Elastic Recoil Pressure Arising from Surface Tension in Hamster Lungs Treated with Intratracheal Bleomycin

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Lung elastic recoil pressure arising from surface tension (Ps) was evaluated in normal and bleomycin-treated lungs. Twenty two male golden hamsters were separated into a control group (group A, n = 10) and a bleomycin group (group B, n = 12). Group B was given a single intratracheal instillation of bleomycin and group A was given normal saline as a control. Thirty days after instillation, three cycles of static air- and saline-filled pressure-volume curves (P-V curve) were measured. Ps was graphically derived from air- and saline-filled P-V curves. The Ps of groups A and B were compared at the same lung volumes. Mean body weight and nose-to-tail length of the two groups did not differ significantly. Total lung capacity, defined as lung air volume at a transpulmonary pressure of 25 cmH2O, was significantly smaller in group B (mean ± s.e. was 4.08±0.20 ml) than in group A (mean ± s.e. was 5.23±0.12 ml) (p <0.01). The Ps of group B was significantly larger than that of group A at all lung volumes studied (p <0.01-0.05). These results indicate that the lung elastic recoil pressure component due to the surface tension was increased by intratracheal instillation of bleomycin in hamsters.

pulmonary fibrosis; surface tension; lung pressure-volume curve.

Lungs treated with bleomycin have been used as a model because of their similarity, in many respects, to human idiopathic pulmonary fibrosis (Snider 1978 a, b). The administration of bleomycin is associated with injury to the alveolar epithelium (Aso et al. 1976; Daskal et al. 1976; Adamson and Bowden 1979), of which the type II cells are regarded as the producers of the constituents of a continuous film lining the alveoli. Bleomycin causes a reactive proliferation of type II cells with an overproduction of surfactant (Fasske and Morgenroth 1983), changes in the distribution of type I and type II cells as well as alteration of lamellar inclusion bodies within type II cells (Aso et al. 1976) with possible changes in surfactant or its secretion (Daskal et al. 1976). In view of these pieces of evidence we hypothesized that lung elastic recoil pressure arising from surface
tension (Ps) might change in lungs treated with bleomycin.

Most previous publications describing effects of bleomycin on the pulmonary surfactant system have focused on the morphological or biochemical aspects. The present study was performed to evaluate change in elastic recoil pressure arising from surface tension (Ps), i.e., functional effect of intratracheally-administered bleomycin on the surfactant system, in hamster lungs. Both air- and saline-filled static pressure-volume curves were measured in each animal. The Ps was graphically obtained using the method reported by von Neergaard (1929).

**MATERIALS AND METHODS**

Twenty two normal male golden hamsters at postnatal week seven weighing 69.4 ± 6.8 (mean±s.d.) g were separated into two groups; a control group (group A, n=10) and a bleomycin group (group B, n=12). To group B, a single dose of bleomycin (0.5 mg in 0.5 ml of saline per 100 g body weight) was administered intratracheally under intraperitoneal pentobarbital sodium anesthesia (7 mg/100 g body weight). To group A, a single dose of 0.5 ml of saline per 100 g of body weight was instilled intratracheally. Thirty days after instillation of bleomycin or saline, lung pressure-volume curves were measured.

Pentobarbital anesthesia was followed by tracheal cannulation and tracheal exposure. Tracheal cannulae were made of polyethylene tubing (internal diameter, 1.5 mm; outer diameter, 2.6 mm; length, 5 cm). The cannula had a hump near its tip which seated on the glottis. A ligature was tied around the trachea with the cannula in order to prevent air or saline from leaking. The anterior and lateral sides of the thorax were widely removed. The lung was not removed from the thorax to avoid pleural injury. The vertebral column was severed at the lumbar-thoracic junction. The lung-thorax preparation was degassed in a vacuum chamber for about 10 min until the lung appeared like liver. The chamber contained a beaker of water. The evacuation pressure was finely controlled through a needle valve so that the water in the beaker did not boil.

Immediately after degassing, pressure-volume curves (P-V curves) with air were determined in a semi-closed plastic box containing water. The water was heated so that the air inside was kept at near 37°C, and saturated with water vapor. Lungs were inflated by a series of 5 sec infusions delivered by a glass syringe mounted in a variable-speed infusion-pump followed by a 5 sec stress relaxation time. After the transpulmonary pressure (Ptp) had reached 25 cmH₂O, the lungs were deflated by a series of 5 sec withdrawals followed by a 5 sec stress recovery time. To avoid airway closure, Ptp was not reduced to zero cmH₂O. Three infusion-withdrawal cycles were obtained. The micro-injector operation resulted in lung volume change of 0.33 ml/5 sec. The air volume at the Ptp of 25 cmH₂O was defined as total lung capacity (TLC).

