Dextran restores albumin-inhibited surface activity of pulmonary surfactant extract

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Kobayashi, Tsutomu, Keisuke Ohta, Katsumi Tashiro, Kazuo Nishizuka, Weimin Chen, Shigeo Ohmura, and Ken Yamamoto. Dextran restores albumin-inhibited surface activity of pulmonary surfactant extract. J. Appl. Physiol. 86(6): 1778–1784, 1999.—We examined the effect of dextran (molecular weight 71,000) in counteracting the surfactant inhibitory action of plasma albumin. The surface adsorption time of 0.5 mg/ml modified natural surfactant (MNS; porcine lung extract consisting of phospholipids and hydrophobic surfactant proteins) with 7.5 mg/ml albumin decreased from 681 to 143 s by addition of dextran at a concentration of 10 mg/ml (P < 0.01). The minimum surface tension of 2.0 mg/ml MNS with 30 mg/ml albumin decreased from over 21 mN/m to below 3 mN/m when dextran was added at a concentration of 10 mg/ml (P < 0.01). Surfactant-deficient newborn rabbits given 10 ml/kg of a liquid containing 2.0 mg/ml MNS with 30 mg/ml albumin had a mean tidal volume of 5 ml/kg after 5 min of mechanical ventilation, but, in those animals given the liquid containing 10 mg/ml dextran also, the volume was >13 ml/kg (P < 0.05). Although the underlying mechanism remains to be elucidated, we conclude that dextran restores the albumin-inhibited surface activity of MNS.

METHODS

Preparation of test liquids. MNS was prepared from the alveolar lavage fluid of recently slaughtered pigs. The cell debris; water-soluble proteins, including hydrophilic SPs; and neutral lipids were removed by centrifugation, chloroform-methanol (2:1, vol/vol) extraction, and acetone precipitation, respectively. The remainder, which consisted of 98% phospholipids (by weight), 0.9% other lipids, and 1.1% hydrophobic SPs, was used as MNS after evaporation of the organic solvent. More detailed isolation methods and chemical compositions of MNS are presented elsewhere (13). All preparations of MNS used in the present study were combined together. They were then suspended in 0.9% saline at a concentration of 8.0 mg/ml, first by repeated suction and expulsion with a syringe and next by incubation in an ultrasonic bath (47 kHz, 90 W, Branson 3200, Yamato, Tokyo, J apan) at room temperature for 15 min.

The dry powder forms of rabbit plasma albumin (A-0764, Sigma Chemical, St. Louis, MO) and of dextran with an average molecular weight of 71,000 (D-1537, Sigma Chemical) were dissolved in 0.9% saline. The ionizable calcium content of dextran determined by the electrode method (ABL 620, Radiometer, Copenhagen, Denmark) was negligible (<0.001%). Using the suspension of MNS and the above two solutions, we prepared various test liquids containing MNS at concentrations of 0–2.0 mg/ml, albumin at 0–30 mg/ml, and dextran at 0–20 mg/ml in 0.9% saline. They were incubated at 37°C for 2 h and then stored at −20°C.

Measurement of surface properties. The surface adsorption time of the test liquids having a MNS concentration that was adjusted to 0.5 mg/ml was measured by the surface aspiration method (9). About 50 ml of the test liquid were kept at 37°C in a round Teflon trough (6.2 cm in diameter) and were then stirred by vibration for 60 s at 800 cycles/min (cpm) from the outside by using a vibrator (EV 210N, National, Tokyo, J apan). The stirring was stopped, and a platinum plate connected to an electrobalance (type UL, Shinkoh, Osaka, J apan) was then dipped into the test liquid to determine the surface tension. Twenty-five seconds after the stirring was discontinued, the surface of the test liquid was quickly aspirated for 5 s with a fine catheter (1.6-mm ID). The surface

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adoption time, that is, the interval between the end of the aspiration procedure and the moment when the decreasing surface tension reached 28 mN/m (4–5 mN/m above the equilibrium value of MNS), was used as an expression of the surface adsorption rate. In each measurement, the aspirated volume (5.5–6.2 ml) and the depth (1.8–2.1 mm) of the test liquid were calculated from the weight, and the surface tension values were corrected for the buoyancy of the platinum plate.

