Surface Activity of Phosphatidylcholines as the Basis for Surfactant Replacement Therapy

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Using the methods of surface balance we compared the effects of different phosphatidylcholines and synthetic surfactant Exosurf on the equilibrium surface tension at the air-water interface. It was found that dipalmitoyl-lecithin, the principal component of natural surfactant, and its synthetic substitutes can be replaced by other phosphatidylcholines, in particular, by modified soybean phosphatidylcholine phospholipon. It was demonstrated that equilibrium surface tension is an informative characteristic in the assessment of potential components for synthetic surfactants.

Key Words: *surface tension; surfactant; phosphatidylcholine; dipalmitoyl-phosphatidylcholine; Exosurf*

Dipalmitoyl-lecithin (DPL) is the major lipid component of aleveolar surfactant (SF) responsible for reduction of surface tension (ST). During expiration SF monolayer contracts together with alveoli, its proteins and unsaturated phospholipids come down to hypophase and contracted monolayer consists almost exclusively of DPL [3]. At 37°C it forms a dense film reducing ST at the air-water interface almost to zero [3,6]. Natural SF proteins facilitate spreading, absorption, and stabilization of the monolayer along the air-water interface [3,7].

The absence of SF in severe pulmonary pathology (respiratory distress-syndrome) can be compensated by SF-replacement therapy [6]: exogenous SF is sprayed into the airways and forms a film in alveoli similar to that formed by DPL. In Russia, there are no SF substitutes ready for use (the development of such preparations on the basis of an amniotic fluid is now under way [2]) and patients are treated with Exosurf (Wellcome), a very expensive protein-free drug containing synthetic DPL. Protein-containing natural substitutes must be carefully purified and sterilized and are still more expensive [5]. Taking the above into account it was reasonable to investigate the possibility of developing a new, available protein-free drug for SF substitution therapy.

When searching for a compound capable of substituting expensive DPL in synthetic SF, we compared ST of Exosurf and different phosphatidylcholines (PC).

MATERIALS AND METHODS

ST was measured at 37°C by Wilhelmy technique using a platinum plate with a K 10ST Digital tensometer (Kruss GMBH). Test lipid aliquote (2.28 mg/ml dissolved in 2-5 μl chlorophorm:ethanol) was carefully layered on 0.9% NaCl (15.2 cm^2) and changes in ST were recorded.

Equilibrium ST (ST_e) was determined as the lowest constant ST value and expressed in mN/m [1,9].

We compared the properties of chromatographically pure yolk PC (Kharkov), soybean PC (Nattermann), DPL (Sigma), phospholipon (Rhone-Polenec Rorer), and Exosurf (Wellcome).

RESULTS

ST_e was measured using optimal surface concentration of PC on salt hypophase determined in preliminary

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experiments with yolk PC tested in concentrations of 0.30, 0.45, and 0.75 μg/cm². During the first seconds after application of PC on the surface of NaCl water solution, ST decreased below the initial value (70 mN/m) recorded at 37°C from pure water surface. Starting from PC concentration of 0.45 μg/cm², ST reached a plateau (ST_e=25 mN/m) and further increase in PC concentration had no additional effect on this parameter (Fig. 1), which corresponded to monolayer saturation. It can be assumed that the excess of lipids formed drops which did not affect ST_e. This optimal concentration (0.45 μg/sm²) was used in the experiments with other PC.

DPL and phospholipon were characterized by the lowest ST_e (21.7±0.34 and 21.4±0.32 mN/m, respectively), which suggests their similar efficiency as the components of SF preparation. ST_e for yolk and soybean PC (25.2±0.18 and 26.6±0.18 mN/m, respectively) although significantly higher, also fall within the physical limits of ST_e for noncontracted monolayer [3.4]. Exosurf was characterized by intermediate ST_e (23.7±0.32 mN/m), hence it formed a film with higher ST_e than its component DPL, which can be attributed to the effects of its other components hexadecanol and tyloxapol improving spreading and absorption of DPL.

To assess the possibility of replacing DPL (at least, partial) with more available PC, we analyzed different PC mixtures. ST_e was directly proportional to the content of each component in the mixture showing no "shielding' effects (Fig .2). It implies that substitution of DPL in SF with more unsaturated PC would increase ST_e and therefore decrease the therapeutic effect of the preparation, which is not acceptable.

At this stage of the analysis, phospholipon, a relatively inexpensive product of natural soybean lecithin containing 86% stearic and 14% palmitic acids offers the best prospects. However, it is necessary to investigate all its properties before making the conclusion that it can substitute DPL in artificial SF. ST_e can be used as an initial criterion for assessing potential substances in SF replacement therapy.

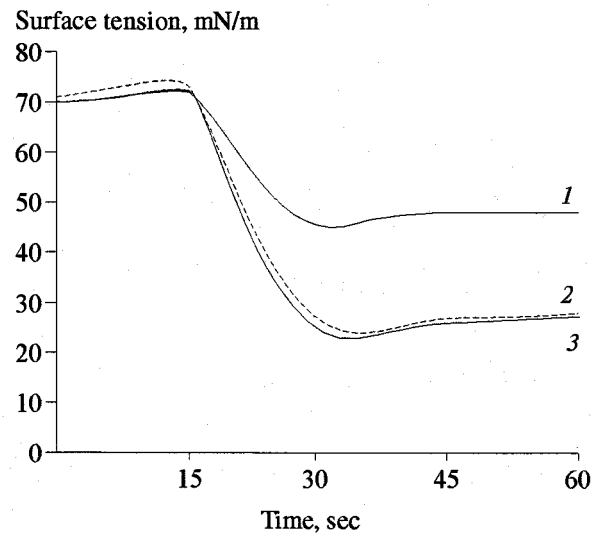

Fig. 1. Effect of yolk phosphatidylcholine in concentrations of 0.30 (*1*), 0.45 (*2*) , and 0.75 (*3*) μg/cm² on equilibrium surface tension.

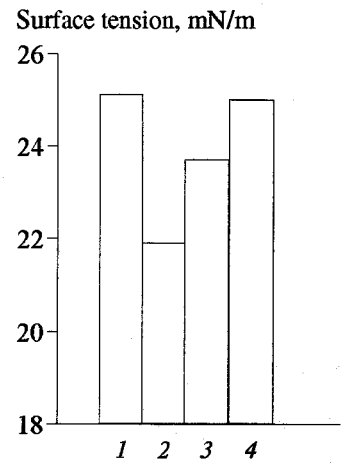

Fig. 2. Equilibrium surface tension of yolk phosphatidylcholine (YPC) and dipalmitoyl-lecithin (DPL) mixtures. *1)* YPC; *2)* DPL; *3)* YPC:DPL (1:1); *4)* YPC:DPL (4:1).

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