A second degassing was followed by measurement of three infusion-withdrawal cycles with isotonic saline in a bath heated to 37°C. Degassing was performed as described above except that the open end of the tracheal cannula was placed under degassed saline with the animal inclined at an angle of about 20 degrees, head down. Upon repressurization, saline (rather than air) entered the cannula. The tracheal cannula was connected to a fixed stopcock attached to the saline bath. The saline bath was filled up to the level of the tracheal cannula. Therefore, the carcass with the lung nearly in situ floated horizontally in the saline bath. Saline at near 37°C was injected and withdrawn in the same way as for the air P-V curves. The volume of saline to be injected was determined by the previously obtained air P-V curve, i.e., the saline volume injected on the first inflation equaled the air volume injected on the first inflation. The transducer was zeroed at the tracheal cannula level.

The tracheal pressure was monitored through a side arm near the tracheal cannula by
a pressure transducer (TP-101T, Nihon Kohden, Tokyo), and recorded on a direct writing recorder (RECTI-HORIZ 8S, San-ei Sokki, Tokyo). The volume was monitored by a potentiometer (CPP-35, Midori Sokki, Tokyo) coupled to the movement of the glass syringe, and recorded by the recorder described above. Error in the volume measurement was less than 0.04 ml. Air- and saline-filled P-V curves for each animal were constructed by sight-fitting to the pressure and volume points obtained from the last parts of the stress-recovery time on the third deflation limb. Mean air- and saline-filled P-V curves within each group were obtained by averaging the pressures at corresponding absolute lung volumes. Zero volume was set to the volume of the degassed lung. The measured volume for air P-V curves was corrected for gas compression. Ps was obtained by subtracting Ptp on saline-filled P-V curves from Ptp on air-filled P-V curves at each corresponding lung volume in each animal. In the analysis of surface tension, only the region of the P-V curves at lung volumes greater than 50% of total lung capacity were used to avoid the effects of airway closure and gas trapping (Hildebrandt 1978).

Data were subjected to the Student's unpaired t-test for all possible comparisons. Probability values p < 0.05 were considered statistically significant.

RESULTS

Ten animals in group A and twelve animals in group B were subjected to the final analyses. Mean body weight and nose-to-tail length did not differ significantly between the treated and control groups (Table 1). Total lung capacity was significantly larger for the control group than for the bleomycin group (p < 0.01). The comparative analyses between the groups were performed at the lung volumes between 2.5 and 4.0 ml.

In Fig. 1, mean air-filled P-V curves are shown with one standard error bars. Transpulmonary pressures consisting of the tissue component of elastic recoil pressure (Pt) and Ps were significantly higher in the bleomycin group at all lung volumes (p < 0.01 between 2.5 and 3.75 ml, p < 0.05 at 4.0 ml). The means of the saline-filled P-V curves also differed significantly between the two groups. The tissue recoil pressure was one to two cmH2O higher in the bleomycin group at lung volumes between 2.5 and 4.0 ml (Fig. 2) (p < 0.01 between 2.5 and 3.75 ml, p < 0.05 at 4.0 ml).

The means of the Ps are shown in Fig. 3. The mean Ps between 2.5 and 4.0 ml was significantly higher for the bleomycin group than for the control group (p < 0.05 at 2.5, 2.75 and 4.0 ml, p < 0.01 between 3.0 and 3.75 ml).

<table>
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<tr>
<th>Table 1. Body weight, nose-to-tail length and total lung capacity of control hamsters and hamsters treated with bleomycin</th>
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<tr>
<td>Control hamsters (n = 10)</td>
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<td>Body weight (g)</td>
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<td>Length (mm)</td>
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<td>TLC (ml)</td>
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Values are means ± s.e. TLC and length denote total lung capacity and nose-to-tail length, respectively. n is the number of the data.
Fig. 1. Mean air-filled P-V curves. Data points are means±s.e. Open circles represent the control group and closed circles the bleomycin group. Total elastic recoil pressure for the bleomycin group was significantly higher than that for the control group at lung volumes between 2.5 and 3.75 ml (p<0.01) and at 4.0 ml (p<0.05). The number of the data n = 10 for the control group and 12 for the bleomycin group at all lung volumes. Ptp, transpulmonary pressure on air-filled P-V curve.