The surface property of the test liquids having a MNS concentration that was adjusted to 2.0 mg/ml was measured by the pulsating-bubble method (3). The test liquid was placed in the sample chamber (25-µl capacity) of a pulsating bubble apparatus (Pulsating Bubble Surfactometer, Electro-netics, Buffalo, NY) and heated to 37°C. A bubble (0.40-mm radius) in connection with the ambient air was created in the liquid within 0.2 s and was left for 10 s in a static condition. After this 10-s period, the bubble was pulsed between radii of 0.40 and 0.55 mm at a speed of 40 cpm. Continuously monitoring the pressure across the air-liquid interface, we calculated the surface tension at the end of the static condition period (end-SCP) and values at 10 min after the start of pulsation, according to Laplace’s law (surface tension = initial pressure·radius/2). The shape of the pulsating bubble deviated from spherical when the surface tension value decreased below 3 mN/m. The calculated surface tension values at these low levels, therefore, were not accurate, but we avoided this inaccuracy. The surface tension values at the minimum and maximum bubble sizes were defined as \( \gamma_{\text{min}} \) and \( \gamma_{\text{max}} \), respectively.

Main animal experiment: Bioassay of test liquids containing MNS. A total of 28 immature newborn rabbits were delivered by hysterotomy from 6 pregnant does at a gestational age of 27 days (26 days 19 h to 27 days 2 h, term = 31 days) and were immediately tracheotomized under anesthesia with intraperitoneal pentobarbital sodium (0.5 mg). They were then randomized into three groups: the dextran-albumin-surfactant (DAS), albumin-surfactant (AS), and control groups. In the DAS group, 10 ml/kg body wt of the test liquid containing dextran (10 mg/ml), albumin (30 mg/ml), and MNS (2.0 mg/ml) in 0.9% saline were given via the tracheal cannula before the first breath was taken. In the AS group, the test liquid containing albumin (30 mg/ml) and MNS (2.0 mg/ml) in 0.9% saline was administered in the same way as in the DAS group. In the control group, no test liquid was given via the tracheal cannula.

After the above procedures, the animals were transferred to a system of multiple plethysmographs (capacity = 10 animals) kept at 37°C. Next, they were relaxed with intraperitoneal pancuronium bromide (0.02 mg) and subjected in parallel to pressure-controlled ventilation. The respirator unit (Servo 900B, Siemens-Elema, Solna, Sweden) delivered 100% oxygen at a frequency of 40 breaths/min with a 50% inspiration time. Throughout the ventilation period of 20 min, the peak inspiratory pressure (PIP) was set at 25 cmH\(_2\)O, and a positive end-expiratory pressure of 2.5 cmH\(_2\)O was applied to the common tube of the respirator circuit by using a variable resistor (model E037E, Siemens-Elema). These ventilatory conditions were precisely maintained by continuous monitoring of the circuit pressure with an electric manometer (TP-400T, Nihon Kohden, Tokyo, Japan).

Individual tidal volumes were recorded at the end of each 5-min interval by means of a pneumotachograph that consisted of a specially designed flow-resistant tube, a differential pressure transducer (model TP-602T, Nihon Kohden), and an integrator unit (model AR-601G, Nihon Kohden) and that was capable of detecting a volume change of 0.02 ml. Electrocardiograms were recorded immediately after the period of ventilation, and animals showing QRs complexes at a frequency of over 100 beats/min were considered survivors.

