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STUDIES OF THE LUNGS IN DIABETES MELLITUS

II. PHOSPHOLIPID ANALYSES ON THE SURFACTANT FROM BRONCHO-ALVEOLAR LAVAGE FLUID OF ALLOXAN-INDUCED DIABETIC RATS

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Summary

Present experiments were undertaken to analyze the phospholipid content and composition in the surfactant from broncho-alveolar lavage fluid of alloxan-induced diabetic rats. The diabetic rats 4 weeks after alloxan treatment had significantly less phospholipid contents in the white layer from broncho-alveolar lavage fluid than the control. As to the phospholipid composition, the white layer of the diabetic rats contained significantly less phosphatidyl choline, and more sphingomyelin and lysophosphatidyl choline compared with the control. These results suggest that diabetes mellitus may induce a disturbance in the synthesis of pulmonary surfactant.

Introduction: It is well known that diabetes mellitus induces abnormal metabolisms, not only in carbohydrates, but also in lipids and proteins. Recent investigations suggest that diabetes mellitus affects mechanical and biochemical functions of the lungs. Decreases in elasticity and in total lung capacity have demonstrated in men with juvenile diabetes mellitus(1). Maternal diabetes predisposes newborn infants to respiratory distress syndrome(2). Diabetic rat lungs show depressed glucose oxidation(3), and a reduced rate of glucose incorporation into neutral lipids and phospholipids(4). We have demonstrated

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that arterial oxygen tensions and pulmonary carbon monoxide diffusing capacities in diabetic patients are often lower than those in a corresponding age group(5, 6). Furthermore, our recent study has demonstrated that diabetes mellitus produces significant morphological alterations of the lung in diabetic rats, which indicate disorders in the pulmonary capillaries and in the metabolism of pulmonary surfactant(7).

However, few data is available concerning biochemical changes of the lungs, especially surfactant, in diabetes mellitus. Therefore, the present experiments were undertaken in an attempt to analyze phospholipid contents and compositions in surfactant obtained from broncho-alveolar lavage fluids of alloxan-induced diabetic rats, and to see whether the biochemical changes of the lungs in the diabetic rat are correlate with the morphological alterations observed in our previous study(7).

Materials and Methods

Experimental animals: Sixty male rats of Wistar derived strain, weighing 100 to 200 mg were used. Diabetes was induced after a 24 hour-fasting period by an intraperitoneal injection of a single dose of alloxan monohydrate(Katayama Chemical, 200 mg/kg of body weight). In each experiment, half of the animals subjected to alloxan treatment were selected at random from groups of uniform age and the remainder were used as normal controls. The control rats received an equal volume of isotonic saline without alloxan. The animals were fed ad libitum and were sacrificed 4 weeks after alloxan treatment.

Development of induced diabetes and histological examinations were described in the previous study(7).

Preparation of broncho-alveolar lavage fluid: The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital(50 mg/kg of body weight) and killed by exsanguination from abdominal aorta. The pulmonary blood vessels were perfused with normal saline via the right ventricle by an injection-syringe. The trachea was cut, and a plastic cannule was inserted into the trachea and tied tightly with a thread. Pulmonary washing was carried out in the lung tissue in situ. 10-15 ml of saline solution was infused into the lungs through the tracheal catheter and aspirated gently, and the same fluid was returned to the lungs 3 times. Pulmonary washings were carried out 5 times and then collected. The volume recovered was 70-80 % of the fluid infused.

Separation of surfactant from lavage fluid: The lung washing fluids were centrifuged in a Hitachi 10-2 roter at 1,000 rpm for 10 min at 4°C to remove cells and cell debris as a sediment and recovered the supernatant. The precipitate was suspended in the original volume of saline and recentrifuged under the same conditions, again recovering the supernatant. The two supernatants were combined and centrifuged at 27,000 x g for 40 min at 4°C in a Hitachi 55P-7 following the methods of Pawlowski et al.(8). The precipitate(white layer) was used as the alveolar surfactant fraction.

Table 1. Protein and phospholipid contents of surfactant from broncho-alveolar lavage fluid

	control	diabetic(4 weeks)
number	14	18
body weight, g	297.2 ± 50.1	176.0 ± 43.0*
blood glucose, mg/dl	137.2 ± 22.6	445.6 ± 42.3*
dry lung weight, g	0.253 ± 0.041	0.171 ± 0.039**
protein, mg	0.202 ± 0.093	0.234 ± 0.101
protein/dry wt.,($\times 10^{-3}$)	1.18 ± 0.60	1.41 ± 0.58
phospholipid, mg	0.599 ± 0.160	0.424 ± 0.123
phospholipid/dry wt., ($\times 10^{-3}$)	2.72 ± 0.52	1.94 ± 0.63**

Values are mean ± standard deviation. * $P < 0.01$ compared with control. ** $P < 0.05$ compared with control.

