, mediated through the glucocorticoid receptor [7]. An important part of the anti-inflammatory effects of glucocorticoids is repression of inflammatory mediators, particularly cytokines, and inhibition of inflammatory cell migration [8]. It has previously been shown that fluticasone propionate almost totally inhibits swine-dust-induced IL-6 and IL-8 release from epithelial cells *in vitro* [9]. Additionally, the inhibition by fluticasone propionate of the dust-induced IL-6 and IL-8 release from epithelial cells is, in part, mediated by inhibition of nuclear factor (NF)-κB

activation [10]. However, the precise molecular mechanism of glucocorticoid action is unclear in this context.

Glucocorticoids are thus capable of influencing different indices of the inflammatory process. In addition to the beneficial effects in asthma, inhaled glucocorticoids are also a remedy for other inflammatory airway diseases, such as severe chronic obstructive pulmonary disease. It is, however, not clear whether or not this treatment alters the airway responses to heavy exposure to organic dust. The aim of the present study was, therefore, to investigate whether 2 weeks of treatment with inhaled and intranasally administered fluticasone propionate alters the inflammatory response and the effect on bronchial responsiveness induced by exposure in a swine barn. The airway pulmonary tissue concentration of the steroid during inhalation treatment would be expected to be in the same order of magnitude [11] as that previously found to abolish cytokine release from epithelial cells *in vitro* [9].

Materials and methods

Subjects

A total of 16 healthy nonsmoking subjects (12 males) with a mean age of 27 yrs (range 21–46) participated in the study. In order to be considered eligible for participation, all subjects

were required to show normal results on physical examination, chest radiography and spirometry. They had to have no history of asthma or allergic diseases, as evaluated by a questionnaire. The study was approved by the ethics committee of the Karolinska Institute (Stockholm, Sweden) and was performed with the informed consent of all subjects.

Study design

The subjects were randomised to either receive fluticasone propionate (500 μg *b.i.d.* by inhalation and 100 μg intranasally once daily, 50 μg in each nostril; n=7, one female) or placebo (twice daily by inhalation; n=8, two females) for 10–14 days in a single-blind manner. The exposure took place after the last dose (∼1 h) while weighing pigs for 3 h in a swine barn, containing 700–900 pigs. The participants assisted the farmer and guided the pigs through weighing boxes. Analysis of nasal lavage (NAL) fluids, serum samples and bronchial methacholine responsiveness was performed at different time points before and after exposure, as follows.

Symptoms

After exposure, symptoms of shivering, headache, malaise, muscle pain and nausea were evaluated by the use of a questionnaire. The symptoms were graded according to severity on a scale of 1–5 (1: no symptoms; 5: severe symptoms). Only grades 4 and 5 were classified as significant. Oral temperature was measured directly before exposure, immediately after exposure and over the next 10 h at 2-h intervals.

Nasal lavage

NAL was performed before the start of medication, after 2 weeks of treatment (1 h before exposure) and 7 h after the start of exposure. The NAL procedure described by PIPKORN *et al.* [12] and BASCOM *et al.* [13] was used with minor modifications. The subject flexed their neck 45° backwards and closed the soft palate while 5 mL 0.9% sodium chloride was instilled into one nostril using a needleless syringe. After 10 s, the neck was flexed forwards and the liquid expelled into a plastic basin, which was placed on ice during processing. The procedure was repeated on the other side. The volume of the combined lavage portions was measured and centrifuged for 10 min at $200 \times g$ at 4°C and the supernatant frozen at -70°C until analysis. The pellet was resuspended in 0.5 mL 9 mg·mL^{-1} NaCl containing 0.1% human serum albumin and the total number of recovered cells were counted in a Bürker chamber. Cytocentrifuge-prepared slides were stained with May-Grünwald Giemsa stain and 300 cells were assessed for differential cell counts. Less than 100 cells were considered too few cells for an accurate differential count.

Serum

Blood samples were collected before medication, 1 h before exposure and 4, 7 and 24 h after the start of exposure. Blood samples were allowed to coagulate at room temperature for 1 h before centrifugation ($1,550 \times g$ for 10 min) and the serum was dispensed into several aliquots, which were kept at -70°C until analysis. Each sample underwent only one freeze/thaw cycle before assay.

