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Swine dust exposure is a model for rapid induction of non-allergic neutrophil inflammation in the nasal mucosa of healthy volunteers, and the symptoms as well as the microcirculation are modified by nasal lavage*

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SUMMARY

Background: *The pathophysiological mechanism of non-allergic rhinitis is not clear and there is a lack of models in healthy volunteers. It has previously been shown that swine dust exposure is an excellent method for inducing inflammatory changes in the lower airways. We have shown earlier that exposure to swine dust increases the histamine sensitivity of the nasal mucosa as measured by rhinostereometry. In this study the aim was to investigate the effects of swine dust exposure on nasal symptoms as well as the microcirculation. Furthermore, the effect on nasal lavage was investigated.*

Method: *Seventeen subjects were exposed to swine dust during a three-hour period of work in a swine house. Nasal symptoms and the nasal mucosal response to histamine before and after exposure to swine dust were evaluated by laser Doppler flowmetry and nasal lavage.*

Results: *Exposure to swine dust increased nasal symptoms and levels of neutrophils, IL-8 and albumin. The increase in nasal symptoms and the microcirculation were modified by nasal lavage. CMBC correlated inversely with an increase in albumin ($p= 0.018$, $R= -0.95$).*

Conclusions: *Swine dust exposure is a useful model for inducing nasal inflammation in healthy volunteers. Furthermore, nasal lavage modifies subjective as well as objective parameters, which should be considered when designing studies. Nasal lavage may be useful in the treatment of workers in a swine dust environment.*

Key words:

INTRODUCTION

Rhinitis is a complex disease with many different etiologies and affects more than 25% of the population [(1)]. Persistent non-allergic inflammation has been reported to be as common as allergic inflammation [(2)]. However, there has been a lack of in vivo models in humans for investigating the pathophysiological mechanisms. It has been demonstrated that it is possible to initiate a strong neutrophil upper and lower airways inflammation in healthy subjects by using a model with healthy subjects working for three hours in a swine confinement building containing about 700 animals. Airborne swine dust contains particles from crushed feed, swine dander and micro-organisms from feces [(3)], -e.g. mainly gram+, such as *Enterococci*, but also gram- bacteria [(4)], and endotoxins, that can cause a strong neutrophil inflammation of the airways. We have previously used this model in a study, where the histamine sensitivity in the nasal and bronchial mucosa was investigated, and found that exposure to swine dust for three hours greatly increased the degree of nasal mucosal swelling during a histamine challenge test, as measured by rhinostereometry [(5)].

Nasal provocation can be performed with different irritating agents including histamine [(5,6)], methacholine [(6)], phentolamine [(6)] capsaicin [(7)], cold air [(8)] hypertonic saline [(9)] etc. Histamine has a direct effect on blood vessels inducing vasodilation and nasal congestion, but also activates H1 receptors on sensory C-fiber afferents setting up an axon reflex with the subsequent

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release of substance P and CGRP [10]. Thus histamine is an ideal substance for nasal provocation in a model of non-allergic inflammation since it can detect both vascular and nerve-induced nasal hyper-reactivity.

Symptom scores as well as objective measurements can be useful in order to evaluate nasal inflammation. Symptoms in rhinitis are nasal blockage, rhinorrhea, sneezing, facial pain or fullness as well as hyposmia. However, in chronic rhinitis the dominating symptom is often nasal blockage. Objective measurements of nasal congestion include acoustic rhinometry, rhinomanometry, PNIF and rhinostereometry. Furthermore, nasal lavage can be useful to evaluate cellular infiltration and signalling as well as albumin leakage.

The aims of the study were to evaluate:

1. Whether exposure to swine dust would
   a) increase nasal blockage, neutrophil infiltration and IL8 levels
   b) induce microcirculatory changes
   c) induce changes in CMBC correlating with albumin in the lavage.
2. If nasal saline lavage performed before the challenge test would modify the results.

