ANALYSIS OF FATTY ACIDS FRACTIONS OF PHOSPHOLIPIDS AND NEUTRAL LIPIDS FROM BRONCHOALVEOLAR LAVAGE FLUID (BALF) IN PATIENTS WITH OCCUPATIONAL LUNG DISEASES (OLD)

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INTRODUCTION

The pulmonary surfactant has caught the attention of numberless investigators since its discovery was reported by Pattle[1] and the primary characteristics of the substance were first described by Clements.[2]

Basically the pulmonary surfactant reduces surface tension of the interface air-liquid of the alveolus, maintaining mechanical stability and avoiding alveolar collapse particularly at small pulmonary volumes.[3,4,5] Other functions of this complex substance are the help on removal of particles from airways and on digestion of bacterias intra and extra cellulars, the inhibition of pulmonary edema and the transudation of fluids into the alveolus.[7,8,9]

Dipalmitoyl phosphatidylcholine (DPPC) is the predominant molecule of the pulmonary surfactant and of it depends, in great part, the tensio-active function of this compound.[3,5,6] The exact functions of the remaining components such as insaturated phosphatidylcholine, other phospholipids, neutral lipids, and specific proteins are incompletely studied.[3,10]

The pulmonary surfactant is produced in the pneumocyte type II, synthetized in its endoplasmic reticular system and transferred, by mechanisms not fully known, to the lamellar bodies and then secreted to the alveolar surface.[3,11]

Several enzymatic mechanisms are involved in the synthesis of phospholipids and there is evidence of a certain hormonal regulation—androgenes, corticosteroids, tiroxine and insulin.[11,3]

In the control of the secretion there seem to be involved cholinergic and B adrenergyc mechanisms. Chemical mediators such as prostagl; dines, physical factors such as the distortion of the alveolar membrane and hyperventilation can also interfere in surfactant secretion.[11,12]

The importance of surfactant defects is well established in the pathogenesis of diseases like neonatal and adult respiratory distress syndrome[13,14] and less well established in other situations of respiratory suffering in man.[5,6]

Since on occupational lung diseases there are important immunologic and inflammatory mechanisms occurring in the surface of the alveolus including changes in the alveolar microatmosphere, thickness and rigidity of the membranes and distortion of the structure,[15,16,17] an hypothesis was sought that this factor could condition changes in the composition of the pulmonary surfactant.

In order to attempt to prove it, we studied the lipidic composition—phospholipids, neutral lipids and fatty acids—of the extracellular compartment of the pulmonary surfactant, obtained through bronchoalveolar lavage in 15 individuals: 5 normal, 5 pigeon breeders and 5 silicosis patients.

MATERIAL AND METHODS

Patients

We studied 5 patients with silicosis and 5 with pigeon breeder's lung, diseases confirmed through usual criteria.

Both groups were homogeneous concerning age and sex. None of the patients smoked cigarettes and none was submitted to corticotherapy.

All were submitted to a clinical and functional study, a standard thorax X-ray and bronchoalveolar lavage.

As controls we used 5 normal volunteers, non-smokers with ages not differing significantly of those of the patients.

Bronchoalveolar Lavage

All individuals studied were submitted to BAL in one of the sub-segments of the middle lobe. Four fractions of 50 ml of saline at 37°C were instilled with a syringe adapted to the fibroscope and after a few seconds the liquid was retrieved. The mucus was separated, total cell count was performed by hemocitometry. The cellular component was separated by centrifugation at 500 G for 15 min. at 4°C. The supernatant was dried in a vacuum oven −50°C to −60°C for several hours and stored in a steam of nitrogen for further study.