Additional animal experiment: Bioassay of test liquids not containing MNS. Eight immature newborn rabbits were delivered from one pregnant doe at a gestational age of 27 days. They were anesthetized and tracheotomized in the same manner as in the main animal experiment. Next, five of them received, via the tracheal cannula, 10 ml/kg body wt of 0.9% saline containing 10 mg/ml dextran alone. The other three received the same volume of 0.9% saline containing 30 mg/ml albumin alone. Their tidal volumes were measured under the same conditions as described in the main animal experiment.

Statistical analysis. Values of surface adsorption time, animal body weight, and tidal volume were expressed as means ± SD, and their differences were assessed by one-way or two-way analysis of variance followed by Scheffé’s method. Differences in survival rate were assessed by Fisher’s exact test. Data of surface tension were presented as median and range, and the differences were examined by Dunn’s test. In all assessments, levels of \( P < 0.05 \) were considered significant.

RESULTS

Figure 1 shows representative original tracings obtained in the surface adsorption measurements. The surface tension of the test liquid consisting of 0.5 mg/ml MNS alone was elevated by the surface aspiration procedure to 70 mN/m, which is almost equal to the surface tension of clean normal saline at 37°C, and then decreased to the equilibrium value of ~23 mN/m. The tracings of the test liquids containing MNS and albumin constantly showed an S-shaped curve, with comparatively rapid reduction of the surface tension both at the beginning and at the end of the adsorption period and with a period of slower reduction in the middle. In the first one-half of the adsorption period (from the end of the surface aspiration to the moment at which the surface tension decreased to ~51 mN/m), the shape of the tracing was similar to that of albumin alone in 0.9% saline. Furthermore, the surface adsorption rates of the test liquids with 10 mg/ml dextran were clearly faster than those without, when the other constituents were identical. A solution of albumin alone showed an equilibrium surface tension of 51 ± 1 mN/m within the examined concentration range (0.25–7.5 mg/ml).

Figure 2 shows the relationship between the albumin concentration and the time taken for surface adsorption of MNS having a concentration that was fixed at 0.5 mg/ml. When dextran was not present, the relationship was formed of two distinct parts, one with a steep slope (–580 s·mg\(^{-1}\)·ml\(^{-1}\)), seen at albumin concentrations of ≤0.25 mg/ml, and the other with a slope of –53 s·mg\(^{-1}\)·ml\(^{-1}\), seen at albumin concentrations of ≥1.5 mg/ml. The time needed for adsorption was lengthened from 78 ± 6 to 681 ± 17 s when the albumin concentration was increased from 0 to 7.5 mg/ml. With 10 mg/ml dextran, however, the time needed for adsorption was 44 ± 4 s when albumin was absent. By increasing the albumin concentration to 7.5 mg/ml, the time was lengthened to 143 ± 20 s but was approximately one-fifth of that without dextran.
Figure 3 presents the relationship between the dextran concentration and the surface adsorption time. All the data shown in this figure except that for MNS alone were obtained from the test liquids containing MNS at 0.5 mg/ml and albumin at 7.5 mg/ml. The time needed for the surface adsorption was shortened with increasing dextran concentrations in a fashion similar to an exponential curve and reached $10^2 \times 6 \times 7$ s when the dextran concentration was increased to 20 mg/ml.

Figure 4 shows the surface tension at the end-SCP (A) and the $\gamma_{\min}$ and $\gamma_{\max}$ values (B) that accompanied changes in the dextran concentration. All the test liquids containing MNS at 0.5 mg/ml and albumin at 7.5 mg/ml. The time needed for the surface adsorption was shortened with increasing dextran concentrations in a fashion similar to an exponential curve and reached $10^2 \pm 7$ s when the dextran concentration was increased to 20 mg/ml.

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when the dextran concentration was increased to 10 mg/ml and above, the median values of the surface tensions at the end-SCP decreased to \(<28\) mN/m and the \(\gamma_{\min}\) values fell to within a range of 0.5–3.0 mN/m. The \(\gamma_{\max}\) also decreased with increasing dextran concentrations and reached a value similar to that obtained with MNS alone when the dextran concentration was \(\geq 10\) mg/ml.