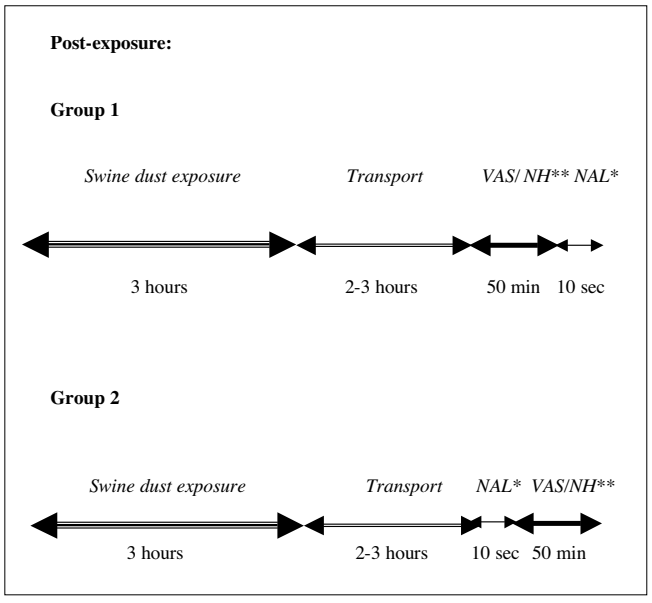

Fig. 1 Study design.

To evaluate the inflammatory effects on the microcirculation, Group 1 was subjected to nasal histamine challenge before the nasal saline lavage, and to evaluate the effects of the nasal lavage on the microcirculation, Group 2 had lavage before the histamine challenge test. This order was used during the pre- and post-exposure measurements. The post-exposure measurements occurred 2-3 hours after exposure. The pre-exposure challenge test was performed in the same order for Group 1 and Group 2 respectively.

*VAS/NH = Visual Analog Scales evaluating nasal blockage performed immediately before the nasal histamine challenge test. **NAL = Nasal lavage.

MATERIAL AND METHODS

Study group

We studied 17 healthy non-smokers, who were students or employees in the hospital. They had had no previous exposure to farm dust, or history of chronic rhinitis, allergy, asthma or signs of airway disease on physical examinations of the nose and lungs. Before the tests, the subjects had had no airway infections for at least 30 days. All gave their informed consent. The local ethics committee approved the study.

Study design (Figure 1)

Subject testing started within 2-30 days after inclusion. The subjects were divided into two groups in order to evaluate the local nasal effects of nasal lavage. In Group 1 (4 men and 4 women, mean age 25, range 18-32 years) the nasal histamine challenge test was done first and was followed within 10 minutes by a nasal lavage. In Group 2 (4 men and 5 women, mean age 27, range 17-32 years), the order was reversed. We measured the mucosal microcirculatory response in the challenge tests with a laser Doppler flowmeter connected to a rhinostereometer and the albumin, cells and IL-8 concentrations in the lavage. One week after the initial tests the subjects were exposed to swine dust for three hours in a swine confinement building housing about 700-900 pigs. During the exposure, the participants helped to guide the pigs through the weighing boxes, a procedure that causes a considerable amount of dust. Two to 4 subjects were exposed on each weighing occasion and dust exposure measurements were made at the same time. Two to 3 hours after the exposure, the measurements were made again in the same order as before exposure.

Dust exposure measurements

Measurements of inhalable (<10 μm) and respirible (<5 μm) dust and of endotoxin were made in two other subjects who were exposed on the same occasion, using the equipment described elsewhere [5,11].

Nasal lavage

We performed nasal lavage with 0.9% NaCl at 37°C [12], but with minor modifications [5]. The subjects were seated with their necks flexed backward about 45 degrees, and 5 ml was then instilled into one nostril, using a syringe without a needle. After 10 seconds, they bent forwards and the liquid ran out into a plastic basin. The volumes of the lavage from each nostril were measured and centrifuged at 200 g for 10 min at 4°C and the supernatant frozen at −70°C, pending the analyses. The pellet was resuspended in 0.9% NaCl with 0.1% human serum albumin. The cells were counted in a Bürker chamber and the cell concentration in the lavage fluid recovered was calculated. The concentration of human serum albumin in the nasal lavage supernatant was measured, using an inhibition enzyme-linked immunosorbent assay (ELISA) [13]. The lower detection limit of the assay was 25 ng/l.

