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Pulmonary Uptake of Liposomal Phosphatidylcholine upon Intratracheal Administration to Rats

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Liposomes composed of dipalmitoylphosphatidylcholine (DPPC) labeled with L-α-[dipalmitoyl-1-^{14}C]-phosphatidylcholine ([^{14}C]-DPPC) and cholesterol (molar ratio 7:2) were injected into rats intratracheally. The lungs took up about 90% of administered [^{14}C]-DPPC and 50% of liposomal DPPC was retained for over 24 h. In contrast, uptake by the liver and kidney after intravenous administration was high and uptake by the lung was negligible. Uptake of [^{14}C]-DPPC by lung lamellar bodies was higher than that by other lung subcellular fractions (mitochondria, microsomes and soluble fractions). From the results of histochemical investigation, it was found that the liposomes were taken up uniformly by the lung.

Keywords——liposomes; dipalmitoylphosphatidylcholine; pulmonary uptake; neonatal respiratory distress syndrome; lamellar body; horseradish peroxidase

Neonatal respiratory distress syndrome (RDS) is related to alveolar lining surfactant deficiency.[1] The major lipid component of this surfactant is phosphatidylcholine (PC), especially dipalmitoylphosphatidylcholine (DPPC).[2] This disaturated PC is stored in the lamellar bodies of type II alveolar epithelial cells. It is then secreted to participate in forming a surface-active lining at the air-alveolar surface that stabilizes the lung and aids respiration.[1] At present, the usual treatment approach for RDS is supportive neonatal intensive care methods,[1] in conjunction with assisted ventilation and end positive pressure for hypoxia and ventilatory failure. Other approaches have recently been attempted, *i.e.*, to deposit surfactant onto the alveolar surface by tracheal instillation, using positive ventilation of aerosolized PC extracted[3] from lungs and synthetic PC.[3b]

It is well known that phospholipid, when suspended in an excess of aqueous solution, spontaneously forms liposomes.[4] Liposomes have been widely used as drug carriers for targeting various substances such as enzymes, hormones and antitumor drugs in the treatment of disorders.[5]

In the present study, we tested the pulmonary uptake of DPPC administered intratracheally and the maintenance of DPPC in the lung as a preliminary experiment to evaluate the possibility that administration of supplementary surfactant might serve as a prophylaxis against RDS.

Experimental

Materials——L-α-[Dipalmitoyl-1-^{14}C]-phosphatidylcholine ([^{14}C]-DPPC) was purchased from Japan Radioisotope Association (Tokyo, Japan). Horseradish peroxidase (HPO) and DPPC were obtained from Sigma Chemical Co. Cholesterol (CHO) was the product of Wako Pure Chemicals. All other chemicals were commercial reagent-grade products.

Preparation of Liposomes——Liposomes were prepared from combinations of DPPC and CHO (molar ratio DPPC: CHO, 7: 2), using the method described by Bangham *et al.*[6] The lipid containing 30 μmol DPPC labeled with [^{14}C]-DPPC (0.4 μCi) and 8.67 μmol CHO were dissolved in a 100 ml round-bottomed flask and, after evaporation, the lipid layer of the wall of the flask was dispersed with 4 ml of buffered saline solution (pH 7.1) by mechanical shaking with a vortex mixer (Thermonics Co., Ltd.) for 2 h. This suspension was sonicated using a 2.6 cm titanium probe at 250 μA in a sonicater (Nihonseiki, Model G50022-4) for 1 min in an ice-bath. The suspension was shaken, and separation of liposomes was effected by centrifugation at 105000×*g* for 90 min.

Administration——Liposome suspension (0.1 ml) labeled with [^{14}C]-DPPC was administered directly into the respiratory systems of rats by a modification of the procedure described by Enna and Shanker.[7] Male Wistar rats, weighing approximately 150 g, were anesthetized with pentobarbital, and a cannula was inserted into the trachea in each rat. Liposomes were given as an intratracheal bolus by means of a syringe needle-plastic tubing arrangement positioned so that the tip of the tubing was at the tracheal bifurcation.

