Bronchoalveolar Lavage Alterations in Pulmonary Embolism

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The objective of this study was to determine quantitative and qualitative surfactant alterations, proteins, and platelet activating factor (PAF) in bronchoalveolar lavage (BAL) fluid from patients with pulmonary thromboembolism (PTE) with respect to ventilated patients without PTE. Patients with PTE underwent BAL at the most affected lung area on the first and tenth days of PTE diagnosis. Total proteins and albumin, total lipids, individual phospholipid classes, PAF and PAF-acetylhydrolase (PAF-AcH) activity were determined in BAL fluid. Total proteins and albumin were found to be increased in both successive samples of patients with PTE when compared with the control group ($p < 0.001$ and $p < 0.05$, respectively). Total phospholipids, though, were elevated on the first day, but they decreased on the tenth day, in comparison with the control groups ($p < 0.05$). Alterations in the percentage of individual phospholipid classes were observed in both successive samples of BAL fluid when compared with those in the control subjects. PAF and PAF-AcH were detected in high levels on the first day ($p < 0.001$), which were reduced on the tenth day ($p < 0.05$). An inverse correlation between PAF levels and $Pa_{O_2}/F_{I_{O_2}}$ ratio was observed. Finally, the percentage of macrophages decreased and the percentage of neutrophils increased during the course of PTE. In conclusion, pulmonary embolism is associated with alterations in lung surfactant and inflammation in lung tissue, expressed by an increase in PAF and in neutrophils. Nakos G, Kitsiouli EI, Lekka ME. Bronchoalveolar lavage alterations in pulmonary embolism. AM J RESPIR CRIT CARE MED 1998;158:1504–1510.

Pulmonary thromboembolism (PTE) is the clinical consequence of the occlusion of pulmonary arteries by thomboemboli. The events that occur after arterial occlusion are complex, involving mechanical and reflex effects of vascular occlusion as well as the release of various vasoactive and bronchoactive mediators (1). Basic dysfunction of PTE is expressed by abnormalities in pulmonary gas exchange. Mechanical obstruction in the pulmonary vascular bed initially leads to unperfused or poorly perfused, but still ventilated, lung units. The ventilation of unperfused or hypoperfused alveoli results in a high ventilation-perfusion ratio ($\dot{V}_A/\dot{Q}$) abnormalities cannot justify the hypoxemia occurring during PTE. Moreover, the lack of correlation between clinical findings and embolized pulmonary vascular bed indicates once more that the clinical consequences of pulmonary embolism are not due only to mechanical obstruction (2). Experimental data suggest that the mechanisms primarily involved in the development of abnormal gas exchange after pulmonary embolism are shunting, low $\dot{V}_A/\dot{Q}$, and possibly a decrease in mixed venous P_{O_2} ($P\bar{v}_{O_2}$) (3, 4). The shunting is primarily intrapulmonary and, secondly, right-to-left intracardiac at the atrial level (5). The etiology of the intrapulmonary shunt and $\dot{V}_A/\dot{Q}$ abnormalities after pulmonary embolism are not well understood. Atelectasis and high permeability or hydrostatic pulmonary edema have been suggested to play a role in the development of intrapulmonary shunt and even in low $\dot{V}_A/\dot{Q}$ ratio. The release of mediators from platelets and other cells has been implicated in atelectasis and pulmonary edema by producing bronchoconstriction, vasoconstriction, increased alveolar-capillary membrane permeability, and loss of surfactant activity (2, 6–8). Furthermore, PTE is associated with a partial inhibition of hypoxic pulmonary vasoconstriction, although the inhibited hypoxic pulmonary pressor response does not always deteriorate V_A/Q mismatching in PTE (9).

Platelet-activating factor (PAF) (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), a potent phospholipid mediator in inflammatory and allergic reactions, is known to be implicated in acute, but also in long-term, pulmonary effects. It is produced by a variety of cells, including inflammatory endothelial and epithelial cells (10). Recent studies strongly suggest that PAF also plays an immunoregulatory role affecting cytokines production (11, 12). Its metabolic inactivation involves cleavage of the acetyl group by a specific PAF-acetylhydrolase (PAF-AcH) (13).

