

Pulmonary surfactant given prophylactically alleviates an asthma attack in guinea-pigs

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Summary

Background Previous studies have indicated that the increased airway resistance that develops in asthma may partly be due to a surfactant dysfunction. If so, it might be possible to alleviate the acute signs following an allergen challenge by prophylactically instilling into the airways a well functioning pulmonary surfactant.

Objective The study was planned and enacted to test the above hypothesis.

Methods The lung function (airway resistance, tidal volume, minute ventilation, and dynamic compliance) of 22 immunized guinea-pigs was studied for 30 min following a challenge. Ten of the animals had received a tracheal instillation of 0.5 mL calf lung surfactant extract (CLSE, 35 mg/mL) prior to the challenge.

Results The animals receiving the dose of 17.5 mg surfactant were less affected by the challenge than were the controls. Only one of them died following the challenge, whereas four of the 12 controls succumbed. Lung function was significantly less affected among the nine surviving animals treated with surfactant prior to the challenge than among the eight surviving controls ($P < 0.01$) and also their blood gases ($p\text{CO}_2$ and $p\text{O}_2$) were less influenced ($P < 0.05$).

Conclusion The study indicated that the symptoms developing after a challenge, which to some extent simulate those of asthma, can be alleviated by a prophylactic airway instillation of pulmonary surfactant.

Keywords: airway inflammation, airway resistance, surfactant dysfunction, asthma, surfactant prophylaxis, pulsating bubble surfactometer

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Introduction

Pulmonary surfactant is required to prevent alveoli from collapsing at end expiration. This concept, first presented by Clements [1], is widely accepted and has been the basis for a successful use of natural surfactant, extracts thereof, or synthetic preparations, to prevent and treat the neonatal respiratory distress syndrome (RDS). Pulmonary surfactant is thus of vital importance for normal alveolar function, but there have been publications [2–7] indicating that a surfactant is required not only in the most terminal part of the airway, but also in

the narrow section through which air is conducted to the alveoli. For maintenance of conducting airway patency a well functioning pulmonary surfactant would seem to be required.

It was demonstrated [5,6] that when a glass capillary was made very narrow in a short section, a small volume of liquid ($0.5 \mu\text{L}$) instilled into the narrow section would block the lumen of the capillary. If the liquid consisted of a suspension of well functioning pulmonary surfactant, it would not return to the narrow section once it had been extruded; the capillary would thus remain patent. If, on the other hand, albumin had been added to the surfactant suspension or it had been exposed to conditions likely to be present in the airways of an asthmatic patient, it was found to lose its ability to maintain openness of the glass capillary.

Recent studies from this laboratory [8] indicate that

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the *in vitro* situation referred to above applies also to the airways of the lung, which suggests that the increased airway resistance, so characteristic of asthma, might partly be due to a surfactant dysfunction. The augmented resistance might be caused by a blockage of many of the most peripheral conducting airways. This concept was supported by a recent study [7], showing that when guinea-pigs, immunized against ovalbumin, were challenged by being forced to inhale the aerosolized antigen, they developed serious breathing problems and, most importantly, they acquired a severe surfactant dysfunction. Chemical analysis of the guinea-pigs' pulmonary surfactant demonstrated that the phospholipid content and composition had not been affected by the challenge, but surfactant inhibiting plasma proteins had invaded the airways, causing the crucial dysfunction.

It has previously been demonstrated that plasma proteins may inhibit the activity of pulmonary surfactant [9–12] but by raising the surfactant concentration the activity may be regained [11,12]. For this reason it would seem possible to alleviate an acute asthma attack by supplying into the lumen of the airways a well functioning pulmonary surfactant preparation. With an appropriately planned and controlled, but small, clinical trial this concept was tested and found to be true [13]. If surfactants were to be supplied prior to an expected attack it might be more effective since it might prevent symptoms from appearing, or making them less serious. This paper is the report on a study which tests the hypothesis that, by instilling pulmonary surfactant prophylactically into the airways of immunized guinea-pigs, the respiratory distress, brought on by a challenge, will be alleviated.

Materials and methods

Immunization

Male guinea-pigs (Buckberg Labs, Liberty, NY, USA), weighing approximately 650 g, were studied according to a protocol which had been approved by the University Animal Care Committee. The animals ($n = 22$) were immunized according to the method described by Dunn *et al.* [14] and Popa *et al.* [15]. Briefly, 0.35 mL of a 5% (w/v) solution of ovalbumin (Grade III, Sigma, St Louis, MO, USA) was injected into each hind limb. The procedure was repeated after 7 days. Five weeks after the initial injection of ovalbumin the animals were prepared for antigen inhalation.