Fig. 2. Mean saline-filled P-V curves. Data points are means±s.e. Open circles are the control group and closed circles the bleomycin group. Tissue recoil pressure was significantly higher for the bleomycin group than for the control (p<0.01 between 2.5 and 3.75 ml, p<0.05 at 4.0 ml). n = 10 for the control group and 12 for the bleomycin group at all lung volumes. Pt, tissue elastic recoil pressure derived from saline-filled P-V curve.
DISCUSSION

The present study demonstrates that the elastic recoil pressure arising from pulmonary surface tension is significantly higher in hamster lungs treated with bleomycin. There have been several reports concerning bleomycin-induced morphological or biochemical alterations in surfactant secretion systems (Bedrossian et al. 1973; Aso et al. 1976), with possible impairment of surfactant production. However, this is the first paper describing the effects of intratracheally administered bleomycin, which is in wide use for inducing experimental pulmonary fibrosis, on in situ function of surfactant.

Conventionally, Ps has been graphically derived by subtracting Pt obtained on saline-filled P-V curves from Ptp on air-filled P-V curves (von Neergaard 1929). Using this methodology, Picken et al. (1974) reported the effects of corticosteroid on surface tension. In our analysis we assumed that the surface area of the saline-filled lung was the same as that of the air-filled lung at a given lung volume. The assumption of equal surface areas in air- and saline-filled lungs at a given lung volume was criticized (Bachofen et al. 1979; Gil et al. 1979) and it was reported that the difference between Ptp in air-filled lung and Pt obtained in saline-filled lung is the sum of surface tension, which we attempted to obtain, and the tension of tissue distorsion caused by surface tension (Hoppin and Hildebrandt 1977; Wilson 1981). Although this simple interpretation proposed by von Neergaard (1929) is not completely agreed, we believe that this method for obtaining the contribution of surface tension can provide us with useful information about the

![Fig. 3](image_url)  
Fig. 3. A plot of mean Ps (lung recoil arising from the surface tension) against lung volumes. Symbols represent values of the bleomycin group significantly larger than the control group (*p < 0.05, **p < 0.01). n = 10 for the control group and 12 for the bleomycin group at all lung volumes. Open circles denote the control group and closed circles the bleomycin group.
in situ function of lung surfactant.

Surfactant production is enhanced by various chemical and mechanical stimulants. Aso et al. (1976) reported a significant increase in total phospholipids in alveolar lavage fluid in mice treated with intraperitoneal bleomycin. This may indicate that dipalmitoyl-phosphatidyl-cholin, the principle component of surfactant, was also increased in the bleomycin treated lung, although total phospholipid content is not the perfect index of the amount of active surfactant. In a fibrotic lung where severely or mildly involved areas coexist, all alveoli are not evenly inflated. Some alveoli may be deformed by the stress applied by the surrounding lung (Mead et al. 1970). When these deformed alveoli have type II cells capable of producing surfactant, phospholipid release might be stimulated by distension of type II cells (Nicholas and Barr 1981). These two mechanisms discussed above were probably active in the lungs of the bleomycin group in the present study, with a resultant increase in surfactant in the alveolar spaces.

Lamellar bodies, the major storage sites of surfactant phospholipid, are also increased by bleomycin. Aso et al. (1976) reported an increase in the number of lamellar bodies within type II cells in mice given bleomycin intraperitoneally. Unpublished data from our laboratory also shows that both the number of lamellar bodies per type II cell and the volume density of lamellar bodies were significantly increased in hamsters thirty days after being given bleomycin intratracheally, compared with the control. We do not clearly understand what this increase in the number and volume of lamellar bodies within type II cells means. However, for the present time it is not unreasonable to understand this as increase in surfactant materials in the alveolar spaces.

If the two observations discussed above, the increase in total phospholipid and increase in the number and volume density of the lamellar bodies caused by bleomycin, imply the increase of functionally intact surfactant in the alveolar spaces, $P_s$ should be lowered in bleomycin group. However, the result was reverse. Then the present results may suggest that the surfactant secreted into the alveolar spaces became less potent for some reason. Surfactant might be deactivated by substances released from damaged alveolar tissues (Taylor and Abrams 1966; Colacicco et al. 1976) or free radicals produced by bleomycin (Burger et al. 1979; Sugiura 1979).

References