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Phospholipasic and Prophospholipasic Activities in Bronchoalveolar Lavage Fluid in Severe Acute Pulmonary Disease With or Without ARDS

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Abstract. Bronchoalveolar lavages were performed in 21 patients undergoing mechanical ventilation: Group I: coma due to sedative overdose (11 cases), without pulmonary impairment, serving as control group; Group II: severe acute pulmonary disease without ARDS (5 cases); Group III: patients with ARDS (5 cases). In the recovered fluid we measured: total proteins (P) and phospholipids (PL), phospholipasic (PLase) and prophospholipasic (PPLase) activities. In ARDS group, considerable increase of P, ($p < 0.001$), and a doubling of PL ($p < 0.02$) was found. Total PLase activity was present in all three groups, with a higher mean level in Group III ($p < 0.01$). PPLase activities were low or undetectable in four patients of this group. PLase/PL ratio was increased in acute respiratory insufficiency, with or without ARDS, suggesting an increase of surfactant catabolism. The decrease of this ratio when pulmonary function improved, or its increase after deterioration suggest that it is related to changes of surfactant.

Key words: Bronchoalveolar lavage – Acute pulmonary disease – ARDS – Phospholipasic activities – Prophospholipasic activities

Introduction

Pulmonary surfactant is a lipoprotein lining the alveoli and is credited with an important role in maintaining the integrity of alveoli. Optical and electron microscopic studies have shown surfactant destruction in the lungs of patients who died from an adult respiratory distress syndrome (ARDS) [1, 2]. We could not, however, find any information regarding its biochemical degradation during the evolution of the ARDS.

By adjusting the technique of bronchoalveolar lavage (BAL) for patients undergoing mechanical ventilation we have been able to measure the total proteins (P) and the phospholipids (PL) together with phospholipasic (PLases) and prophospholipasic activities (PPLases) in the BAL fluid of patients suffering from ARDS.

Methods

Twenty-one patients underwent mechanical ventilation with a constant volume ventilator via a cuffed endotracheal or tracheostomy tube. They were divided into three groups.

Group I included 11 previously healthy subjects (ten women and one man) with coma due to sedative overdose. The mean age was 31.5 years with a range of 19 to 66 years. FIO_2 was below 0.40 and PaO_2 was above 95 torr. Chest X-rays were normal. All patients recovered in less than 48 h.

Group II included four men and one woman with severe acute pulmonary disease; PaO_2 was above 100 and below 500 torr while FIO_2 was 1.0; X-rays showed localized parenchymal infiltration, unilateral in Patients 12 – 15, and bilateral in Patient 16. The mean age was 60 years with a range of 41 to 71 years. Patients 14 and 15 had unilateral lung contusion; patients 12 and 13 had bacterial sepsis (E. *coli*, *Clostridium perfringens*) with unilateral pneumonia; Patient 16 had bilateral aspiration pneumonia. Patients 14 and 15 recovered and Patients 12, 13 and 16 died. Post mortem examination was done in Patient 13. Macrophagic alveolitis without hyaline membrane was present.

Group III included four men and one woman with ARDS. ARDS was defined by 1) PaO_2 of less than 100 torr while FIO_2 was 1.0, 2) a chest X-ray showing

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diffuse bilateral parenchymal infiltrates, 3) a compatible underlying disease. Four patients had septicemia (*Streptococcus pneumoniae* Patients 17 and 18; *Staphylococcus aureus* Patient 19; *Pseudomonas aeruginosa* Patient 21). Patient 20 had an aspiration pneumonia. The mean age was 52 years with a range of 29 to 71 years. All patients died. Post mortem examinations were done in Patients 17, 19 and 20. In these three patients hyaline membranes were present.

The bronchial catheter (Medi-Tech B 1365, Biotrol Lab., Paris) was introduced through a Cobb's connector without interrupting ventilation and positioned under fluoroscopic control using a special handle (Medi-Tech). Sterile isotonic saline at room temperature was injected by aliquots of 50 ml and immediatly aspirated. After centrifugation at 4000 rpm the supernatant was removed and stored at −20°C.

Group I

Duration of the ventilation before BAL ranged from 0.5 to 20 h (mean 4.5 h). The bronchial catheter was positioned in the middle or right lower lobe. The volume injected ranged from 100 to 180 ml (mean: 140 ml). The percentage of aspirated volume ranged from 0.08 to 0.50 (mean: 0.21) of the injected volume. No adverse reaction was observed, arterial pressure and chest X-rays did not change.

