Comparison of Distribution Characteristics of Phosphatidylcholines and Surfactant Preparation

T. S. Zakharova, E. S. Fortinskaya, M. M. Kochetova, T. I. Torkhovskaya, and E. M. Khalilov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 129, No. 4, pp. 411-413, April, 2000
Original article submitted May 27, 1999

Using the methods of surface balance we compared surface activity of different natural phosphatidylcholines. Dipalmitoylphosphatidylcholine, the principal component of natural surfactant, is characterized by less rapid spreading on the air-water interface compared to unsaturated yolk and soybean phosphatidylcholines, while the absolute rate of surface tension reduction was the same for all test lecithines. The surface activity of modified saturated soybean phosphadtidylcholine (Phospholipon) was similar to that of dipalmitoylphosphatidylcholine. The relationship between saturation of fatty acids in the molecule of phosphatidylcholine and the time of spreading on the air-water interface revealed in this study allows to use this parameter together with equilibrium surface tension for evaluation of surface activity when testing synthetic surfactant constituents.

Key Words: *surface tension; surfactant; phosphatidylcholine; dipalmitoylphosphatidylcholine; Exosurf*

Protein-free surfactants for replacement therapy of respiratory distress-syndrome are based on expensive synthetic dipalmitoylphosphatidylcholine (DPPC) [2], the principal component of natural pulmonary surfactant. DPPC is resistant to oxidation, possesses surface activity properties, and contains specific proteins facilitating its spreading and absorption, which ensure function of natural surfactant. Protein-free surfactants for replacement therapy can be developed not only by imitating natural compounds. Similarly to DPPC, exogenous surfactant should form a lipid film and this effect dependson biophysical and spreading factors differing from those acting *in vivo* [2]. Surfactant film are usually evaluated *in vitro* by surface absorption and spreading, film contractility, and related changes in surface tension (ST) during contraction cycle [2].

We have previously found that equilibrium surface tension (ST_E) represents an informative parameter for comparative assessment of surface properties of different phosphatidylcholines (PC) [1]. In this study we further compared natural PC, DPPC and Exosurf.

Laboratory of Biochemistry and Autoanalysis, Institute of Physicochemical Medicine, Russian Ministry of Health, Moscow. ***Address for correspondence:*** tz-post@mail.ru. Zakharova T. S.

MATERIALS AND METHODS

ST was measured at 37°C by the Wilhelmy technique on a K10ST Digital tensometer (Kruss GmbH) using a platinum plate. An aliquote containing 6.84 μg PC in 3 μl chlorophorm:ethanol (9:1) mixture was carefully layered onto 0.9% NaCl in a round container with a 4.4 cm^2 (0.45 $\mu g/cm^2$) surface area [1], and changes in ST were recorded for 30 sec. Measurements were stopped after ST_E was achieved.

ST reduction rate was expressed in mN/m/sec.

Surface spreading time (SST), *i. e.* the time from lipid application onto salt hypophase to ST_E was determined for each PC.

We compared the properties of chromatographically pure yolk PC (Kharkov), soybean PC (Nattermann), DPPC (Sigma), Phospholipon (Rhone-Poulenc Rorer), and Exosurf (Wellcome).

0007-4888/00/0004-348$25.00

RESULTS

All test lipids caused a linear decrease in ST within the entire SST. Figure 1 shows a typical ST reduction curve. After application of Phospholipon, ST decreased for 16 sec with a constant rate of 3 mN/m/sec from the initial value of 70 mN/m (ST of saline at 37°C) to 21.4 mN/m (ST_E for phospholipon [1]).

The rate of ST reduction was the same (3 mN/m/sec) for all test lipids. This index did not depend on the level of fatty acid saturation and remained unchanged even after addition of Exosurf components tyloxapol and hexadecanol to DPPC.