Liposome Distribution and Uptake by Lung Subcellular Fractions——The anesthetized animals were killed by decapitation at intervals after administration of the liposomes, and several tissue (lung, liver, kidney, spleen and heart) were removed and weighed wet. [^{14}C]-DPPC levels in isolated tissues were measured with an Aloka LSC-704 liquid scintillation spectrometer after being treated with Protosol (New England Nuclear) and diluted directly with a scintillator (Aquasol 2, New England Nuclear). [^{14}C]-DPPC levels in the lung subcellular fractions (lamellar bodies, mitochondria, microsomes and soluble fractions) were also measured by the method described above. One gram of lung was homogenized in 3 ml 0.33 M sucrose–0.01 M Tris-HCl buffer (pH 7.4) in a Potter–Elvehjem homogenizer. The homogenate was filtered and mixed with 3 volumes of 0.25 M sucrose solution. Then, it was isolated by the sucrose gradient method[8] and analyzed for [^{14}C]-DPPC.

Histochemical Study——The distribution of liposomes within the lung was visualized as follows. Liposomes containing HPO, an enzyme that can be used to generate a histochemical stain, were prepared.[9] Liposomes containing HPO were intratracheally administered to rats. After 5 min, the animals were killed, then the lungs were excised and frozen quickly. Sections were cut across the largest frontal diameter of the lung at a thickness of 8 μm and stained with 3,3-diaminobenzidine-tetrahydrochloride by a modification of the method of Kaplow.[10]

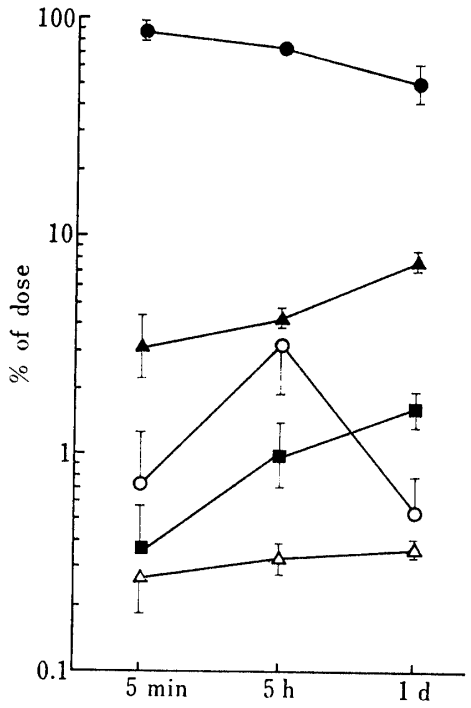

Fig. 1. Distribution of Liposomal [^{14}C]-DPPC at 5 min, 5 h and 1 d after Pulmonary Administration to Rats

●: lung, ▲: liver, ○: spleen, ■: kidney, △: heart.

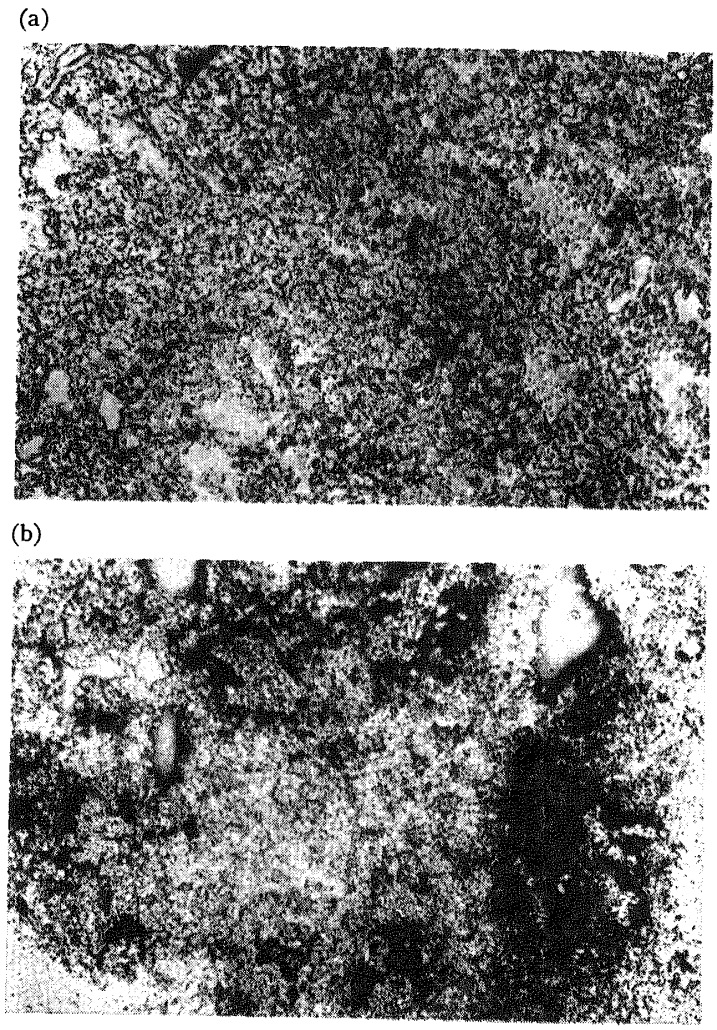

Fig. 2. Distribution of HPO-containing Liposomes in the Lung of Rats 5 h after Administration (Magnification × 100)

(a) No HPO administered.
(b) Liposomes with 10 mg HPO administered.