Analysis

TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF) levels in serum and NAL fluid were analysed using commercial high sensitive sandwich enzyme immunoassay kits (Quantikine^{TM}; R&D Systems Europe, Abingdon, UK). The detection ranges were 0.5–32 pg·mL^{-1} for TNF-α and 1–64 pg·mL^{-1} for GM-CSF. IL-6 and IL-8 and RANTES (regulated upon activation, normal T-cell expressed and secreted) were measured using an ELISA developed in the authors' laboratory, together with an enzyme-amplified detection system when necessary. The IL-6 analyses were described in detail by EK *et al.* [14] and the lowest standard concentration was 0.125 pg·mL^{-1}. For analyses of IL-8 and RANTES, commercially available antibody pairs, standards and Quantikine^{TM} serum controls at three different concentrations (R&D Systems Europe) were used, and the lower detection limits were 25 and 2.34 pg·mL^{-1}, respectively. Albumin in NAL fluid was quantified by ELISA, as previously described [4]. The lower detection limit of the assay was 0.11 μg·mL^{-1}. α$_2$-Macroglobulin in NAL fluid was quantified using an ELISA as previously described [6]. The lower detection limit of the assay was 0.5 pg·mL^{-1}. Analysis of leukotriene (LT) E$_4$ in NAL fluid was performed with an enzyme immunoassay using polyclonal antisera and an acetylcholinesterase-linked tracer, as previously described [15]. The detection limit was 7.8 pg·mL^{-1}. For all analyses, duplicates were measured and an intra-assay coefficient of variation of <10% and inter-assay coefficient of variation of <20% were accepted. Absorbance was measured using a Thermomax 250 reader (Molecular Devices, Sunnyvale, CA, USA).

Lung function tests and methacholine provocation

Lung function (forced expiratory volume in one second (FEV1) and vital capacity) was measured using a wedge spirometer (Vitalograph®; Medical Instrumentation, Buckingham, UK) according to the criteria of the American Thoracic Society [16]. Local reference values were used [17, 18]. Peak expiratory flow was measured with a mini-Wright peak flow meter (Clement Clarke, London, UK); the best of three blows was registered.

Bronchial responsiveness was assessed by methacholine challenge. Inhalation of diluent was followed by inhalation of doubling methacholine concentrations, starting at 0.5 mg·mL^{-1} and up to 64 mg·mL^{-1} or until there was a 20% fall in FEV1, as previously described [19]. The results were expressed as the provocative concentration (PC20) or cumulative dose (PD20) of methacholine causing a 20% fall in FEV1. In one subject pre-exposure FEV1 did not fall by 20% at the maximum concentration. This subject was assigned a PC20 of 65 mg·mL^{-1} for nonparametric statistical calculations.

The methacholine provocation and lung function tests were performed ∼1–2 weeks before medication and 4 h after cessation of exposure in the swine house.

Exposure measurements

Total dust and endotoxin levels were measured in inhalable and respirable dust as previously described [14].

Statistics

Oral temperatures and lung function data are presented as mean±SEM/SD, as indicated, and comparisons were made

using ANOVA and/or a t-test. Data on bronchial responsiveness, cell count, soluble products in NAL fluid and serum (except for IL-6) are given as median (interquartile range), and comparisons were made using the Wilcoxon signed-rank-sum test and Mann-Whitney U test. Serum levels of IL-6 were compared by ANOVA and t-tests after confirmation of normal distribution of pre-exposure data (Z-score). A p-value of <0.05 was considered significant. Spearman rank coefficients (rho) were calculated for correlations. Concentrations below the detection limits were assigned a fixed value slightly lower than the lower detection limit for statistical comparisons.

Results

One subject in the fluticasone group was excluded from all analyses due to an upper airway infection that commenced during the pre-exposure treatment period. In addition to severe symptoms of airway infection, all inflammatory marker levels measured were highly elevated 1 h prior to exposure compared to the pre-treatment values obtained 2 weeks previously in this subject.

Symptoms

Five of the eight participants in the placebo group and two of the seven in the fluticasone group experienced symptoms (grades 4 and 5) following exposure. Two participants in the placebo group experienced shivering, one headache and two malaise, and the two individuals in the fluticasone group experienced malaise.

Oral temperature was 35.9 ± 0.5 and 35.9 ± 0.3°C (mean±SD) immediately before exposure in the placebo and fluticasone groups respectively (fig. 1). The temperature increased after exposure in both groups to a maximum 9–11 h after exposure (p<0.01). The increase in temperature was significantly lower in the fluticasone group than in the placebo group (F=3.5, p=0.007).