PROCESSING AND ANALYSIS OF LIPIDS

Extraction of Lipids

The total lipids were extracted from the dried supernatant lavage fluid according to Bligh and Dyer method.[25]

Chromatographic Methods

Total lipids were separated by column chromatography on silica acid-Kieselguhr (BDH), in neutral lipids (NL) and phospholipids according to Cmelik and Fonseca.[26]

The neutral lipids (cholesterol, cholesterol esthers fatty acids and triglycerids fractions) were separated by thin layer chromatography (TLC) on silica gel G plates (Merck Chemical Co.) using petroleum ether/ethyl/acetic acid (70:30:1) as developer.

Phospholipids were separated by TLC on silica gel H (Merck) and Florisil (BDH 10-200 mesb) plates using chloroform/methanol/water (65:25:4) as a solvent.

NL fractions were hydrolyzed with a 5% potassium metoxide solution. After the extraction of the non-saponifiable part, fatty acids were extracted from the acidified solution with ethyl ether.

Phospholipids were bydrolyzed with HCl 6N in sealed tubes immersed in a boiling water bath for 4-6h.

Fatty acids were converted into methyl esters with a methanolic solution of Boron trifluoride BF_3[27] and analyzed on a Perkin Elmer 900 gas-chromatograph with a dual flame ionization detector. The columns were 2 m long with an 1/8 incb o.d. and were packed with 20% Diethylenoglycol Succinate (DEGS) and chromosorb W (DMCS) 80–100 mesh. The analysis was performed at programmed temperature (140°–170°C) with an increasing rate of 2°C minute, followed by isothermal operation. The nitrogen flow rate was 35 ml/min. Peak's identification was determined by comparing their relative retention time with that of known standards. The relative percentage of the peak areas was evaluated by an integrator from Hewlett Packard's 3.380 A).

Spots on analytical plates were visualized by spraying with concentrated sulfuric acid containing 0.1% of potassium dichromate and subsequent charring at 140°C. Spots of the phospholipids were identified by the use of respective standards and phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were identified too by spraying the plates with Dragendorff and Ninhydrin reagents respectively.

Total cholesterol was determined by the use of a modified Lieberman-Buchard reagent.[28] Pbosphorus analysis was performed by the method of Fiske and Subbarow[29] as modified hy King.[30]

Total proteins were assayed by Lowry's method.[31]

RESULTS

Study of phospholipids revealed some differences on both groups of patients in relation with controls (Table I). Therefore on pigeon breeders a significant decrease of phosphatidylcholine was found as well as phosphatidylglycerol on silicosis patients.

On the other hand, in both groups we found significant increases of phosphatidylethanolamine and sphingomyelin.

As for neutral lipids, the fact that cholesterol was abundant in the 3 groups prevailed. Also in the 3 groups free fatty acids were detected (Table II).

In the composition on fatty acids of phospholipids, significant differences on both groups of patients were also detected by comparison with the controls (Table III), mainly the important decrease of C16:0 and increase of fatty acids insaturated in C16, C18 and C20. To be noted also the significant increase of arachidonic acid on pigeon breeders. From this results that the ratio between saturated and insaturated fatty acids is inferior to unity on patients and superior to 2 on controls (Table IV).

From the analysis of the composition in fatty acids of the neutral lipids once more highlights the important decrease of palmitic acid and the increase of insaturated fatty acids in patients, mainly in C16 and C18 (Table V). The ratio saturated/insaturated fatty acids results once again less than unity on patients and greater than 2 on controls (Table VI).

DISCUSSION

The 3 groups of individuals studied being comparable, it becomes evident that on patients with occupational diseases of the lung there are significant changes on the composition of the pulmonary surfactant.

On the whole, the changes encountered on both groups of patients are similar, suggesting partially common metabolic paths. This is in accordance with other data of the study of BAL liquid referring either to cellular elements or to chemical mediators.[17,19,20,21,22,23,24]

One of the more relevant conclusions of this study is the decrease of the phospholipids that form the molecules with tensio-active function generally accepted, especially phosphatidylcholine, as it is clear on Table I.[5,3] In parallel there are alterations of many other phospholipids, whose meaning is difficult to determine since its functions have not yet been clarified.