Lung function testing and blood gas analysis

To evaluate lung function and blood gases during spontaneous breathing, before and after a challenge,

the immunized guinea-pigs were anesthetized with sodium pentobarbital injected into the peritoneal cavity. As described in a previous article [7] intra-oesophageal pressure was measured. The trachea was exposed and a tube, PE 240, was inserted and connected with a Fleisch pneumotachograph (Gould, Hato Rey, Puerto Rico). The flow and pressure signals were recorded with a polygraph (Model 79D, Grass, Quincy, MA, USA). Flow was integrated to tidal volume, and minute ventilation was calculated from tidal volume and frequency of ventilation. Dynamic compliance, C_{dyn} , was calculated by dividing the expiratory volume with the change in oesophageal pressure, and total resistance of the lung, R_L , was estimated by dividing the change in oesophageal pressure with expiratory flow rate.

Through a catheter in the left jugular artery blood pressure was recorded and blood samples were collected for examination with a gas analyser (ABL 300, Radiometer, Copenhagen, Denmark) immediately before and about 1 min after the challenge.

Surfactant preparation used for airway instillation

As previously described [16,17], a chloroform-methanol extraction of cell free bronchoalveolar lavage fluid from freshly killed calves can be used as raw material for a surfactant preparation. After the chloroform has been removed under a flow of nitrogen, the dried extract is suspended in 0.15 M sodium chloride. This suspension of calf lung surfactant extract (CLSE) is given a phospholipid concentration of 35 mg/mL. It is autoclaved in sealed vials in which it remains until needed. CLSE consists mainly of lipids (>97%) but it also contains the lipophilic surfactant associated proteins, SP-B and SP-C. Since 1984 [18,19] CLSE has been utilized clinically to prevent and treat neonatal RDS. It was used for this study since it has a composition very close to that of natural surfactant, and it is produced under standardized conditions (ONY Inc., Amherst, NY, USA).

Surfactant instillation

The 22 immunized guinea-pigs to be challenged were randomly divided into two groups. Twelve animals served as controls and 10 received 0.5 mL of the CLSE suspension described above. The surfactant was instilled through the tracheal tube. The procedure took about 2–3 min and, to make it more likely that there would be an even distribution of the surfactant to all lung lobes and to peripheral sections of the lung, the hind part of the body was lowered 30° and the animal was slowly rotated during the instillation.

Aerosol challenge

After anaesthesia had been instituted for the surgical procedures and following the instillation of surfactant to 10 animals, values of pulmonary function and of blood gases were obtained during a 30 min period. Following this acquisition of baseline values, all animals were, for a period of 60 s, forced to inhale a mist of the antigen they had previously been immunized against. An 0.08% (w/v) ovalbumin solution was nebulized with a DeVilbiss 65 ultrasonic nebulizer (DeVilbiss, Somerset, PA, USA).

Statistical analysis

Student's *t*-test was used for a comparison of two mean values. For a comparison of more than two groups a one-way analysis of variance (ANOVA) was used, followed by Duncan's multiple range comparison test. Results are given as means $\pm$ SEM. A *P*-value <0.05 was considered to indicate a significant difference.

Results

The CLSE suspension instilled intratracheally caused transient dyspnoea and coughing. However, within 30 min those symptoms had disappeared. Each immunized animal, the controls as well as the surfactant treated, reacted vigorously and almost instantaneously (within 1 min) to the challenge. The symptoms were of varying severeness and were those previously described [7,14,20–22].