Nasal lavage fluid

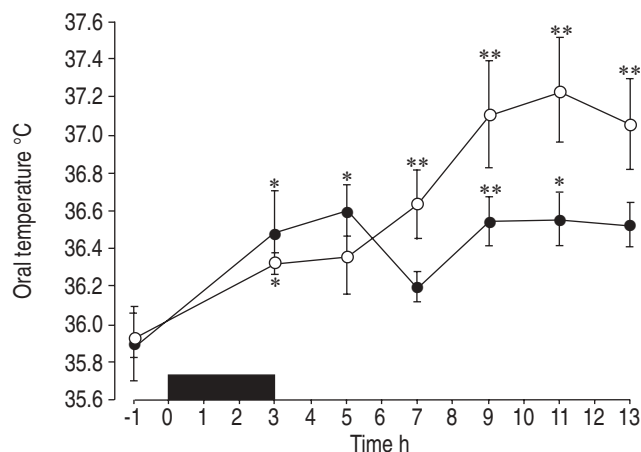In the placebo group, exposure caused a significant increase in total cell (p=0.01) and neutrophilic granulocyte

Fig. 1. – Oral temperature in healthy subjects before and after exposure (■) in a swine house. The subjects were treated with placebo (○; n=8) or fluticasone propionate (●; n=7) for 2 weeks prior to exposure. Data are presented as mean±SEM. The increase in body temperature was significantly smaller in the fluticasone group than in the placebo group (F=3.5, p=0.007). *: p<0.05; **: p<0.01 *versus* pre-exposure values.

concentration (p=0.02), whereas no significant changes in total cell (p=0.5) or neutrophil concentration (p=0.18) were found in the fluticasone group (fig. 2, table 1). There was, however, no significant difference between the groups with regard to total cell (p=0.3) or neutrophil concentration (p=0.4).

There was a significant increase in IL-6 and IL-8 and TNF-α levels after exposure, whereas LTE$_4$, albumin and α_2-macroglobulin levels increased significantly only in the placebo group (fig. 2). There were significantly lower increases in the fluticasone group with regard to IL-8 (p=0.02), TNF-α (p=0.03) and albumin (p=0.02). GM-CSF concentrations in NAL fluid (1 h before and 7 h after exposure) were below the detection limit (<1 pg·mL^{-1}) in both groups. The RANTES concentration in NAL fluid was below the detection limit (<2.3 pg·mL^{-1}) in 14 of the 16 subjects before medication, in 13 of the 16 subjects before exposure and in 10 of the 16 subjects after exposure.

There were significant correlations between post-exposure increases in levels of albumin and of α_2-macroglobulin (rho=0.79, p=0.003), IL-8 (rho=0.90, p=0.0007), TNF-α (rho=0.84, p=0.004) and LTE$_4$ (rho=0.92, p=0.004) (fig. 3).

Serum

The concentration of IL-6 in serum increased significantly in both groups (p<0.05), with a lesser increase in the fluticasone group (F=3.2, p=0.03) (fig. 4). In the placebo group (but not in the fluticasone group), there was a correlation between the maximum increase in serum IL-6 concentration and the maximum increase in body temperature (rho=0.79, p=0.04). The serum concentration of TNF-α 1 h before exposure was 2.0 (1.9–2.2) pg·mL^{-1} in the placebo group and 2.6 (1.5–2.8) pg·mL^{-1} in the fluticasone group, and did not change after exposure. The RANTES concentrations 1 h before exposure were 71 (51–83) ng·mL^{-1} in the placebo group and 71 (67–79) ng·mL^{-1} in the fluticasone group, and did not change after exposure. Exposure or medication did not significantly affect the serum concentrations of TNF-α or RANTES. GM-CSF concentrations in serum were below the detection limit (<1 pg·mL^{-1}) in all but one subject.

Lung function

Exposure caused a slight but significant decrease in vital capacity and FEV1 in all subjects, and there was no significant difference between the groups (table 2).

Methacholine provocation

Bronchial responsiveness to methacholine increased significantly in both groups after swine house exposure, with no significant difference between the groups (p=0.4) (fig. 5). In the placebo group, the PD20 fell from 1.90 (1.24–5.23) mg before to 0.25 (0.08–0.35) mg after exposure (p=0.01) and, in the fluticasone group, from 1.39 (0.52–2.34) mg before to 0.12 (0.08–0.15) mg after exposure (p=0.02). Thus bronchial responsiveness to methacholine increased by 3.2 (2.8–4.1) doubling concentration steps in the placebo group and 2.5 (1.5–3.9) doubling concentration steps in the fluticasone group.