In the main animal experiment, 11, 9, and 8 immature rabbits were utilized in the DAS, AS, and control groups, respectively. Two animals utilized in the DAS group (2 of 11) developed pneumothorax, but none did in the AS and control groups. After excluding the animals with pneumothorax, we found no significant differences in body weight among the groups (29.6 ± 5.3, 30.7 ± 4.2, and 31.4 ± 4.2 g in the DAS, AS, and control groups, respectively). All the animals in the DAS and AS groups survived during the ventilation period, but four of the eight animals in the control group did not survive (\(P < 0.05\) vs. DAS and AS groups). Figure 5 is an original tracing of the tidal volume measurement obtained from five immature newborn rabbits at 5 min after the start of the ventilation. Although the animals were delivered from one pregnant doe and were identically ventilated, the tracing indicates that the tidal volumes in the animals of the DAS group were clearly larger than those of the AS and control groups.

Figure 6 shows that the tidal volumes of both the DAS and AS groups increased with the duration of ventilation (\(P = 0.02\)). The volumes of the DAS group were 13.4 ± 6.8 and 28.3 ± 6.4 ml/kg at 5 and 20 min after the start of ventilation, respectively. The corresponding values in the AS group were 5.0 ± 4.6 and 12.8 ± 7.0 ml/kg. Thus the DAS group exhibited significantly larger tidal volumes than did the AS group (\(P < 0.05\)), when the changes with time were taken into consideration. The tidal volumes of the control group remained \(<3\) ml/kg throughout the ventilation period.

In the additional animal experiment, no animal receiving the solution of 10 mg/ml dextran alone or 30...
mg/ml albumin alone developed pneumothorax. Their body weights (30.2 ± 2.7 g) were not significantly different from those of the main animal experiment, but their tidal volumes were ≤3 ml/kg throughout the ventilation period.

DISCUSSION

In the present study, the surface adsorption time of 0.5 mg/ml MNS prolonged by albumin was significantly shortened when dextran (average molecular weight 71,000) was added. In the presence of dextran at a concentration of 10 mg/ml or higher in a test liquid containing 2.0 mg/ml MNS and 30 mg/ml albumin, surface tension at the end-SCP and \( \gamma_{\text{min}} \) were significantly lowered to the same levels as for MNS alone. Immature newborn rabbits receiving the test liquid with 10 mg/ml dextran developed larger tidal volumes than when dextran was not present. We incubated MNS in an ultrasonic bath to make the suspension uniform. Although it is possible that this procedure alters the properties of MNS, we believe that it is valid to discuss the present results, because the influence of ultrasound should be the same for all the test liquids.

Holm et al. (6) have suggested that the mechanism by which albumin inhibits the surface adsorption of surfactant molecules is a competitive process: albumin is adsorbed onto the air-liquid interface and occupies space in competition with surfactant molecules. In our surface-adsorption study, the surface tension of the test liquid containing MNS and albumin decreased in a fashion similar to that of albumin alone until the moment at which the equilibrium surface tension of albumin (−51 mN/m) was reached (Fig. 1, III and IV). This may support the competitive process. However, the surface tension of the test liquids containing both MNS and albumin decreased faster when the adsorption process approached its end point. In addition, the gradient of the surface adsorption time curve depending on the albumin concentration clearly differed between the concentrations of ≤0.25 mg/ml and ≥1.5 mg/ml (Fig. 2). These findings can hardly be explained by the competitive process alone. Our data may have been influenced by diffusion resistance and settling of the surfactant molecules (28), because we did not stir the test liquids during the measurement. Even when these factors are taken into consideration, the present findings suggest that some process other than competition may also be involved in the mechanism of inhibition related to albumin.