Group II

BAL was performed at an average of four days after the onset of the pulmonary disease with a range of one to nine days. The duration of ventilation before the BAL ranged from 1 to 48 h (mean: 29 h). The bronchial catheter was positioned in the area which appeared abnormal as described above. The injected volume ranged from 120 to 350 ml (mean: 220 ml). The percentage of aspirated volume ranged from 0.08 to 0.35 (mean: 0.16).

Group III

BAL was performed for two to twenty days (mean: seven days) after the onset of the ARDS. The duration of ventilation before the BAL ranged from 1 to 24 h (mean: 20 h). The bronchial catheter was positioned in an abnormal area and the injected volume ranged from 100 to 400 ml (mean: 214 ml). The percentage of aspirated volume ranged from 0.08 to 0.30 (mean: 0.22).

The following measurements were made on the cellular free supernatant of centrifuged lavage fluid:

- Total proteins by Lowry's method [3]. Results were expressed in μg per ml of lavage fluid.
- Phospholipids by Delsal and Manhouri's method [4]. Results were expressed in μg per ml of lavage fluid.
- Phospholipasic activities, similar to A2 phospholipases observed by Franson [5] were determined as previously described [6]. Total phospholipasic activities (PLases) were obtained by addition to BAL fluid of lysed platelets (prophospholipases activator) in an incubation medium of optimum pH 8.5 containing calcium. The substrate used was an ultrasonicate of ^{14}C phosphoatidylethanolamines whose specific activity was 6000 d.p.m. per nmole. Results were expressed in nmoles per hour and per ml of BAL fluid.
- Prophospholipasic activities (PPLases) were the result of the difference between measures with and without platelet cofactor addition. Results were expressed in percent of total phospholipasic activities.
- Thin layer chromatography of neutral lipids was performed on BAL fluid according to Skipski [7] in order to appreciate qualitatively the esterification level of cholesterol.

BAL fluid was also examined in the clinical microbiology laboratory:

- A rough quantitative estimate for leukocytes and macrophages was made on gram stains of BAL fluid by applying the following scoring system under 10× magnification: 0.0 cell per microscopic field; 1+, 1 to 10 cells per microscopic field, 2+, 10 to 24 cells per microscopic field; 3+, 25 to 50 cells per microscopic field. We checked that score 0 means that there are less than 100,000 cells per ml.
- Quantitation of bacterial growth was based on the results of classical streaking of the original plates as follows: 1+, light growth in the primary streaking zone only; 2+, heavy growth in the primary streaking zone only; 3+, growth in the primary and secondary streaking zones; and 4+, growth in the primary, secondary and tertiary streaking zones.

Statistical comparisons were made by unpaired student's t test.

Results

1. Total Proteins

Total proteins amounted (mean ± 1 SD): in Group I (Table 1) 150 ± 100, in Group II (Table 2) 300 ± 250, in Group III (Table 3) 2450 ± 1400 μg per ml. The

increase was significant between Group I and III ($p < 0.001$) and between Group II and III ($p < 0.01$). There was no significant difference between Group I and II.

2. *Phospholipids*

Mean levels ± 1 SD of phospholipids were: in Group I (Table 1) 24 ± 8.5, in Group II (Table 2) 27 ± 10, in Group III (Table 3) 48 ± 25 µg per ml. Difference was significant only between Groups I and III ($p < 0.02$).

3. *Prophospholipasic Activities (PPLases)*

There was in BAL fluid an inactive form of phospholipases (PPLases) convertible to an active form by the addition of lysed platelets. They were substantial but with a wide range in nine patients of Group I (Table 1). The percentage ranged from 0 to 0.74 (mean 0.31). In Group II (Table 2), three patients had no PPLases and two patients had a level above 0.25.

PPLases activities were low or undetectable in four patients of Group III (Table 3) range 0 – 0.5. It reached 0.51 in one patient of this group.

Table 1. Leukocytes, macrophages, bacteria, total proteins (P), total phospholipids (PL), phospholipasic (PLase) and prophospholipasic (PPLase) activities in BAL fluid of patients with sedative overdose coma (Group I)