Exposure measurements

The concentration of inhalable dust, assessed by the use of personal samplers, was 27.2 (19.8–36.4) mg·m^{-3}, 842 (396–1,197) ng·m^{-3} of which was the endotoxin concentration.
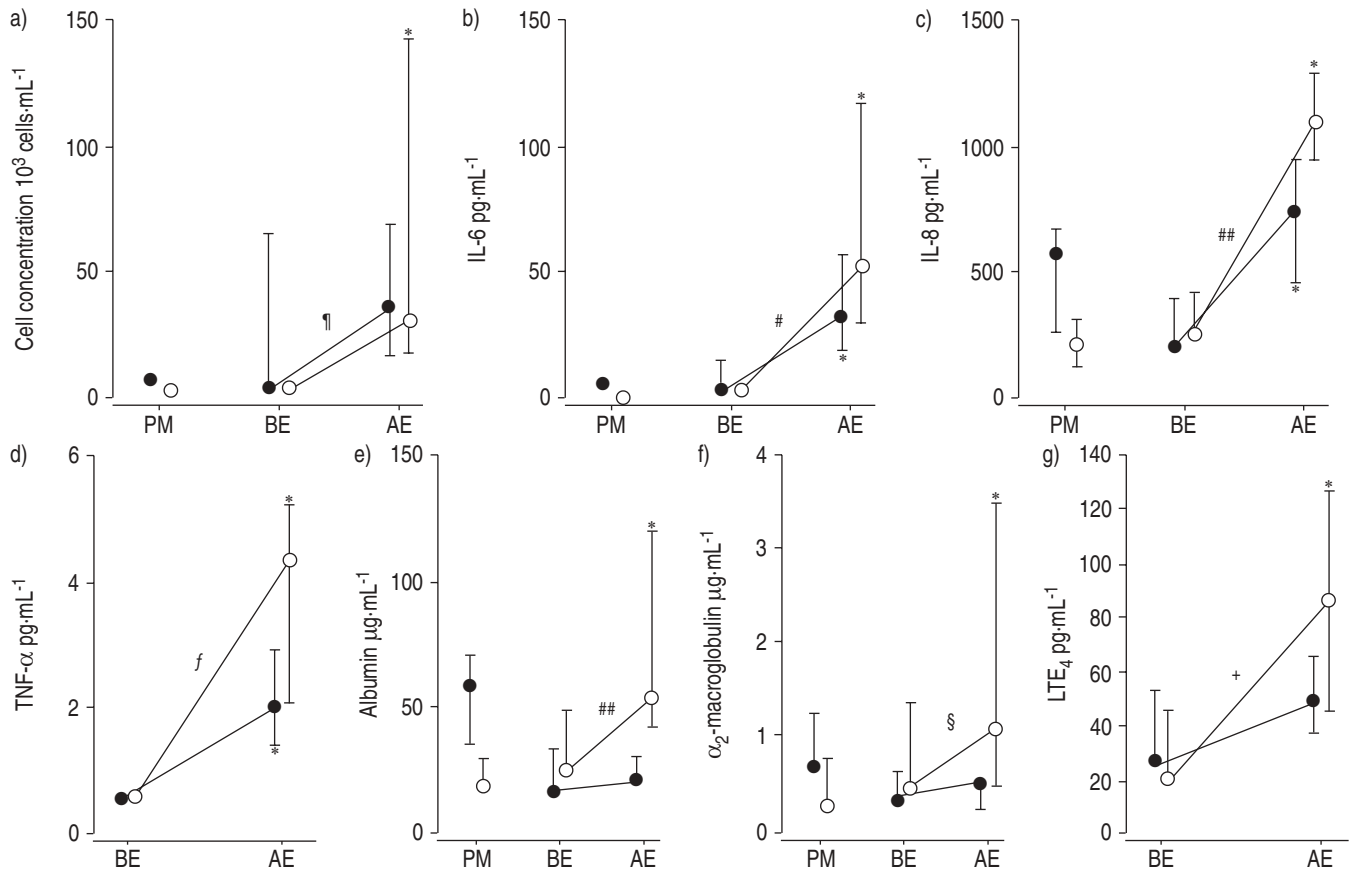
Fig. 2. – Analysis of nasal lavage fluid from healthy subjects before and after exposure in a swine house: a) cell concentration, and b–g) concentrations of b) interleukin (IL)-6, c) IL-8, d) tumour necrosis factor (TNF)-α, e) albumin, f) α_2-macroglobulin, and g) leukotriene (LT)E$_4$. The subjects were treated with placebo ($\bigcirc$; n=8; n=6 for LTE$_4$ analysis) or fluticasone propionate ($\bullet$; n=7; n=5 for LTE$_4$ analysis) for 2 weeks before exposure. Owing to the limited volume of lavage fluid, only 11 LTE$_4$ analyses could be performed. Data are presented as median (interquartile range). Fluticasone propionate treatment did not significantly alter concentrations of cells IL-6, IL-8, albumin or α_2-macroglobulin before exposure. PM; premedication; BE: before exposure; AE: after exposure. *: $p<0.05$ versus pre-exposure values; #: $p=0.6$; ¶: $p=0.3$; +: $p=0.07$; §: $p=0.06$; f: $p=0.03$; ##: $p=0.02$ difference between groups before and after exposure.