Determination of protein and phospholipid contents of alveolar surfactant: Each white layer was resuspended with 0.5 ml distilled water containing 0.1 % NaN_3 and 0.01 % EDTA, and used for protein and lipid analysis. Proteins were determined by the methods of Lowry et al.(9) using human serum albumin(Behring Inst., W.Germany) as a atandard. Total lipids were extracted according to the method of Folch et al.(10) with chloroform-methanol(2:1, by volume). Samples of the chloroform-methanol extract were taken to dryness under nitrogen and analyzed for phosphorus content according to the colorimetric method of Bartlett(11), and further analyzed by thin layer chromatography to determine the per cent composition of each constituent phospholipid. Samples on a 0.25 mm thick Silica gel G plate were developed in a solvent system of chloroform-methanol-water(65:25:4) at room temperature. Seven area were identified after exposure to iodine vapor: the origin, lysophosphatidyl choline, sphingomyelin, phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine and the other. Each spot was marked and scraped into a separate tube for phosphorus determination as described by Parker and Peterson(12).

Results

In Table 1 are presented the protein and phospholipid contents of surfactant from broncho-alveolar lavage fluids. The blood glucose levels in diabetic rats showed much higher values than that of control, and body weight and dry lung weight of diabetic rats were significantly lower than those of the control. The total amounts of protein and phospholipid in 4 week diabetic rats were similar to the control. However, the total amount of phospholipid per dry

Table 2. Phospholipid composition of surfactant from broncho-alveolar lavage fluid

	control	diabetic(4 weeks)
	10	13
% distribution of individual phospholipids		
% PC	83.2 ± 4.0	73.6 ± 7.3 *
% PE	6.60 ± 1.87	7.20 ± 2.24
% SPH+LPC	3.36 ± 1.01	5.09 ± 1.71 *
% others	6.87 ± 4.86	14.14 ± 5.09

Abbreviation used are: PC;phosphatidylcholine, PE;phosphatidylethanolamine, SPH;sphingomyeline, LPC;lysophosphatidylcholine, others; origin + polyphosphatides +unknown. * $P < 0.05$ compared with control.

lung weight in diabetic rats was significantly lowered, showing 71 per cent of that of the control value($P < 0.05$), although the differences of the amount of protein per dry lung weight were not statistically significant. The phospholipid composition of the white layer from broncho-alveolar lavage fluid is shown in Table 2. Phosphatidyl choline(PC) was the major component comprising 83 % in the control rats. Compared with the control, the phosphatidyl choline decreased to 73 per cent, while sphingomyeline(SPH) and lysophosphatidyl choline(LPC) increased to 150 per cent($P < 0.05$). The other fractions of phospholipid composition showed no significant differences between control and 4 week diabetic rats.

Discussion

Our previous experiments had demonstrated that the alloxan-induced diabetes mellitus produced significant morphological alterations in the rat lung(7). The most affected cell type in the parenchyma of the diabetic rat lung was the granular pneumocyte, which is considered to be main cellular source of pulmonary surfactant(13, 14, 15, 16). Surface-active material is considered to accumulate

in the white layer collected from mammalian lung washings(17, 18). Therefore, we examined the phospholipid analyses of the white layer from broncho-alveolar lavage fluid in the diabetic rats.

Protein and phospholipid contents, and phospholipid compositions of the lungs in the control rats were similar to those reported by others(17, 18, 19, 20). On the other hand, in the diabetic rat lung, phospholipid content(per dry lung weight) decreased to 71 per cent of that of the control($P < 0.05$), although the protein content showed no difference between the control and diabetic rats. Furthermore, as to phospholipid composition, there were a decrease of phosphatidyl choline(PC) and an increase of sphingomyeline(SPH) and lysophosphatidyl choline (LPC) in diabetic rats. Several reasons explaining the present results can be offered: 1) the synthesis of alveolar PC is reduced in diabetic rats.; 2) the transport of these phospholipids into the alveolar lumen is inhibited.; 3) newly synthetized PC are destroyed more rapidly in diabetic rats.; 4) the removal of alveolar phospholipid, especially SPH and LPC, is depressed in diabetic rats. In lung tissue, de novo synthesis of PC mainly occures via the CDP-choline (cytidine diphosphate-choline) pathway(21). It has been suggested that saturated PC is synthesized from LPC via a reacylation pathway, such as Marinetti's pathway or the acyltransferase pathway(22, 23). The present results suggest that diabetes mellitus may induce a disturbance of these pathways in the synthesis of pulmonary surfactant in the lung.