Patient No.	Sex	Age (years)	Leukocytes	Macrophages	Bacteria. Number of colonies	P µg/ml	PL µg/ml	PLase nm/h/ml	PPLase in % of PLase	PLase/PL
1	F	18	+	0[b]	*P. aeruginosa*	180	35	27	74	0.77
2	F	28	+ +	0	*S. aureus* + *H. influenzae* +	460	29	14	0	0.44
3	F	49	+ + +	+	no growth	60	31	10	30	0.30
4	F	21	+	+ +	Oropharyngeal flora[a]	150	22.5	11	18	0.49
5	M	34	0	0	Oropharyngeal flora	60	12	9.5	10	0.79
6	F	66	0	0	*S. aureus* + + +	140	12	15	40	1.2
7	F	51	no data	no data	no data	120	18	15	6	0.83
8	F	20	0	0	Oropharyngeal flora	120	20.5	17	17	0.85
9	F	19	0	0	*S. aureus* + + +	240	20	14.5	31	0.73
10	F	20	+	0	no growth	120	39	15	66	0.38
11	F	21	0	+	Oropharyngeal flora	130	27.5	18.5	46	0.65
Mean ± 1 SD		31.5				150 ± 100	24 ± 8.5	15 ± 4.5		0.67 ± 0.25

[a] Oropharyngeal flora: Corynebacteria, Neisseria, non hemolytic Streptococci
[b] When score 0 is observed it means that there are less than 100,000 cells per ml

Table 2. Leukocytes, macrophages, bacteria, total proteins (P), total phospholipids (PL), phospholipasic (PLase) and prophospholipasic (PPLase) activities in BAL fluid of patients with acute pulmonary disease without ARDS (Group II)

Patient No.	Sex	Age (years)	Leukocytes	Macrophages	Bacteria. Number of colonies	P µg/ml	PL µg/ml	PLase nm/h/ml	PPLase in % of PLase	PLase/PL
12	M	71	+ + +	0	*S. aureus*	290	13.5	8	25	0.59
13	M	54	0	0	*S. aureus* + + + +	450	20	50	0	2.5
14	F	71	+ + +	+ + +	*K. pneumoniae* + + + +	120	30	48	0	1.6
15	M	64	+ + +	+ +	*K. pneumoniae* + + + *E. coli* + + +	50	32	26	0	0.81
16	M	43	no data	no data	no data	700	39	87	35.8	1.73
Mean ± 1 SD		60				300 ± 250	27 ± 10	44 ± 29		1.5 ± 0.75

Table 3. Leukocytes, macrophages, bacteria, total proteins (P), total phospholipids (PL), phospholipasic (PLase) and prophospholipasic (PPLase) activities in BAL fluid of patient with ARDS (Group III)

Patient No.	Sex	Age (years)	Leukocytes	Macrophages	Bacteria Number of colonies	P µg/ml	PL µg/ml	PLase nm/h/ml	PPLase in % of PLase	PLase/PL
17	F	47	+ +	0	*P. aeruginosa* + + + +	4000	82	390	1.5	4.7
18	M	75	no data	no data	*S. pneumoniae* + + + +	3000	28.5	80	5	2.8
19	M	55	+ + +	0	*P. aeruginosa* + + + *S. aureus* +	1660	35	85	4.5	2.8
20	M	52	0	+	*S. aureus* + *E. cloacae* +	350	27	29	51	1.1
21	M	29	+ + +	0	*P. aeruginosa* 06 + +	3200	63	240	0	3.4
Mean ± 1SD		52				2450±1400	48 ± 25	165 ± 150		3 ± 1.3

4. Total Phospholipasic Activities (PLases)

They were present in all three groups. The levels were in a rather narrow range in Group I with a mean value of 15 ± 4.5 nmole per hour per ml (Table 1). The levels were more scattered in Groups II and III (Tables 2 and 3) with respective mean values of 44 ± 29 and 165 ± 150. The differences are significant between Groups I and II ($p < 0.01$) and between Groups I and III ($p < 0.01$).

5. PLases/PL Ratio

The mean values in Group II (Table 2) (1.5 ± 0.75) and in Group III (Table 3) (3 ± 1.3) were above those in Group I (Table 1) (0.67 ± 0.25) ($p < 0.01$ and $p < 0.001$).

This ratio was reevaluated during the course of the illness in four patients. In Patients 14, 15 and 19, when pulmonary function improved, the ratio decreased to half of the initial level or even less. In Patient 21, whose state became worse, the ratio increased from 3.4 to 4 (Fig. 1).

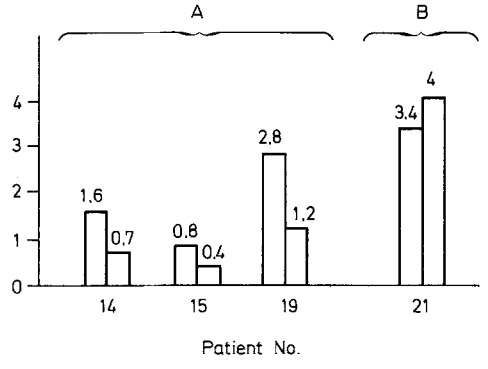

Fig. 1. Evolution of PLase/PL ratio in four patients. **A** Improvement of the pulmonary function in Patients 14 and 15 of Group II and in Patient 19 of Group III. **B** Worsening of the pulmonary function in Patient 21 of Group III

6. Esterification Level of Cholesterol

Thin layer chromatography of neutral lipids failed to demonstrate esterified cholesterol in all the patients.