The concentration of respirable dust was 0.98 (0.72–1.27) mg·m^{-3}, 33 (15–51) ng·m^{-3} of which was the endotoxin concentration [14]. There were no significant differences in exposure between the groups.

Discussion

In the present study, it has been demonstrated that 2 weeks of fluticasone propionate treatment significantly attenuated the systemic effects induced by exposure to organic dust in a swine barn. Thus, the increase in body temperature and serum IL-6 levels after exposure were significantly lower in the fluticasone group than in the placebo group. Furthermore, intranasally administered fluticasone propionate had a major influence on the exposure-induced plasma leakage into the upper airways and significantly attenuated the nasal IL-8 and TNF-α response. However, fluticasone propionate inhalation did not alter the increased bronchial responsiveness to methacholine induced by exposure.

Table 1. – Nasal lavage fluid cell densities before and after exposure in a swine house#, in subjects treated with placebo or fluticasone propionate¶

	Premedication	1 h before exposure	7 h after exposure	p-value+
Neutrophils 10^6 cells·mL^{-1}				
Placebo	0.2 (0.0–4.1)	0.0 (0.0–1.1)	17.9 (10.4–159.5)	0.02
Fluticasone propionate	0.0 (0.0–0.9)	0.3 (0.0–16.7)	31.1 (15.3–66.8)	0.18
Epithelial cells 10^6 cells·mL^{-1}				
Placebo	1.1 (0.1–2.0)	1.2 (0.0–7.6)	1.6 (0.2–7.1)	0.99
Fluticasone propionate	0.0 (0.0–2.0)	2.3 (0.3–13.3)	1.9 (1.0–4.2)	0.3