Even more interesting are the profound modifications on the composition of fatty acids related with phospholipids knowing that, for instance, the tensio-active properties of PC depend of the fatty acids in positions α or β.[7]

It is true that for a normal surfactant function a minimal quantity of DPPC is required and that some variations in fatty acids composition do not interfere with this function.[4] On these patients it is a field requiring further research, so much for the fact that we recognize that, in exposed individuals without the disease, the composition in fatty acids did not vary from controls; suggesting that the alterations follow the surge of the disease.

One word about the great amount of arachidonic acid found in pigeon breeders without forgetting that it is on the basis of leucotrienes, Prostaglandins and Paf-acether, mediators involved in the disease.[17,22,24]

Also in relation to the composition in neutral lipids, the alterations are profound and its meaning remains uncleared, given the lack of knowledge associated with its functions.[5,18] However we would like to point out that triglycerids are the preferential form of stock of the fatty acids which, by subsequent oxidation or sterification form other lipids.[18] Also the relative decrease in fatty acids found on some patients may contribute to explain the susceptibility to acquire respiratory infections which is widely accepted.[8,9]

So we find important disturbances on the metabolic path of the surfactant synthesis. What does it mean?

Table I
Bronchoalveolar Lavage

	PIGEON %	CONTROLS %	SILICOSIS %
PHOSPHATIDYLCHOLINE	52,1±2,5 * ↓	59,6±2,9	65,8±5,4 N.S.
PHOSPHATIDYLGLYCEROL	16,3±2,2 N.S.	23,4±5,6	9,9±3,5 *** ↓
PHOSPHATIDYLINOSITOL + PHOSPHATIDYLSERINE	9,2±4,2 N.S.	9,8±2,1	8,0±2,5 N.S.
PHOSPHATIDYLETHANOLAMINE	10,4±2,7 *** ↑	4,9±1,5	7,5±1,0 ** ↑
CARDIOLIPINE	6,1±1,6 N.S.	4,9±0,9	4,8±1,6 N.S.
SPHINGOMYELINE	7,0±1,3 **** ↑	1,3±0,6	7,9±3,1 ** ↑

* $p < 0,05$
** $p < 0,02$
*** $p < 0,01$
**** $p < 0,001$

Table II
Bronchoalveolar Lavage

	PIGEON	CONTROLS	SILICOSIS
CHOLESTEROL	++++	++++	++++
FREE FATTY ACIDS	+	+	+
TRIGLYCERIDES	++	++	++
CHOLESTEROL ESTER	+++	+++	+++

We have already mentioned that in these diseases there are changes in the structure and in the microatmosphere of the alveolus that may explain its origin.[11,12] Also, we must not forget that the alveolar macrophage (AM) which, in these patients, is permanently activated producing enzymes and chemical mediators whose interaction with the Pneumocite type II is not clarified.

In parallel the cellular membrane of the activated AM is a productive source of lipidic molecules[22,24] which may contribute for the constitution of the surfactant. In the end it is possible that the Pneumocite type II may be directly injured; Schuyler[21] found in the bronchoalveolar lavage fluid of unusual silicosis patients an a burdance of Pneumocites type II so far unexplained.

In the pathogenesis of occupational diseases of the lung there are mechanisms that may cause modifications in the synthesis and secretion of the surfactant.

We believe however these changes to be more than an epiphenomenon. It is probable that they condition functional changes of the pulmonary surfactant and therefore enable a positive loop of amplification of the processes in course.

Let us not forget that in the acute phases of the disease there is interstitial edema an alveolar transudation and that in other phases alveolar collapse appears. What is the role of surfactant alterations in these processes? Can these changes disturb its basic role in the muco-ciliar stair?

CONCLUSIONS

In patients with occupational diseases of the lung there are important alterations in the lipidic composition of the pulmonary surfactant. Future research will be necessary to establish up to which point these changes disturb its functions contributing to the clinical-pathological picture of the diseases.