Interleukin-8 was measured in duplicate with an ELISA method

using commercially available antibody pairs (capture antibody (MAB208), detection antibody (BAF208) and standard (208-IL-101), R&D Systems Europe, Abingdon, U.K.). The lower detection limit of the assay was 25 ng/l.

Nasal patency

Before starting the challenge tests, the subjects evaluated their degree of nasal stuffiness by using Visual Analogue Scales, VAS [14]. Each score was a 100-mm solitary line ranging from 0 (nostril completely clear) to 100 (nostril completely blocked). Each nostril was closed one at a time, while the subject breathed through the other nostril to estimate the patency. To obtain the value for stuffiness, the values on the right and left side were added and divided by two.

Measurements of the microcirculation

The laser Doppler apparatus connected to a rhinostereometer

The recording device of the rhinostereometer consists of a surgical microscope placed on a micrometer table. The table is attached to a frame and can be moved in 3 perpendicular directions defining a 3-dimensional coordinate system, in which the nasal cavity is placed. To maintain the distance between the area to be studied during the test and the microscope, the patient bites down on an individually-cast tooth splint fixed to the frame. The eyepiece of the microscope is fitted with a horizontal millimeter scale. Since the microscope has a small depth of focus, minor changes in the position of the medial side of the inferior turbinate can be recorded in the plane of focus along the millimeter scale of the microscope [15].

The laser Doppler flowmetry apparatus measures the microcirculation in the superficial part of the nasal mucosa. Light with a wavelength of 780 nm is transmitted to the tissue via a fiber optic probe. When the light strikes the moving blood cells, it undergoes a change in wavelength (Doppler shift), which is received by the specific fibers. A computer analyzes the data. The magnitude and frequency distribution of these changes are directly related to the number (CMBC) and mean velocity of moving blood cells in the volume measured, i.e., the blood perfusion. The results are given in arbitrary units, and therefore, the perfusion is expressed in arbitrary perfusion units (PU). PU cannot be given in ml/min/100g tissue, although there is a linear relationship between PU and ml/min/100g tissue [16]. Since the LDF apparatus is calibrated in the same way on all occasions, comparisons could be made between the measurements in each subject as well as between the subjects.

A specially designed probe with an outer diameter of 1.6 mm and a fiber separation of 0.5 mm was used, which has fibers containing efferent and afferent reflected light from the tissue. The end surface is angled 15 degrees from the line of sight so that it stays parallel to the surface of the mucosa. The rhinostereometer, equipped with a micromanipulator to which the laser probe was attached, enabled continuous adjustments of the laser probe end with an accuracy of 0.1mm, while keeping the measuring distance within 0.3 mm, in accordance with the criteria for precision in all 3 dimensions [17]. This equipment permits simultaneous recordings of changes in the congestion and the microcirculation of the same human nasal mucosa [17,18]. The measuring depth of the laser Doppler flowmeter is affected by the type of tissue, the wavelength, and fiber separation, and cannot be exactly determined. In human skin, when using a wavelength of 780 nm, the measuring depth is estimated 0.5-1 mm [19]. In the nasal mucosa, the measuring depth has been estimated to be at least 1 mm [20].

Laser Doppler flowmetry measurements

Before the test, the subject was acclimatized in the examination room for at least 30 minutes. Acclimatization was defined as when the position of the part of the mucosa surface being studied with the rhinostereometer was stable- i.e., no change in position exceeded 0.2 mm in three consecutive measurements, each separated by one minute.

The laser Doppler flowmeter measurements recorded were defined as the baseline values of the microcirculation measurements on the test day.

Nasal histamine challenge test

Most histamine tests were performed on the right side. If there were technical reasons (i.e., septal deviation) the left side was used instead. After the microcirculatory baseline values were recorded, 0.14 ml histamine-free isotonic saline containing phosphate buffer and 0.9 % bensylic alcohol (control) was applied only to the area studied (unilaterally). Approximately every 10^{th} minute, immediately after recording the microcirculation for 15-30 seconds, the same area was challenged with 0.14 ml histamine chloride in stepwise increasing concentrations (0.5, 1, 2 and 4 mg/ml, representing 0.07, 0.14, 0.28 and 0.56 mg histamine, respectively). The laser Doppler flowmetry recordings were made only on the challenged side 5 and 10 minutes after each saline or histamine application.