Discussion

The percentage of fluid aspirated was similar in all three groups, with a mean value of 20%. This volume is smaller than reported in other studies; 55% in normal people and 35% in patients with bronchopulmonary disease [8, 9]. The following reasons may explain these discrepancies:

- Unlike others who used fiberoptic bronchoscopy, we could not aspirate under visual control which allows for a more accurate collection of BAL fluid [10].
- In our study, mechanical ventilation was never interrupted during BAL. Patients often coughed and rejected some fluid into the ventilator tubes. Weinsten et al. [11] also performed 53 BALs under mechanical ventilation with a mean aspirated volume of 35%. This percentage, although larger than ours, is smaller than the mean percentage in subjects breathing spontaneously and without pulmonary disease.

In order to minimise the error inherent to fluid losses we expressed PLases as a ratio of the amount of PL in the recovered fluid.

Several biochemical studies have been performed on the BAL of normal subjects. Proteins levels ranged from 34 to 188 μg/ml (mean: 84 μg/ml) [12, 13]. Total phospholipids ranged from 14 to 35.5 μg per ml (mean 24.5 μg per ml) [14, 15]. The similarity of these results with ours validates our method and allows us to consider Group I as a control group. Indeed the mean age of this group is less than in Group II and III. But the results in the three older patients of Group I (mean age 55 years) are within the range of those obtained in younger patients.

The important increase of P, PL and PLases in Group III in whom the mean time of ventilation is shorter than in Group II eliminates the duration of ventilation as a causal factor of the biochemical modifications.

The increase of total proteins in the patients suffering from acute pulmonary disease without ARDS was moderate and not significant compared to Group I. Other authors have reported a wide range of total proteins (50 to 665 μg/ml) in bronchopulmonary diseases (asthma, fibrosis, infections, allergic alveolitis) [13, 16].

In ARDS we observed a high quantity of proteins, 16 times greater than in Group I. There are two possible explanations for this:

- Proteins are abundant in edema fluid when alveolar capillary membranes are damaged. According to two recent studies the edema fluid to plasma proteins ratio exceeded 0.6 in cases of permeability edema. This ratio was below 0.5 in cardiogenic edema [17, 18]. The membrane damage allows the escape of proteins in permeability edema. When edema results primarily from high pressure, the membranes remain an effective barrier to proteins.
- In ARDS, cell proteins may be released from destroyed interstitial cell and alveolar epithelium.

In asthma, fibrosis, and pneumonia, phospholipids ranged widely from 15 to 191 μg per ml [8, 19]. In ARDS we documented a two-fold increase above control level of phospholipids vs a 16-fold increase of proteins. The fluid PL probably do not originate from plasma for two reasons:

- in the plasma, PL are included in the lipoproteins whose large size prevents escape even through a damaged membrane
- plasma cholesterol is essentially esterified. But we have not found any esterified cholesterol in BAL fluid of ARDS.

The PL in the BAL fluid seems to be released from destroyed type II pneumocytes which contain a great deal of PL [20]. The increased total phospholipasic activity found in our study may limit the increase of fluid PL.

There was an increase of total phospholipasic activity in Groups II and III. Post heparin phospholipase and bacterial phospholipase cannot explain this increase because they are A1 phospholipases [21, 22].

The A2 phospholipases might come from destroyed type II pneumocytes and macrophages which contain a great deal of PLases [6]. They also can originate from plasma. In man, plasmatic phospholipases are essentially inactive. They are converted to active A2 form by a platelet factor [23]. This factor might be produced by damaged capillary endothelium during severe pulmonary disease. So the phospholipases might be converted locally and escape through alveolar capillary membranes.

Very few studies have measured PLases in BAL fluids. In BAL fluid recovered from alveolar proteinosis, and asthma, A2 PLases were present [24].

We found no, or low, PPLases activity in four patients of Group III. PPLases could be activated by a platelet factor expelled into the alveoli or by a trypsinlike protease (cellular or bacterial) [25]. However trypsin was not found in BAL fluid [12].

PLases/PL ratio increased in acute respiratory insufficiency without ARDS, suggesting an increase of surfactant catabolism. The decrease of this ratio when pulmonary function improved and its increase after deterioration are another argument for its relation of the changes of surfactant.

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