In the control group ($n = 12$) severe breathing

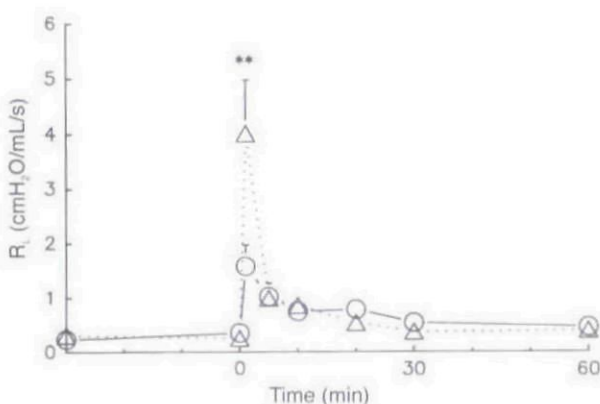


Fig. 1. Without a prophylactic surfactant treatment 30 min prior to the challenge, the total resistance of the lung, R_L , increased drastically following a challenge, which was started at time 0. The values are the means of the surviving animals: eight untreated and nine treated. The two asterisks indicate that the difference was significant ($P < 0.01$). $\Delta \cdots \Delta$. Untreated. $\circ \cdots \circ$. Surfactant-treated.

problems developed and mean blood pressure dropped from 61 ± 5 to 44 ± 4 mmHg ($P < 0.05$). Four of the 12 controls died, and this mortality of one-third was identical to what we observed in a previous study [7] where nine of 27 immunized animals died shortly after they had been challenged. Immediately following the challenge, airway resistance increased from 0.27 ± 0.04 to 4.02 ± 0.97 cm H₂O/mL/s ($P < 0.01$, Fig. 1), and tidal volume, minute volume, flow rate and dynamic compliance were significantly reduced ($P < 0.01$, Fig. 2). Surviving animals quickly recovered from this acute asthmatic reaction and 30 min after the antigen inhalation their lung function had returned to baseline values.

The controls' PaCO₂ increased from 43 ± 2 to 52 ± 3 mmHg and the value of PaO₂ dropped from 89 ± 8 to 20 ± 3 mmHg ($P < 0.01$).

In the surfactant treated group ($n = 10$) the acute asthmatic reaction was attenuated. Airway resistance was increased but only about a third as much as in the controls ($P < 0.01$, Fig. 1). The drop in tidal volume, minute volume, flow rate, and dynamic compliance was significantly less than in the control group ($P < 0.01$, Fig. 2). Blood pressure was minimally affected and only one of the 10 animals died.

In the group of guinea-pigs treated with surfactant, the challenge hardly affected PaCO₂ and the reduction in the value of PaO₂ was less than that observed in the controls ($P < 0.05$, Fig. 3).

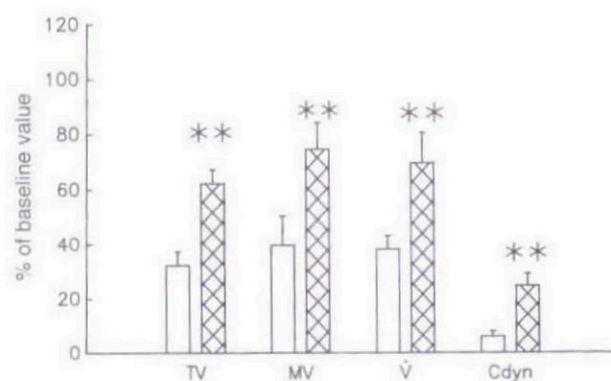


Fig. 2. Bar graphs showing how, immediately after the challenge, tidal volume (TV), minute volume (MV), flow rate (V), and dynamic compliance (C_{dyn}) diminished and became only a small percentage of the baseline values. Among the animals receiving surfactant the effect was alleviated, and the two asterisks indicate that the difference in response was significant ($P < 0.01$). The values ($M \pm SE$) were obtained shortly after the challenge from the surviving animals: eight untreated ($\square$) and nine treated ($\blacksquare$).

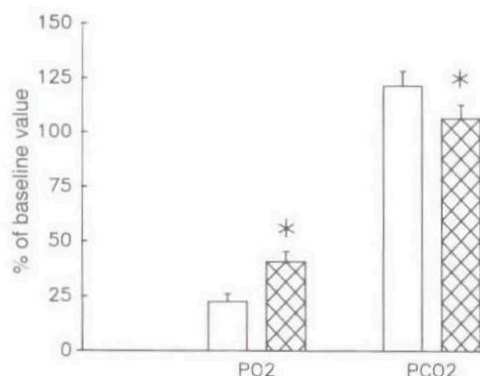


Fig. 3. Blood gases deteriorated after the challenge, but less among the animals treated with surfactant ($n = 9$) (▨) than among those receiving no prophylactic treatment ($n = 8$) (□). The values are those obtained immediately after the challenge. The asterisk indicates a significant difference ($P < 0.05$).