Statistical analysis

StatView™SE + Graphics® (Abacus Concepts Inc.) was used for the statistical analyses. Wilcoxon´s signed rank test was used for comparisons within the groups, the Mann-Whitney U test for comparisons between the groups, and these calculations were performed in the Department of Mathematical Statistics at the University of Stockholm.

The correlations were determined with Spearman´s rank order correlation test. The Δ-values for each subject were obtained by a subtraction of the pre-exposure from the post-exposure albumin concentrations in lavage and the pre-exposure from the post-exposure values of the baseline CMBC. Data is expressed as median and range, unless otherwise stated. Values of $p < 0.05$ were considered statistically significant.

RESULTS

The laser Doppler flowmetry measurements were made in all subjects except in one in Group 2, whose post-exposure

records were missing because of technical difficulties with the measurement equipment.

Exposure measurements

The exposures were similar in both groups, i.e., mean concentrations (SD) of inhalable dust 31.4 (9.8) mg/m^3 versus 27.19 (10.4) mg/m^3 (n.s.), of respirible dust 1.34 (0.74) mg/m^3 versus 1.04 (0.62) mg/m^3 (n.s.), and endotoxin contents of 0.086 μg/m^3 (0.048) and 0.074 (0.051) (n.s.) in Groups 1 and 2, respectively.

Airborne respirible dust correlated significantly with the following parameters in the nasal lavage: the increase in IL-8 levels ($R = 0.58$, $p = 0.015$, Spearman), the increase in albumin concentrations ($R = 0.57$, $p = 0.017$), and the increase in cell count ($R = 0.6$, $p = 0.012$).

Symptom scores of nasal blockage

In Group 1, the median pre- and post-exposure evaluations of nasal blockage respectively (assessed immediately before starting the challenge tests) increased from 4.5 (1.5-29) to 27.5 (0-97) (p=0.017). In Group 2 the corresponding pre- and post-exposure median values were 3 (0-18) before and 6 (0.5-39) after exposure (p=0.05). The results are presented in Figure 2.

Nasal lavage

Enough cells to perform cell differential counts were obtained from 9/17 individuals at the pre-exposure lavage, and from 16/17 at the post-exposure lavage. The median neutrophils increased from 27% (3-92%) to 96% (10-100%) after dust exposure. The outcome is further documented in Table 1.

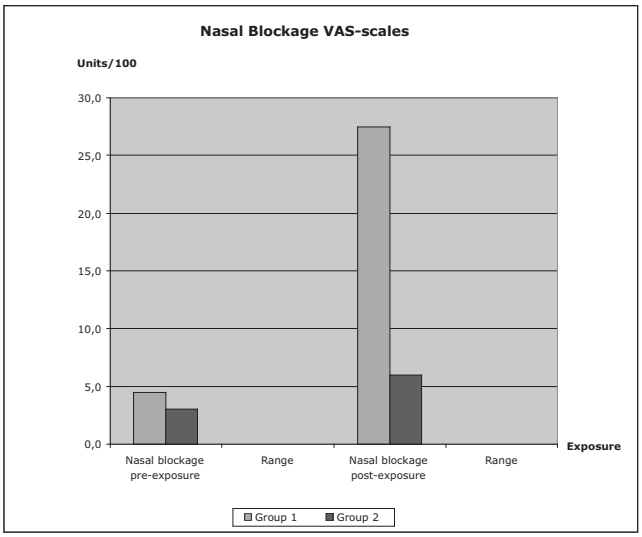

Figure 2. Visual analogue scales: Nasal blockage (median and range).

Immediately before starting the challenge tests, the subjects evaluated their degree of nasal blockage by using visual analogue scales (VAS). Each score was a 100-mm solitary line ranging from 0 (nostril completely clear) to 100 (nostril completely blocked). To obtain the current value for nasal blockage, the values on the right and left side were added and divided by two.