Cell differential counts were not possible in all lavage fluids due to low cell concentrations; therefore, seven premedication lavages and five lavages performed 1 h before exposure were assigned a cell concentration of 0×10^6 cells·L^{-1}. Data from one subject in the placebo group were omitted due to technical problems in one lavage. Data are presented as median (interquartile range) (n=7 for both groups). The cellular reaction was not significantly different between the groups (p=0.4 for both neutrophils and epithelial cells). #: before medication, 1 h before exposure and 7 h after exposure; ¶: for 2 weeks prior to exposure; +: 1 h before exposure versus 7 h after exposure.
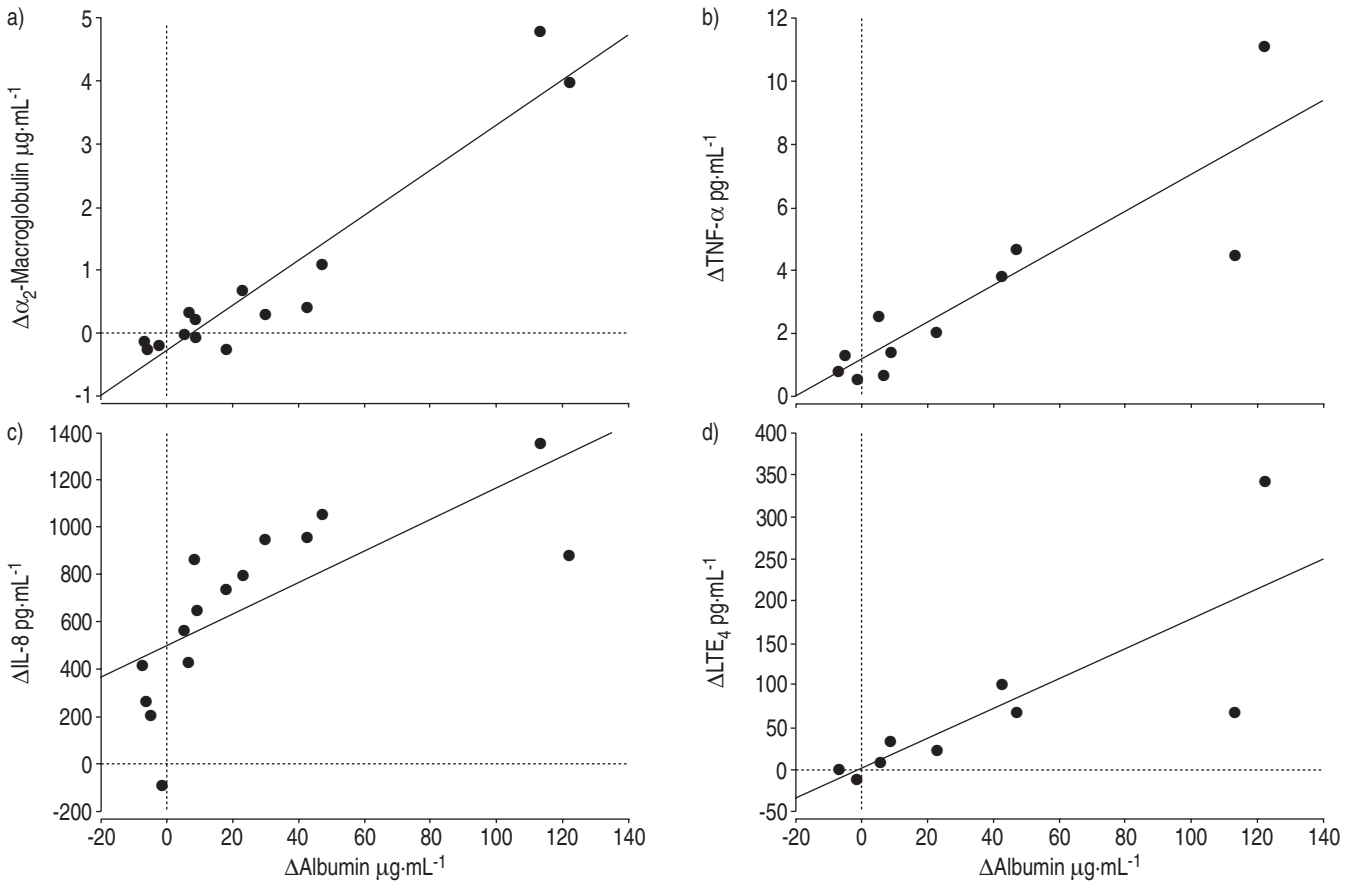
Fig. 3. – Correlations between differences (Δ: after exposure–before exposure) in nasal lavage fluid (whole material) levels of albumin and: a) α_2-macroglobulin (rho=0.79, p=0.003), b) tumour necrosis factor (TNF)-α (rho=0.84, p=0.004), c) interleukin (IL)-8 (rho=0.90, p=0.0007), and d) leukotriene (LT)E$_4$ (rho=0.92, p=0.004) (n=15 (n=11 for LTE$_4$ analysis)).

Exposure in swine houses causes airway inflammation with an increase in levels of inflammatory mediators, such as IL-6, in lavage fluid and blood [4]. Therefore, there is reason to believe that the airway is the source of the elevated IL-6 levels found in blood. IL-6 is an endogenous circulating pyrogen,

Fig. 4. – Interleukin (IL)-6 concentrations in serum from healthy subjects before and after exposure (■) in a swine house. The subjects were treated with placebo (○; n=8) or fluticasone propionate (●; n=7) for 2 weeks prior to 3 h of exposure. Data are presented as median (interquartile range). There is a significant difference between the groups (F=3.2, p=0.03). PM: premedication. *: p<0.05; **: p<0.01 *versus* pre-exposure values (1 h before exposure).

