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Lubricating recovery of damaged pleural mesothelium: effect of time and of phosphatidylcholines

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ABSTRACT

Effect of time and phosphatidylcholines (PCs) on lubrication of damaged mesothelium has been investigated. Marked increase in coefficient of kinetic friction (μ) of pleural specimens after mesothelial blotting and rewetting decreased by 23.4 ± 3.5%, 41.8 ± 3.8%, and 40.5 ± 2.7% after 30 min, 1 h, and 2 h. Hence, damaged mesothelium is able to partially reset lubricating molecules on its surface. Increase in μ of post-blotting Ringer 2 h after addition of unsaturated PCs (3 mg/ml) decreased a little more than after 2 h Ringer. Effects of unsaturated and saturated PCs were similar, contrary to expectation raised by their different percentage in pleural and alveolar lavage. Effect of PCs did not increase at 6 mg/ml, and was nil at 0.4 mg/ml. Increase of μ after short phospholipase treatment decreased by 45.9 ± 2.0% after 2 h Ringer, and a little more after addition of unsaturated or saturated PCs. Hence, PCs, as other phospholipids, have a small effect, likely because of difficulty in resetting their relationships with main lubricating molecules.

1. Introduction

Mills et al. (2005, 2006) provided evidence that the percentage of the various phosphatidylcolines (PCs) species in pleural lavage of dogs and cats are quite different from those in bronchoalveolar lavage. Moreover the sample of pleural lavage of Mills et al. (2005, 2006) was obtained quickly (30s), without contact with air, while in the earlier research by Hills et al. (1982) the sampling of pleural lavage was preceded by a pneumothorax. In the liquid from the bronchoalveolar lavage of Mills et al. (2005, 2006) the main component was dipalmitoil-phosphatidylcoline (DPPC, 59 and 68% in dogs and cats, respectively), a saturated PC with strong surface action (Brown, 1964). Instead, in the liquid from the pleural lavage the main component was stearoyl–linoleoyl–phosphatidylcholine (SLPC, 58 and 46%), an unsaturated PC absent in alveolar liquid; then, there were other unsaturated PCs: PLPC (17 and 31%) and POPC (16 and 13%), and a nearly negligible percentage of DPPC (Mills et al., 2005, 2006). On the whole, the high percentage of SLPC in the pleural lavage, and its lack in the bronchoalveolar lavage, leads to think that SLPC plus other unsaturated PCs may have important boundary lubricating properties in pleural mesothelium.

In a previous research (Bodega et al., 2012) we had made a rough attempt to check whether the addition for 2 and 1/2 h of a mixture of phospholipids without unsaturated PCs (Hills, 1989) was able to reduce the marked increase in the coefficient of kinetic friction (μ) of the pleural mesothelium specimen occurring after its blotting with filter paper, and rewetting with Ringer solution, a condition in which most of the molecules relevant for boundary lubrication should have been removed or damaged (Bodega et al., 2012). The decrease was substantial, particularly if one consider that this mixture of phospholipids might not be appropriate according to the findings of Mills et al. (2005, 2006). It seems, therefore, important to determine whether a mixture of phospholipids rich in unsaturated PCs (particularly SLPC) decreases the marked increase in μ, occurring in post-blotting Ringer, more than the mixture of phospholipids without unsaturated PCs at the same concentration. On the other hand, in order to investigate the functional role of the above mentioned differences in pleural PCs it is important to recall that the spatial relationships between sialomucin and phospholipids on the surface of pleural mesothelium are not known. The former, which has been shown to provide good boundary lubrication (Bodega et al., 2012, 2013), is on the surface of the glycocalyx of pleural mesothelium (Ohtsuka et al., 1997), while the latters, which according to Hills (1992) samples are adjacent to the mesothelial cells, might also occur in other parts of the glycocalyx, as suggested by lamellar layers in the glycocalyx of peritoneal mesothelium (Dobbie and Anderson, 1996).

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The purposes of the present research are the following. (1) To check whether μ of the pleural specimens changes during a period of 2 h after their blotting and rewetting. (2) To determine whether, 2 h after the addition of a mixture of phospholipids rich in unsaturated PCs, the marked increase in μ occurring in post-blotting Ringer decreases more than after the addition of a mixture without unsaturated PCs at the same concentration. To repeat this comparison at another concentration. (3) To determine some of these comparisons in pleural specimens after short phospholipase treatment, which produces a marked increase in μ like blotting, but with a different mechanism (Bodega et al., 2014).

2. Methods

Lung and diaphragm were obtained from 26 rabbits (2.6–3.5 kg b.w.). Animal experimentation was authorized by the Ministry of Health by decree N. 60/03A issued according to Order of the Executive 116/92, in compliance with Directive 86/609/EC. Rabbits were anesthetized with an intravenous injection of 2 ml/kg of a mixture of pentobarbital sodium (Sigma, 12 mg/ml) and urethane (Sigma, 150 mg/ml). They were then heparinised (0.1 mg/kg) and killed by exsanguination. After removal of the skin and superficial muscles, the antero-lateral sides of the rib cage, the lungs (with closed trachea), and the diaphragm were removed, and kept at room temperature (21–28 °C) in Ringer bicarbonate solution (in mM: Na^+ 139, K^+ 5, Ca^{2+} 1.25, Mg^{2+} 0.75, Cl^- 119, HCO_3^- 29, D-glucose 5.6) through which 95% O_2 and 5% CO_2 was continuously bubbled.