The surface adsorption of the more diluted surfactant is more markedly inhibited by albumin (6). In the adsorption measurements, we diluted the test liquids until the MNS concentration became 0.5 mg/ml. This concentration, however, is too low to exhibit physiological events (13). Surface tension at the end-SCP reflects the surface adsorption rate of the surfactant molecules (28). It is clear, therefore, that surface adsorption of the test liquids containing 2.0 mg/ml MNS and 30 mg/ml albumin is also accelerated by the presence of dextran at concentrations of ≥10 mg/ml. To develop the low \( \gamma_{\text{min}} \) or to normalize the ventilation mechanics, surfactant molecules that leave the air-liquid interface during surface compression (expiration) must be replenished rapidly during surface expansion (inspiration) (10, 14).

In the measurements made by the pulsating-bubble method, the test liquids that had an accelerated adsorption rate showed \( \gamma_{\text{min}} \) of 0.5–3.0 mN/m. We suppose that, in the present study also, improvement of the surface adsorption may be one of the factors that reduced the \( \gamma_{\text{min}} \) values.

Dextran is electrically neutral and shows little surface activity by itself. By the addition of dextran at a concentration of 10 mg/ml, the surface tension of 0.9% saline (70 mN/m at 37°C) was little changed (data not shown), but the albumin-inhibited surface adsorption rate of MNS, as well as the \( \gamma_{\text{min}} \) and \( \gamma_{\text{max}} \) values, was clearly restored. Content of calcium ions, which are known to accelerate the surface adsorption of pulmonary surfactant molecules (2, 7, 12), was negligible in the present dextran solution (<0.003 mM). Water-soluble polymers, including dextran, are known to dehydrate the surface of phospholipid vesicles and to cause the vesicles to aggregate (17). We suppose that these processes may be related to the cause of the present phenomena, because dehydration and aggregation of surfactant vesicles can alter the surface adsorption property (8, 28). This is, however, nothing but a speculation. Further studies are needed to elucidate the mechanism by which dextran restores the albumin-inhibited surface activity of MNS.

Fig. 6. Tidal volumes of immature newborn rabbits receiving mechanical ventilation. ●, DAS group (n = 9), which was given test liquid containing 2.0 mg/ml MNS and 30 mg/ml albumin with 10 mg/ml dextran; ○, AS group (n = 9), which was given test liquid containing MNS and albumin at same concentrations as those for DAS group but without dextran; ×, control group (n = 8), which was given no test liquid. Values are means ± SD. *P < 0.05 vs. AS group. Tidal volumes of both the DAS and AS groups increased significantly with time (P < 0.02).
For treatment of RDS patients, it has been recommended to give an exogenous surfactant in doses of 60 mg/kg body wt, in concentrations of 20 mg/ml, and in fluid volumes of 1–4 ml/kg (5). The dose of MNS given to the animals used in the present study (20 mg/kg), as well as the concentration and the fluid volume, is significantly different from the clinical recommendations. In ARDS, a considerable amount of edema fluid enters the alveolar space and dilutes the surfactant. It is reported in animals with ARDS induced by oxygen poisoning that edema fluid sampled from the airways had a surfactant content of 1.0 mg/ml and an albumin content of 40 mg/ml (11). To imitate these conditions seen in ARDS, we decreased the MNS concentration of the test liquid to 2.0 mg/ml, nearly the lowest concentration at which MNS alone can develop 3 mN/m of γmin by itself (13). Furthermore, we increased the volume of the test liquid to 10 ml/kg and adjusted the albumin concentration to 30 mg/ml, a value close to that seen in the edema fluid of the ARDS animals.

Only a very small tidal volume (<3 ml/kg) appeared in the control group and in the animals given the solution of dextran alone or albumin alone. After comparison of these results with the findings in the DAS and AS groups, we can state that the present immature rabbits were lacking in their own pulmonary surfactant, as reported previously (20). One impressive finding in this animal study was that the tidal volumes of both the DAS and AS groups gradually increased with time of ventilation. In most cases of surfactant-replacement therapy, the PIP of the initial few breaths has been elevated to above 30 cmH2O to enhance the distribution of the administered surfactant. We omitted this maneuver because of the risk of pneumothorax in the immature animals, which had already received a large volume of the test liquid in their lungs. We cannot, therefore, deny the possibility that the distribution process took part in the time-dependent increase of the tidal volumes. At least in regard to the AS group, however, further discussions are needed, because preparations having the γmin, as high as the test liquid given to the AS group (>21 mN/m) have hardly brought about a tidal volume in surfactant-deficient immature rabbits (10, 14, 20).