responsible for induction of fever during infection and inflammation [20]; increased levels of IL-6 are found in the blood of patients following injury and correlate significantly with the increase in body temperature [21]. In the present study, a correlation was found between the maximal increase in serum IL-6 level and body temperature after exposure in the placebo group but not in the fluticasone group. Therefore, a causal relationship might exist between the post-exposure attenuation of serum IL-6 level and body temperature in the fluticasone group. Fluticasone propionate, however, did not alter IL-6 release neither in the nose (NAL) nor, as previously demonstrated [14], in the lungs following exposure. One reason for this could be that the major source of serum IL-6 might be the lower airways and BAL was performed too late (24 h after exposure) to detect a possible steroid-mediated effect on airway IL-6 release in that study. Recent unpublished findings in the present study laboratory support such a hypothesis, demonstrating that IL-6 release and expression decline in epithelial cells after a few hours of exposure to swine house dust *in vitro*. It has previously been shown that fluticasone propionate inhibits IL-6 secretion from alveolar macrophages stimulated by lipopolysaccharide and abolish IL-6 secretion from swine dust-stimulated epithelial cells at a concentration (1×10^{-9} M) that can be anticipated to occur in lung tissue during treatment with the doses used in the present study [9, 11]. In addition, it has recently been shown that the transcription factor NF-κB is involved in organic-dust-mediated cytokine release (IL-6 and IL-8) from epithelial cells, and that inhibition of IL-6 and IL-8 release by glucocorticoids is, in part, mediated by inhibition of this
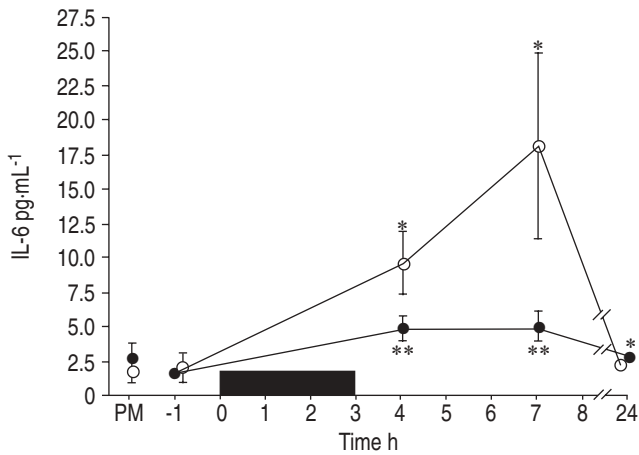
Table 2. – Lung function before and after exposure in a swine house[#] in subjects treated with placebo or fluticasone propionate[¶]

	Subjects n	Pre-exposure VC % pred	Post-exposure ΔVC %	Pre-exposure FEV1 % pred	Post-exposure ΔFEV1 %
Placebo	8	89.1±10.0	-3.6±2.9**	95.1±7.3	-7.8±4.1**
Fluticasone propionate	7	97.1±7.9	-1.9±1.5*	99.9±12.0	-4.9±2.6**
Between-group p-value			0.4		0.4

Data are presented as mean±SD; There was no significant difference between the groups (t-test); VC: vital capacity; Δ: change; FEV1: forced expiratory volume in one second. [#]: before medication and 7 h after exposure; [¶]: for 2 weeks prior to exposure; *: $p<0.05$; **: $p<0.01$ *versus* pre-exposure values.
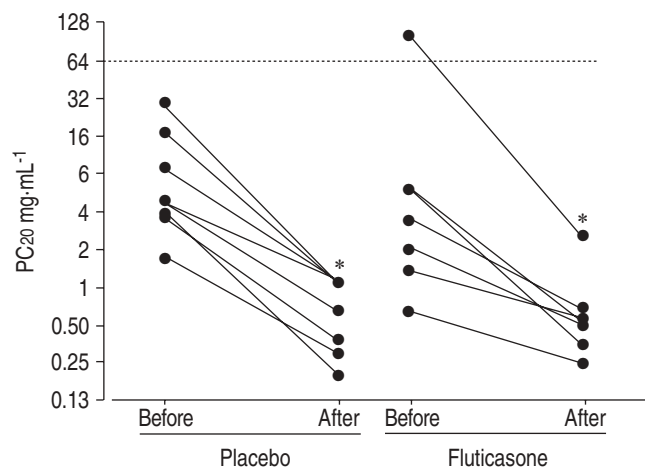