The longest SST (16 sec) was shown by DPPC and Phospholipon. Exosurf displayed an intermediate (15 sec), while yolk and soybean PC the shortest (14 sec) SST. Although DPPC and Phospholipon were characterized by the lowest ST_E values (21.7 and 21.4 mN/m, respectively) [1], they exhibited the longest SST. Since the rate of ST reduction was the same for all PC, these low ST_E were attained due to prolonged SST. Low ST_E of DPPC seems to be a more important parameter for its functioning as the basis of surfactant than long SST. Therefore, we found an inverse relationship between ST_E and SST, which can explain the discrepancy between the therapeutic potency and surface activity of surfactants revealed in *in vivo* tests [3].

Exosurf was characterized by intermediate SST. As shown previously, this drug is characterized by intermediate ST_E between the values typical of saturated and unsaturated PC [1], which can be attributed to the presence of other components in Exosurf.

It should be noted that there is no universal index characterizing surfactant spreading on the air-water interface. The commonly used term, the rate of spreading along the surface [6], means either the proper rate of ST reduction after application of test substance [6] or the time until ST decrease to the value 2 mN/m above ST_E [4]. Sometimes the process of surfactant spreading is characterized by a conventional parameter, such as ST value attained during certain interval [5]. Taking into account the ambiguity of these parameters characterizing different aspects of surfactant spreading, we measured both the rate of ST reduction after lipid application to the surface of salt hypophase and SST. It was found that the rate of ST reduction was constant irrespective of tested lipids, while SST

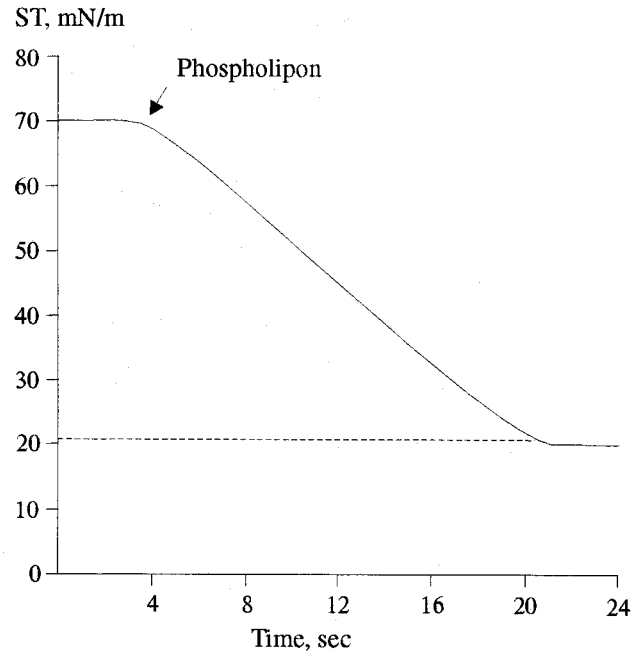

Fig. 1. Changes in surface tension (ST) after Phospholipon application on salt hypophase. Dotted line: equilibrium ST.

depended on fatty acid saturation, and apart from ST_E characterized their surface activity.

Therefore, not only ST_E, but also SST reflect the difference in surface activity properties of PC determined by different lipid composition. The relationship between PC fatty acid saturation, SST and ST_E allows us to use both parameters for assessment of surface activity properties of components of artificial surfactant.

REFERENCES

1. T. S. Zakharova, E. S. Fortinskaya, M. M. Kochetova, *et al., Byull. Eksp. Biol. Med.*, **129,** No. 2, 168-170 (1999).
2. T. Fujiwara and B. Robertson, *Pulmonary Surfactant,* Eds. B. Robertson *et al.,* Amsterdam (1992), pp. 561-592.
3. K. Nohara, P. Berggren, T. Curstedt, *et al., Eur. J. Respir. Dis.,* **69,** 321-335 (1986).
4. B. Sun, *Experimental Studies of Neonatal Surfactant Dysfunction and Surfactant Therapy in Meconium Aspiration Syndrome,* Stockholm (1993), pp. 8-15.
5. A. Takahashi, T. Nemoto, and T. Fujiwara, *Acta Paediatr, Jpn.,* **36,** 613-618 (1994).
6. J. Tanaka, T. Takei, and T. Aiba, *J. Lipid Res.,* **27,** 475-485 (1986).