Results

Fig. 1 showed the uptake of liposomal [^{14}C]-DPPC by various tissues after intratracheal administration. It is clear that about 90% of administered [^{14}C]-DPPC was accumulated in the lung at 5 min after administration. In addition, experiments with liposomes containing HPO indicated that there was intense and widely distributed HPO staining lining the airways of the rats (Fig. 2). At 5 h, 77.9% of [^{14}C]-DPPC remained and even after 24 h, 51.4% of [^{14}C]-DPPC was retained in the lung. The redistribution to the liver, kidney and heart increased with time but that of the spleen increased up to 5 h and then decreased. In contrast with the accumulation in the lung, redistribution to other tissues was very minor.

In contrast, the distribution to various tissues after intravenous administration is shown in Fig. 3. As shown in Fig. 3, it was found that the liver took up a large amount of the administered [^{14}C]-DPPC and other endothelial cells such as kidney and spleen took up more than the heart and lung. This means that the liposomes tend to be taken up by the endothelial systems, in accord with the results of other workers.[11] The uptake by the lung was very small and distribution to the lamellar bodies after intravenous administration was less than that to other subcellular fractions such as mitochondria, microsomes and soluble fractions (these results are not shown).

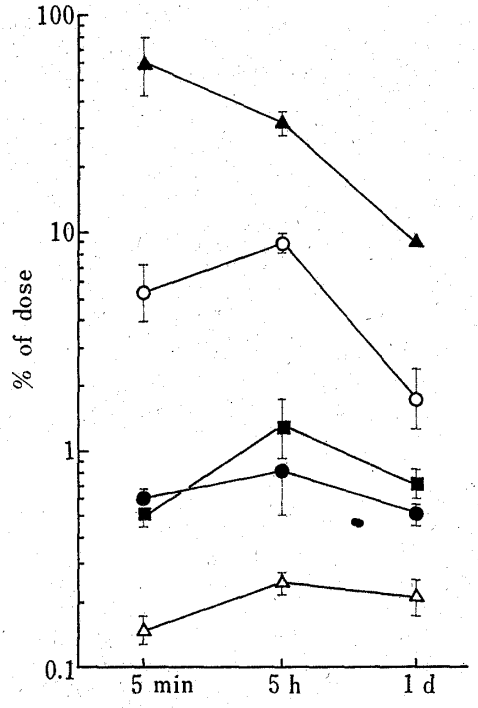

Fig. 3. Distribution of Liposomal [^{14}C]-DPPC at 5 min, 5 h and 1 d after Intravenous Administration to Rats

Key: as in Fig. 1.

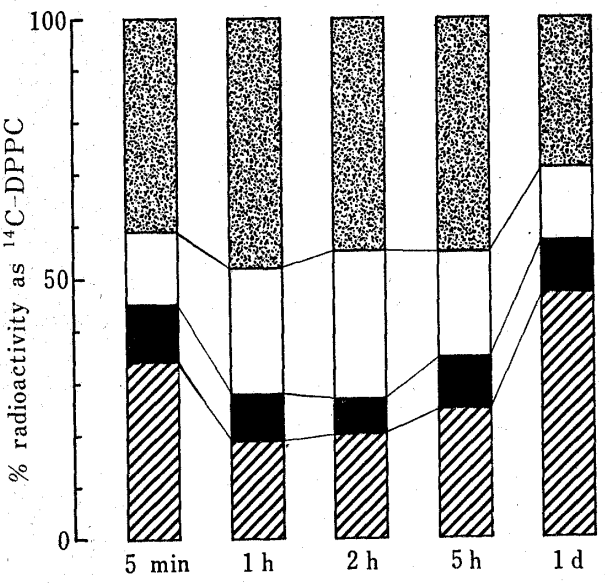

Fig. 4. Uptake of Radioactivity in Lung Subcellular Fractions derived from Liposomal [^{14}C]-DPPC

: lamellar bodies.
: mitochondria.
: microsomes.
: soluble fraction.