Fig. 5. – Bronchial responsiveness to methacholine before medication and 7 h after the start of the exposure in a swine house in healthy subjects pretreated with placebo (n=8) or fluticasone propionate (n=7) during the 2 weeks prior to exposure (·····: highest inhaled concentration of methacholine). The change in bronchial responsiveness calculated from the decrease in doubling concentration steps did not significantly differ between the groups (p=0.4). PC20: provocative concentration of methacholine causing a 20% fall in forced expiratory volume in one second. *: $p<0.05$ *versus* pre-exposure values.

mechanism [10]. These *in vitro* data are in agreement with the finding of lower IL-6 levels following exposure in the subjects who were treated with glucocorticoids. The effect on serum IL-6 might explain the attenuated increase in post-exposure body temperature in the steroid-treated group.

Topical fluticasone propionate treatment inhibited the organic-dust-induced increase in IL-8 and TNF-α levels in NAL fluid following organic dust exposure, which is consistent with previous findings *in vitro* [9, 10]. Neutrophilic granulocytes, which increase substantially in number after exposure [2], may, in addition to epithelial cells, be an important source of IL-8 and TNF-α, since both cell types are capable of producing both cytokines [22]. In a recent study, glucocorticoids were found to be important inhibitors of IL-8 release from neutrophils *in vitro* [23]. Mast cells are also important sources of TNF-α and IL-8 production [24]. Mast cells seem to play a role in the organic-dust-mediated airway inflammatory response since urinary excretion of 9α,11β-prostaglandin F$_2$, a prostaglandin D$_2$ metabolite that is almost exclusively produced by mast cells, increases after exposure [25]. In addition, inhalation of sodium cromoglycate alters the inflammatory reaction to swine dust exposure, probably by interacting with mast cells [26]. It was recently shown that dexamethasone potently inhibits TNF-α production by activated mast cells *in vitro* [27]. It is thus possible that fluticasone propionate treatment attenuates the organic-dust-induced cytokine level increase by interaction with epithelial cells and other cell types, such as mast cells and/or neutrophils.

Topical fluticasone propionate treatment also inhibited the leakage of plasma proteins (albumin and α_2-macroglobulin) into NAL fluid following organic dust exposure. This finding indicates a major effect on plasma leakage induced by exposure and confirms previous data showing that glucocorticoids inhibit plasma exudation in inflammatory airways diseases [28]. It is not known whether the anti-exudative efficacy of glucocorticoids in rhinitis and asthma [29, 30] reflects a direct effect of glucocorticoids on the microvascular wall in human airways or whether it reflects indirect action on cellular inflammatory responses. The possibility of direct action on the microvascular wall has been demonstrated in animal experiments [31]. However, budesonide treatment did not affect the histamine-induced mucosal exudation of albumin in humans and it has, therefore, been claimed that the anti-exudative efficacy of topical glucocorticoids in airway disease reflects the ability of these drugs to suppress cellular mechanisms [32]. A relationship was found between the release of IL-8 or LTE$_4$ and albumin and α_2-macroglobulin into NAL fluid. The increased vascular permeability observed under inflammatory conditions is suggested to be driven by cysteinyl LTs [33] and possibly IL-8, which may also induce plasma exudation [34]. It is thus possible that the IL-8 and LTE$_4$ released after exposure contribute to the plasma exudation observed, and the significant inhibition of exposure-induced IL-8 release by fluticasone propionate might, in part, lead to the inhibition of plasma leakage.

No effect of fluticasone propionate treatment on the increase in methacholine-induced airway responsiveness induced by exposure in a swine barn was found in healthy subjects. A recent meta-analysis on the dose/response relationship between inhaled corticosteroids and bronchial hyperresponsiveness in asthmatic subjects showed that glucocorticoids reduce airway hyperresponsiveness to methacholine by 0.45–3.47 doubling dose steps and that high doses confer greater improvement than low doses [35]. However, the factors predictive of improvement in bronchial hyperresponsiveness with inhaled glucocorticoids are still largely unknown, and the relationship between inflammation and bronchial hyperresponsiveness is not clear. In agreement with the present findings, NIGHTINGALE *et al.* [36] found that inhaled budesonide did not protect against neither the ozone-induced increase in methacholine reactivity nor the increase in sputum neutrophil numbers in normal subjects.

In conclusion, it was found that glucocorticoid treatment attenuated the upper airway response and the systemic response to inhaled organic dust without affecting the increased bronchial responsiveness.

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