Before the first breath was drawn, the alveoli of the newborn are filled with 30 ml/kg of fetal lung liquid, a volume that is similar to the functional residual capacity (24). Into these alveoli, we administered 10 ml/kg of the test liquid. This amount of liquid may disturb the ventilation mechanics and may dilute the administered surfactant. These obstacles, however, gradually diminish if the liquid is absorbed by the pulmonary vascular system during the ventilation period. It is known that the surfactant inhibitory action of plasma proteins is abolished at higher surfactant concentrations, even when the protein-to-surfactant molar ratio is the same (6, 7, 21). This process may be another important cause for the appearance and time-dependent increase of the tidal volume seen in the AS group, and also in the DAS group.

The tidal volume of neither the DAS group nor the AS group seemed to have reached the ceiling value during the ventilation period. We, therefore, cannot deny the possibility that the tidal volumes of both the DAS and AS groups would have become larger than those actually seen in the present study, if the animals had been ventilated for a longer duration. We had to terminate ventilation by the 20th min, because prolongation of the period increased the incidence of pneumothorax. Actually, 2 of the 11 animals in the DAS group developed pneumothorax. Dextran itself may not be to blame for this event, because no animals receiving the solution of dextran alone developed pneumothorax. We believe that the pneumothorax was a result of overinflation of the lung in which compliance had been improved by the replaced test liquid. Where a clinical situation is concerned, we should decrease the PIP after the tidal volume has been normalized. Furthermore, the blood-gas levels and the static pressure-volume curve of the lungs were not examined. We can state, however, from the present findings that, in the presence of albumin, MNS exhibits its biological functions significantly faster when dextran is also present, because animals of both the DAS and AS groups were identical ventilated.

Dextran, the molecular weight of which is 40,000–72,000, has been used clinically as a plasma substitute. Almost the largest of these molecular weights (71,000) was used for the present study because, in our preliminary study, dextran of larger molecular weights showed greater ability to restore the albumin-inhibited surface adsorption (unpublished observations). When the permeability of the alveolar-capillary membrane increased, intravenously infused dextran may leak into the alveolar spaces, as albumin does (19), and may counteract the surfactant inhibitory action of albumin. This speculation, however, is based on the findings of the present study. "Complete" pulmonary surfactant has four SPs, which are classified into the hydrophilic type (SP-A and SP-D) and the hydrophobic type (SP-B and SP-C), and shows stronger resistance against inhibitory action than surfactant preparations not having hydrophilic SPs, such as MNS (1, 25, 27). In addition, fibrinogen, fibrin-degradation products, and hemoglobin, which also have surfactant-inhibitory action, are present in the pulmonary edema fluid of ARDS patients (6, 21). Furthermore, calcium ions, which may change the surface adsorption rate of surfactant molecules (2, 7, 12), are found in pulmonary edema fluid. We need further studies to examine whether dextran can restore the activity of complete pulmonary surfactant when this is inhibited by actual edema fluid appearing in the alveoli.

We conclude that dextran (average molecular weight 71,000) restores the albumin-inhibited surface activity of MNS. At the present time, this conclusion is limited to albumin only, of all the surfactant inhibitors. However, we cannot deny the possibility that the effect of dextran on some surfactant inhibitors other than albumin may also be seen. We consider that the underlying mechanisms of the present phenomena are worthy of
further investigation to improve the therapy of patients whose pulmonary surfactant function has been inhibited by plasma-derived proteins.

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REFERENCES