The apparatus used to measure the frictional force was that described by D'Angelo et al. (2004). It consists of a sliding platform connected through unextensible threads to the core of a differential transformer, and of a balance arm held stationary at its fulcrum by a force transducer. The specimen of diaphragm was fixed with the pleural surface facing upwards to the sliding platform, while that of the lung was fixed with the pleural surface facing downwards to a perspex rod attached to one end of the balance arm. The balance arm was held horizontal, and counterweights added to its other end enabled to change the normal force applied to the tissue from ~0.5 to ~8 g, corresponding to a pressure on the mesothelium from ~0.8 to ~12.9 cm H_2O. The frictional force on the direction of motion was measured by the force transducer. The coefficient of kinetic friction (μ) was computed as the slope of the relationship between the load and the frictional force recorded in the central 40% of the excursion of the sliding platform (Bodega et al., 2013). The sliding velocity was 1.9 cm/s.

For the reasons outlined under Introduction, we first checked whether μ of the pleural specimens changed 2 h after its blotting and rewetting (Bodega et al., 2012) or short phospholipase treatment (Bodega et al., 2014), two maneuvers which produce marked increase in μ and imply damage on the surface of pleural mesothelium. Measurements of μ were made at room temperature (21–28 °C) under the following sequence of conditions. (1) Control with Ringer bicarbonate solution; (2) after mesothelial blotting with filter paper, and rewetting with Ringer, or after short phospholipase treatment (see below); (3) after 2 h incubation in Ringer (see below); (4) after washout with Ringer. Short phospholipase treatment was achieved by adding to the specimens a solution with 7.5 U/ml of phospholipase C (SIGMA P7633), which was blocked after 10 min by ethylendinitrotetracetic acid (EDTA) 5 mM in phosphate buffer (PBS, in mM: Na^+ 144, K^+ 4, Cl^- 131, $H_2PO_4^-$ 1.5, HPO_4^{2-} 8, D-glucose 5.5), and washed out with Ringer (Bodega et al., 2013). For the incubation period a specimen of muscular diaphragm, pinned on a flat cork with the pleural surface facing upwards, was kept in a container immersed in Ringer solution (bubbled with 95% O_2 and 5% CO_2) up to a level that was just enough for its liquid surface to contact the upper border of the cork. The specimen was then covered by Ringer, and the piston with a lung specimen was placed on it. The container and the specimens were then covered by a plastic sheet. After 2 h the piston with its lung specimen was brought back to the balance arm, while the cork with its diaphragm specimen was fixed to the sliding platform, and μ measurements were performed. Measurements of μ of pleural mesothelium were also performed 30 min, and 1 h after its blotting and rewetting.

To study the effect of phospholipids the sequence of experimental conditions was similar to that mentioned above, except for the incubation period in which a mixture of phospholipids was placed on the specimens, instead of Ringer. The following phospholipids mixtures were used. (1) Rich in unsaturated phosphatidylcholine (PCs): stearoyl–linoleoyl–phosphatidylcholine 45%, palmitoyl–linoeyl–phosphatidylcholine 22%, palmitoyl–oleoyl–phosphatidylcholine 13% (AVANTI, 850468, 850458, and 850457, respectively), phosphatidyl–ethanolamine 13%, and sphingomyelin 7% (Sigma, P1348, and S7004, respectively). The percentages of PCs of this mixture were mainly based on the findings of Mills et al. (2005, 2006). Unfortunately, Mills et al. (2005, 2006) did not provide information on other kinds of phospholipids occurring in the pleural lavage. Therefore, for these the only data available are those from Hills et al. (1982), with the limitation mentioned under Introduction, and those from samples of pericardium liquid lavage by Hills and Butler (1985) which seem to have been obtained in a way more close to that of Mills et al. (2005, 2006). We used, however, only half of the percentages found by Hills et al. (1982), because their sample is less close to physiological conditions than that of Mills et al. (2) Rich in saturated PCs: dipalmitoylphosphatidylcholine 49%, dipalmitoylphosphatidylethanolamine 32%, and sphingomyelin 18% (Sigma, P0763, P1348, and S7004, respectively), i. e., similar to that previously used (Bodega et al., 2012) according to Hills et al. (1982). (3) Without PCs: phosphatidyl–ethanolamine 65%, and sphingomyelin 35%. This mixture was used only in the experiments in which the mesothelium was blotted. The concentrations of phospholipids in the suspensions used were: 0.4 mg/ml, 3 mg/ml, or 6 mg/ml in the experiments in which the mesothelium was blotted, and 3 mg/ml in the experiments with short phospholipase treatment.

Occasionally (in both series of experiments, without and with phospholipids) the value of μ after final washout with Ringer was greater than that after blotting and rewetting or after the short phospholipase treatment. This likely indicates that the surface of the pleural mesothelium underwent a damage during the incubation period. To be sure to eliminate these cases we considered only the experiments (more than 90%) in which μ of post-blotting Ringer, or post-phospholipase Ringer, was at least 0.003 (i.e. 3.4-4.1%) higher than that of final Ringer washout.

Linear regressions between frictional force and load were computed with the least squares method and statistical assessment was made by covariance analysis. The results are presented as mean ± S.E. Statistical significance of group mean values was assessed by analysis of variance. The level of significance was taken at $P < 0.05$.

3. Results and discussion

3.1. Time effect

In Table 1 are reported the mean values ± SE of the coefficient of kinetic friction (μ) of the pleural mesothelium under the following conditions: (1) control, (2) after mesothelial blotting with filter paper, and rewetting with Ringer solution (post-blotting Ringer), (3) 2 h later, (4) after washout with Ringer. The value of μ of post-blotting Ringer after 2 h decreased by 0.026 ± 0.002 ($P < 0.01$);