Table 1. Nasal lavage (median and range).

CELLS *(cells/ ml)*	**Pre-exposure**	**Post-exposure**	
Group 1 (n=8)	4 857 (322 – 55 390)	70 334 (7 538 - 258 970)	$p < 0.05$
Group 2 (n=9)	1 668 (572 – 3 176)	19 739 (9 739 – 195 362)	$p < 0.01$
ALBUMIN *(mg/ ml)*	**Pre-exposure**	**Post-exposure**	
Group 1 (n=8)	42.6 (3.6 – 147.1)	102 (33.8 – 198.4)	$p < 0.05$
Group 2 (n=9)	13.5 (7.8 – 26.9)	38.1 (11.4 – 62.9)	$p < 0.05$
IL-8 *(ng l^{-1})*	**Pre-exposure**	**Post-exposure**	
Group 1 (n=8)	300.7 (0 – 790.7)	1 167 (453 – 1 411)	$p < 0.05$
Group 2 (n=9)	127.1 (87.1 – 578.2)	886 (197.5 – 1 756.0)	$p < 0.01$

Microcirculation

Baseline values, (arbitrary units, median and range)

Perfusion: In Group 1 median pre- and post-exposure perfusion was: 267 (59-627) versus 421 (75-731) (n.s).

In Group 2 the corresponding results were 376 (156-503) versus 357 (141-521) respectively (n.s).

CMBC: In Group 1 median pre- and post-exposure CMBC were: 102 (66-123) versus 101 (82-110) respectively, and in Group 2: 107 (90-117) versus 101 (96-108) (n.s.).

Histamine challenge test

Perfusion: see Figure 3.

Group 1 (histamine challenge *before* lavage): There was no significant difference in perfusion between the pre- and post-exposure challenge tests, but also in Group 1 histamine challenge increased the perfusion ($p < 0.05$, Wilcoxons signed rank test).

Group 2 (histamine challenge *after* lavage): The sum of the post-exposure measurements throughout the challenge test was reduced as compared to the corresponding pre-exposure ones ($p = 0.0002$, Wilcoxon´s signed rank test). However, in both challenge tests histamine challenge increased the perfusion as compared to baseline ($p < 0.05$, Wilcoxons signed rank test).

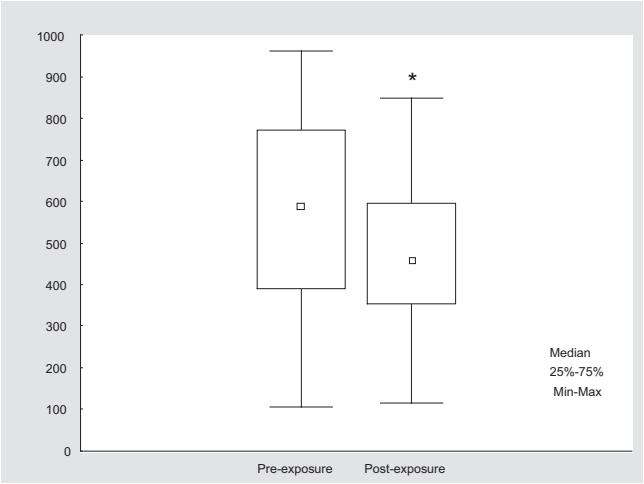

Figure 3. The effects of exposure to swine dust on perfusion during histamine challenge test in Group 2 (histamine challenge *after* nasal lavage). (Median and Range 25%-75%).

In Group 2 the collected post-exposure measurements of perfusion, including baseline, 5 and 10 minutes after challenge with saline and 4 increasing histamine concentrations (11 measurements) were lower than the pre-exposure ones ($p < 0.0002$, Wilcoxon´s signed rank test). This was not true for Group 1.

CMBC:

Group 1: The sum of the post-exposure measurements throughout the challenge test was reduced as compared to the corresponding pre-exposure values ($p < 0.001$, Wilcoxon´s signed rank test). There was no corresponding difference in Group 2, and likewise no significant differences were detected between Groups 1 and 2 in any respect (Mann-Whitney U test).