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Table III

	PIGEON	CONTROLS	SILICOSIS
12:0	1,1±0,2 N.S.	1,4±0,6 N.S.	1,9±1,1 N.S.
14:0	1,1±0,2	2,1±0,8	4,5±0,9 * ↑
14:1	< 1	< 1	1,1±0,9
16:1	5,6±2,2 ↓ ***	59,7±2,8	25,9±2,9 ↓ ***
16:1	< 1	< 1	< 1
16:2	< 1	< 1	< 1
16:3	21,8±1,5 ↑ ***	< 1	13,8±1,3 ↑ ***
18:0] 18:1]	42,9±2,9 N.S.	24,5±7,4	30,2±2,2 N.S.
18:2	< 1 ↓ **	6,1±2,9	3,2±3,3
18:3	4,3±0,9 ↑ **	< 1	13,1±0,9 ↑ **
20:0] 20:1]	< 1	< 1	7,6±1,6 ↑ **
20:4	22,6±4,1 ↑ **	8,7±2,5	4,4±3,6 N.S.

* $p < 0,05$ ** $p < 0,01$ *** $p < 0,001$

Table IV

Ratio Total Saturated Fatty Acids/Total Unsaturated Fatty Acids

PIGEON	CONTROLS	SILICOSIS
0,3 ± 0,1 ← S***	→ 2,3 ± 0,6 ← S***	→ 0,7 ± 0,1

S *** $p < 0,001$

Table V

	PIGEON	CONTROLS	SILICOSIS
12:0	< 1 N.S.	< 1 N.S.	4,2±0,7 N.S.
14:0	< 1 ↓ **	6,4±2,2	4,6±0,7 N.S.
14:1	3,4±2,4 N.S.	3 ± 2 N.S.	1,8±0,4 N.S.
16:0	13,2±9,0 ↓ *	44,9±19,5	23,9±1,2 ↓ **
16:1	2,1±1,9	< 1	< 1
16:2	< 1	< 1	< 1
16:3	8,0±1,44 ↑ **	< 1	13,0±1,2 ↑ **
18:0] 18:1]	11,2±9,9 N.S.	21,8±18,2 N.S.	32,4±9,2 N.S.
18:2	13,7±2,4 ↑ **	< 1	< 1
18.3	< 1	< 1	13,1±0,6 ↑ **
20:0] 20:1]	14,4±3,1	13,8±3,4	< 1 ↓ **
20:4	19,8±7,5 N.S.	11,7±7,7 N.S.	8,1±0,8 N.S.
22:1	8,6±6,8 N.S.	9,25±8,6 N.S.	3,3±1,2 N.S.

S * $p < 0,05$ S ** $p < 0,02$

Table VI

Ratio Total Saturated Fatty Acids/Total Unsaturated Fatty Acids

PIGEON		CONTROLS		SILICOSIS
0,3 ± 0,2	S ***	← 1,9 ± 0,8 →	S **	0,8 ± 0,1

S *** $p < 0,01$ S ** $p < 0,05$

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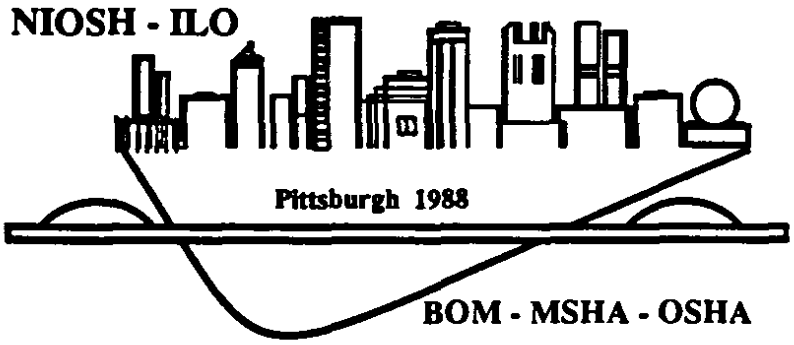

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