Phospholipids are synthesized in the granular endoplasmic reticulum(24). In the lung, organelles known as lamellar inclusion bodies in the cytoplasm of granular pneumocyte are specialized multivesicular bodies, derived from the Golgi apparatus in which surfactant collects before excretion into the alveoli (25). In our previous experiments, morphological changes of the lung induced by diabetes mellitus were marked dilatation of the cisterna of the granular endoplasmic reticulum and dilation of the Golgi saccules in the cytoplasm of the granular pneumocytes. Furthermore, the average number of lamellar inclusion bodies per granular pneumocyte in the diabetic rats decreased to about half of

the control value. Therefore, the decrease in pulmonary surfactant in the diabetic rat may be due to the morphological alterations observed in the previous study(7). A decrease in pulmonary surfactant could be expected to bring collapse of the alveoli. Thus, the morphological and biochemical changes of the lungs induced by diabetes mellitus may probably cause pulmonary dysfunction.

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References

1. Schuyler,M.R., Niewoehner,D.E., Inkley,S.R., Kohn,R. (1976) Am Rev Respir Dis 113,37-41.
2. Robert,M.F., Neff,R.K., Hubbell,J.P., Taeush,H.W., Avery,M.E. (1976) N Engl J Med 294, 357-360.
3. Morishige,W.K., Uetake,C., Greenwood,F.C., Akaka,J. (1977) Endocrinology 100, 1710-1722.
4. Moxly,M.A., Longmore,W.J. (1975) Life Sci 17, 921-926.
5. Sugahara,K., Tsuji,K., Araki,Y. Toyama,N., Morioka,T. (1978) Jap J Anesth 27, 473-478.
6. Sugahara,K., Ushijima,K., Morioka,T., Kato,N., Kawaguchi,K. (1979) Jap J Anesth 28, 1722-1725.
7. Sugahara,K., Ushijima,K., Morioka,T., Usuku,G. Virchows Arch path Anat (in press)
8. Pawlowski,R., Frosolono,M.F., Charms,B.L., Przybylski,R. (1971) J Lipid Res 12, 538-544.
9. Lowry,O.H., Rosebrough,N.J., Farr,A.L., Randall,R.J. (1951) J Biol Chem 193, 265-275.
10. Folch,J., Lee,M., Sloane-Stanley,G.H. (1957) J Biol Chem 226, 497-509.
11. Bartlett,G.R. (1959) J Biol Chem 234, 466-468.
12. Parker,F., Peterson,N.F. (1965) J Lipid Res 6, 455-460.
13. Macklin,C.C. (1954) Lancet 1, 1099-1104.
14. Askin,F.B., Kuhn,C. (1971) Lab Invest 25, 260-268.
15. Goerke,J. (1974) Biochim Biophys Acta 344, 241-261.
16. Kikkawa,Y., Yoneda,K., Smith,F., Packard,B.E., Suzuki,K. (1975) Lab Invest 32, 295-302.
17. Said,S.I., Harlan,Jr., Burke,G.W., Elliot,C. (1968) J Clin Invest 47, 336-343.
18. Finley,T.N., Pratt,S.A., Ladman,A.J., Brewer,L., Mckay,M.B. (1968) J Lipid Res 9, 357-365.
19. Fujiwara,T., Adams,F.H., Nozaki,M., Dermer,G.B. (1970) Am J Physiol 218, 218-225.
20. Välimäki,M., Pelliniemi,T.T., Niinikoski,J. (1975) J Appl Physiol 39, 780-787.
21. Spitzer,H.L., Morrison,K., Norman,J.R. (1968) Biochim Biophys Acta 152, 552-558.
22. Akino,T., Abe,M., Arai,T. (1971) Biochim Biophys Acta 248, 274-281.
23. Frosolono,M.F., Slivka,S., Charms,B.L. (1971) J Lipid Res 12, 96-103.
24. McMurray,W.C., Dawson,R.M.C. (1969) Biochem J 112, 91-108.
25. Chevalier,G., Collet,A.J. (1972) Anat Rec 174, 289-310.