Discussion

We recently found that when immunized guinea-pigs were challenged, by being forced to inhale aerosolized antigen, the activity of the animals' endogenous surfactant was inhibited by plasma proteins leaking into the airway lumen [7]. The results of this paper support the conclusions of our previous publication that the acute symptoms caused by the challenge were at least partly due to a surfactant dysfunction since, when the endogenous surfactant was supplemented prophylactically, the respiratory distress developing was less serious.

When surfactant had been instilled into the guinea-pig's airways 30 min before the challenge, the effect of the ovalbumin inhalation was not as dramatic as it was in the group of controls; the attenuation of R_L and of other assessments of lung function was significant ($P < 0.01$, Figs 1 and 2). It is conceivable that when the surfactant was instilled prior to the challenge a greater number of terminal conducting airways could remain open and this prevented blood gases from being so significantly affected. This in turn might have been the reason why blood pressure did not show a serious decline and only one of the 10 animals died in convulsions, whereas four of the 12 controls succumbed from what seemed to be anaphylactic shock.

It was also noted by Becker [23] that immunized guinea-pigs reacted less to a challenge when pulmonary surfactant had been instilled into the airways. He felt this was due to a sheltering of receptors. Although there is no evidence supporting this hypothesis it cannot be excluded. Since we have data in agreement with the concept that the instilled surfactant exerts

its beneficial effect by increasing the likelihood that narrow conducting airways will remain patent, we feel this is a more likely explanation [5–8].

The immediate and short-lived effect we noted following the challenge has previously been described [7,14,20–22]. It has generally been interpreted as being due to bronchospasm and it simulates but is not equivalent to human asthma. Bronchospasm is a widely accepted and plausible explanation. However, in our previous study we interpreted the increased airway resistance, that followed already one minute after the challenge, as resulting from a protein leakage into the airways [7]. We perceive it is conceivable that the increased airway resistance might be caused not only by a bronchospasm, but might also be due to a blocking of many narrow conducting airways; the invading plasma proteins might have prevented the endogenous surfactant from carrying out its normal function of preserving small airway patency.

A dominating feature of human asthma is the inflammatory reaction. It can be expected to cause an oedematous swelling of tissue in lung parenchyma and airway wall, thereby diminishing airway lumen and consequently increasing resistance to airflow. As part of the inflammation there might also be an increased leakage of plasma proteins into the lumen of the airway. The corticosteroids used for treating asthma are likely to diminish the airway constriction and the protein leakage. That, in turn, would reduce the risk that the airways become blocked by liquid columns [6]. Accordingly, the steroid treatment will counteract the possibility that a serious surfactant dysfunction develops. Furthermore, steroids will stimulate surfactant synthesis, at least in the fetal lung [24–26]. The beta-adrenergic agonists will also have an effect on the surfactant system, since they induce a release of surfactant from the type II cells into the alveolar space [27–29]. It is thus conceivable that the most widely used treatment for asthma will reduce the risk that a surfactant dysfunction will develop.

When the airway lumen has been diminished, due to bronchospasm or to oedema in the airway wall, the necessity of a well functioning surfactant is accentuated. In a previous paper [6] we demonstrated that a surfactant with moderate dysfunction may have the capability of keeping a wide tube open, but not a more narrow one. When immunized guinea-pigs received a surfactant instillation prior to a challenge, they might not have suffered a severe or even a moderate surfactant dysfunction, their terminal conducting airways thereby remaining open, even though they probably became more narrow. On the other hand, in the control animals a surfactant dysfunction, probably

brought on by a protein inhibition, might have caused a liquid blocking of many terminal and narrowing of conducting airways.

The prophylactic dose of surfactant given to the guinea-pigs was 17.5 mg. It is of interest to relate that quantity to the size of the endogenous surfactant pool. For guinea-pigs the pool size is not known, but for sheep it has been reported to be 15 mg/kg bodyweight [30]. If it is of similar size in the guinea-pig the endogenous pool size would be about 10 mg, since the animals weighed approximately 650 g. The dose given may thus seem excessive, but an overdose has not been reported to be harmful, and we wanted to be sure that the dose we gave was large enough. However, we did see a negative effect shortly after the surfactant had been instilled. This was probably due to a transient blocking of the airway lumen, since the surfactant was supplied in a relatively large liquid volume. It would have been preferable if the surfactant had been supplied as an aerosol spray, as it will be in future studies.

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