Figure 4 shows the uptake of liposomal [^{14}C]-DPPC into the lung subcellular fractions after intratracheal administration. Originally, lung surfactant is secreted to the air-alveolar surface from the lamellar bodies of type II alveolar epithelial cells. Lamellar body liposomal [^{14}C]-DPPC uptake was 40—50% of that in all lung subcellular fractions except at 24 h (28.0%). Though the distribution to other fractions (mitochondria, microsomes and soluble fractions) was smaller than that to the lamellar bodies, uptake by soluble fractions at 24 h (47.5%) was comparable to that by lamellar bodies.

Discussion

As the development of RDS is probably due to a prenatal deficiency of surfactant, one form of treatment might be the administration of surfactant through the airways. To date, few studies have been done on pulmonary uptake of liposomes,[12] and little work has been done on the delivery of liposomes to the lung subcellular organelles except for the report of Zachman and Tsao.[12a]

We demonstrated that intratracheally administered liposomal DPPC could be taken up by the lung, especially lung lamellar bodies (Figs. 1 and 4).

Preliminary experiments with liposomes containing HPO as a histochemical tracer indicated that HPO staining material was widely distributed in the bronchi, bronchioles and alveoli in rats given liposomes containing HPO, and these results agree with the results of McCullough *et al.*[13] (Fig. 2) on the distribution of liposomes administered intratracheally in the lung. The amount of [^{14}C]-DPPC taken up by the lung after intratracheal administration was larger than that after intravenous administration (Figs. 1 and 3). As shown in Fig. 1, when liposome suspension was intratracheally administered, 51.4% of [^{14}C]-DPPC administered was retained in the lung even at 24 h. Liposomes can be maintained in the lung continuously by intratracheal administration, and the elimination from the surface of the lung was slow. Uptake to the lung subcellular fraction may increase the content of lung surfactant, as desired. It appears that intratracheal administration is superior to *i.v.* administration in the lung uptake of liposomal [^{14}C]-DPPC.

The results of the present study suggest that the intratracheal administration of supplementary surfactant, liposomal phospholipid, might serve as a prophylactic treatment against RDS. We next intend to determine whether the administration of liposomal phospholipids into the trachea can restore the pressure-volume characteristics of the lung with RDS, and this work is in progress.

References and Notes

1) P.M. Farrell and M.E. Avery, *Am. Rev. Respir. Dis.*, **111**, 657 (1975).
2) T.N. Finley, T.E. Morgan, H.C. Fialkow, and G.L. Huber, *Fed. Proc.*, **23**, 156 (1964).
3) *a*) B. Robertson and G. Enhorning, *Lab. Invest.*, **31**, 54 (1974); *b*) M. Ikegami, T. Hesterberg, M. Nogami, and F.H. Adams, *Pediat. Res.*, **11**, 178 (1977); *c*) R. Nilson, G. Grossmann, and B. Robertson, *Pediat. Res.*, **12**, 249 (1978).
4) A.D. Bangham, M.M. Standish, and J.C. Watkins, *J. Mol. Biol.*, **13**, 238 (1965).
5) G. Gregoriadis and A.C. Allison, "Liposomes in Biological Systems," John Wiley and Sons, Inc., New York, 1980, pp. 25—86; G, Gregoriadis, "Drug Carriers in Biology and Medicine," Academic Press, London, 1979, pp. 287—341.
6) A.D. Bangham, M.W. Hill, and N.G.A. Miller, "Methods in Membrane Biology," ed. by E.D. Korn, Plenum Press, New York, pp. 1—68.
7) S.J. Enna and L.S. Schanker, *Am. J. Physiol.*, **223**, 1227 (1972).
8) F.H.C. Tsao and R.D. Zachman, *Pediat. Res.*, **11**, 849 (1977).
9) M. Karnovsky, *J. Cell. Biol.*, **35**, 213 (1967).
10) L.S. Kaplow, *Blood*, **26**, 215 (1965).
11) for example, G. Gregoriadis, *FEBS Letters*, **36**, 292 (1973); G. Gregoriadis, *Biochem. Soc. Trans.*, **2**, 117 (1974).
12) *a*) D.A. Tyrrell, T.D. Heath, C.M. Colley, and B.E. Ryman, *Biochim. Biophys. Acta*, **457**, 259 (1976); *b*) R.D. Zachman and F.H.C. Tsao, *Pediat. Res.*, **14**, 24 (1980).
13) H.N. McCullough and R.L. Juliano, *J. Nat. Cancer Inst.*, **63**, 727 (1979).