Correlations between albumin levels in lavage and cmbc (fig. 4).

In Group 1 there was a significant inverse correlation between the Δ-albumin levels and the baseline Δ-CMBC in nasal lavage ($R = -0.95$, $p = 0.018$, Spearman´s rank order correlation test). No corresponding correlation was found in Group 2.

DISCUSSION

In this study we have shown that exposure to swine dust induced subjective as well as objective signs of nasal inflammation including increased nasal blockage (Figure 2) and increased levels of neutrophils, IL-8 and albumin in nasal lavage (Table 1). We also found that the exposure induced changes in the microcirculation as well an insterstitial oedema with an increase in albumin levels as albumin leakage (Figure 4, Table 1). Thus exposure to swine dust may serve as a model of non-allergic inflammation in the nasal mucosa of healthy human volunteers. We also have demonstrated that nasal lavage per se seems to affect swine dust exposure-induced patient symptoms as well as objective findings.

Swine farmers have a higher frequency of airway symptoms, and analyses of bronchial lavage have detected signs of a permanent airway inflammation [21,22]. In healthy previously unexposed subjects, a short time exposure to swine dust causes an intense airway inflammation, often accompanied by symptoms of malaise, chills, fever and headache (organic dust toxic syndrome) [3,23]. Analyses of nasal and bronchial lavage after three hours work in a swine confinement building have shown increases in the number of cells (i.e. neutrophils), and in the concentrations of albumin and several cytokines [4,5,23]. A marked increase in the sensitivity to metacholine and histamine in the bronchi has also been found [5,24], which persists above pre-exposure levels for up to one week after three hours' exposure to swine dust [25].

The factors in swine dust that cause airway inflammation are not entirely known [3]. Endotoxin or lipopolysaccharides (LPS) from the fur and bacteria from faeces have been proposed as causative agents, but other components probably also contribute to the inflammatory response [25,26]. In vitro experiments have shown that they are probably not the sole agents, since swine dust induces a more marked inflammatory response than endotoxins [26]. Both gram+ and gram- bacteria and other components of swine dust seem to contribute to the inflammatory reaction after exposure [23,26], and therefore, our data could not be interpreted as solely a consequence of exposure to endotoxins.

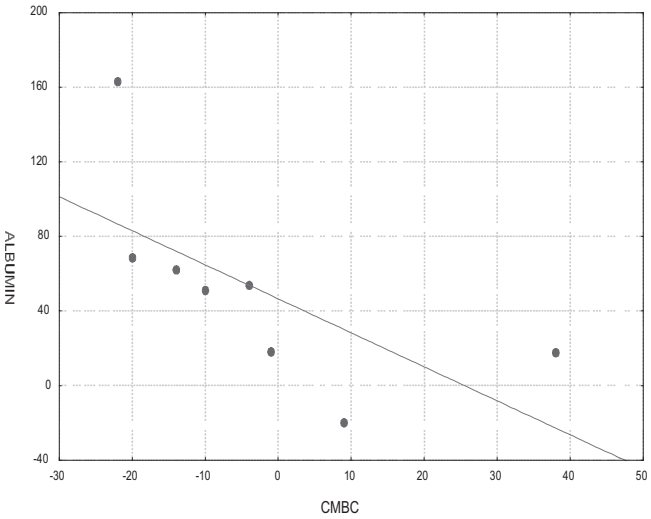

Figure 4. Correlation between increased levels of albumin in nasal lavage and decreased CMBC after exposure to swine dust in Group 1.

In Group 1 (lavage after histamine challenge) the individual Δ-albumin levels and Δ-baseline CMBC levels for the eight subjects of the group showed a significant inverse correlation ($R = -0.95$, $p = 0.018$, Spearman´s rank order correlation test). No such correlations were noted during the rest of the histamine challenge test. In Group 2 (lavage after challenge test), no correlations were found.

The pathophysiological mechanism or mechanisms of non-allergic inflammation is or are not clear and therefore results from our model with swine dust exposure inducing nasal inflammation should be interpreted with care. However, the method has clear-cut advantages compared to induction of a common cold, as it is quick, cheap, and the symptoms are relatively short-lived. After a three-hour exposure period, it is easy to immediately evaluate different aspects of the inflammation, and furthermore, combined studies of the upper and lower airways can also be performed. Consequently, acoustic rhinometry has demonstrated an obstruction in airflow of the nasal cavity due to increased swelling and/or secretion after short time exposure to swines [23]. We have also shown that in the same study group as in this study, the histamine sensitivity was increased in the nasal mucosa (increased swelling of the nasal mucosa as measured by rhinostereometry) as well as in the bronchi, (a decrease in FEV1) after exposure to swine dust [5].

In this study we have shown that symptoms of nasal blockage increased significantly upon swine dust exposure (Figure 2), which makes the model clinically interesting since it indicates that the inflammation is sufficiently strong to influence the patient in real life.

The effects of exposure to swine dust on the symptoms and the microcirculation can be evaluated by the results of Group 1, since the evaluation of the symptom scores and the nasal histamine challenge test were performed *before* the nasal lavage. The exposure to swine dust did not cause any significant changes in the baseline perfusion or in the perfusion during histamine challenge. These results are somewhat confusing, since the symptom scores of nasal blockage increased. Additionally, histamine provocation induced an increased swelling after swine dust exposure as measured by rhinostereometry [5]. Consequently, there were vascular changes in the deeper sinusoidal vessels but not in the superficial nutritional flow. However, the perfusion showed the typical dose-dependent increase after histamine application in both the pre- and post-exposure challenges. In accordance with the increase in nasal blockage and swelling of the mucosa [5], the exposure to swine dust induced a decrease in CMBC during the histamine challenge test. Since an increase in albumin correlated to a decrease in CMBC in Group 1 (Figure 4), these results indicate that during the histamine challenge tests, there was a vascular leakage resulting in an interstitial edema. This is also confirmed by the general increase in albumin levels after the histamine challenge (Table 1).

The effects of the nasal lavage on the symptoms (Figure 2) and on the microcirculation are evaluated by the results in Group 2, since the evaluation of symptoms and the nasal histamine challenge test were performed *after* the nasal lavage (Figure 1). The symptom scores of Group 2 increased only from 3 to 6 mm on a 100 mm scale whereas in Group 1 the symptom scores increased from 4,5 to 27,5 mm (Figure 2). Consequently, the small volume (5+5 ml) of saline used for nasal lavage had a clear-cut effect on nasal symptoms, which is in accordance with earlier studies on wood industry workers [27]. As in Group 1, the histamine challenge increased the perfusion throughout both the pre- and post-exposure tests. However, the increase in perfusion of group two (histamine challenge *after* lavage) was significantly lower during the post-exposure as compared to the pre-exposure challenge test (Figure 3). Therefore, the nasal lavage also affected the microcirculation as well as the nasal patency (Figure 2).

The effect on CMBC under inflammatory conditions seen in Group 1 was not seen in Group 2, probably due to the effect of the nasal lavage as discussed above. Consequently, there was no positive correlation between changes in CMBC and albumin leakage in Group 2.

Our interpretation of these data is that nasal lavage has a significant effect on the evaluation of symptoms as well as objective measurements, which implies that this has to be kept in mind when studies are designed. Furthermore, our data indicate that nasal lavage removes particles and mediators from the nasal mucosal surface, which may reduce inflammatory symptoms, and therefore may be of clinical use to swine farm workers as well as other workers in exposed environments [27].

In conclusion, we have found that exposure to swine dust is a method for inducing nasal inflammation and nasal symptoms in healthy volunteers. Furthermore, we have demonstrated that nasal lavage, even a small volume, affects both subjective and objective parameters.

In real life, the volume of the saline irrigation is much higher than 10 ml, and therefore the anti-inflammatory effects are probably stronger under these conditions. Another, environmental-medicine consequence of these results is that as in wood industry workers [27], nasal irrigation may be a simple method for reducing the airway inflammation in swine farmers.

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