Pulmonary surfactant is a material that lines the interior of the lungs. It lowers surface tension and protects alveoli from collapsing, especially at the end of expiration. Surfactant may also diminish the transudation of fluid into the interstitial and alveolar space (14).

The aim of this study was to determine quantitative and qualitative surfactant, proteins, and inflammatory markers

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TABLE 1

CLINICAL DATA OF THE PATIENTS ONE DAY BEFORE THE EPISODE OF PTE

Patient No.	Age (*yr*)	Sex	Disease	FI_{O_2}	Pa_{O_2}/FI_{O_2}	Crs ($cm^3/cm\ H_2O$)	Rrs ($cm\ H_2O/L/S$)	MV
1	36	M	Cranial trauma	0.21	405	58	8	12
2	28	F	Neuromuscular	0.21	420	70	6	15
3	54	M	Cranial trauma	0.3	380	74	8	14
4	63	M	Cardiac arrest	0.3	360	62	11	10
5	62	M	Cranial trauma	0.3	310	51	14	9
6	70	M	Cranial trauma	0.21	340	64	10	18
7	66	M	Cranial trauma	0.3	370	74	7	13
Mean	54				370	65	9	13
SD	16				37	8.6	2.7	3

Definition of abbreviations: Crs = compliance of the total respiratory system; Rrs = airflow resistance of the respiratory system; MV = days of mechanical ventilation before diagnosis of pulmonary thromboembolism (PTE).

such as PAF and neutrophils alterations in BAL fluid from patients with proven pulmonary embolism.

METHODS

Patients

Seven mechanically ventilated patients with angiographically proven pulmonary embolism were included in this study. The patients had been mechanically ventilated (Siemens-Elema 900 C Servo ventilator; Siemens-Elema, Solna, Sweden) through a cuffed endotracheal tube or a tracheostomy tube (8.5 and 9 mm internal diameter) (Mallinckrodt, St. Louis, MO). The causes that necessitated mechanical ventilation, as shown in Table 1, were noncardiopulmonary diseases. All patients were treated with heparin. Patients who received thrombolytic or mechanical clot fragmentation treatment were excluded from the protocol. Patients with hemorrhagic diathesis were also excluded. Criteria for a positive angiogram were the identification of an obstructed vessel by an embolus or a filling defect (15).

Seven mechanically ventilated patients without cardiopulmonary disease, negative chest radiographs, and $Pa_{O_2}/FI_{O_2} > 300$ were used as a control group (Control A) (Table 2). Six patients with $Pa_{O_2}/FI_{O_2} < 300$, in whom PTE was excluded on the basis of a negative pulmonary angiogram or a normal $\dot{V}$-$\dot{Q}$ scan were used as a second control group (Control B) (Table 2). The chest radiographs in three of the six patients in Control B were compatible with linear alelectasis. In the rest, the chest radiographs were normal.

TABLE 2

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF CONTROL SUBJECTS

Patient No.	Age (*yr*)	Sex	Disease	MV*	FI_{O_2}	Pa_{O_2}/FI_{O_2}
Control A.	Mechanically ventilated patients with $Pa_{O_2}/FI_{O_2} > 300$					
1	7	M	Neuromuscular	13	0.3	360
2	28	M	Cranial trauma	8	0.3	380
3	50	M	Cranial trauma	6	0.3	390
4	37	M	Spinal trauma	7	0.3	460
5	55	M	Neuromuscular	12	0.3	400
6	29	M	Cranial trauma	13	0.3	350
7	75	M	Cranial trauma	17	0.3	410
Mean	40			10		393
SD	22			4		36
Control B.	Mechanically ventilated patients with $Pa_{O_2}/FI_{O_2} < 300$ and without PTE					
1	55	M	Cranial trauma	15	0.4	190
2	36	M	Cranial trauma	7	0.4	230
3	67	M	Tetanus	6	0.4	240
4	52	F	Myasthenia	5	0.4	210
5	38	M	Cranial trauma	8	0.4	270
6	70	M	Cranial trauma	9	0.4	230
Mean	53			8		228
SD	14			3		27

* Days of mechanical ventilation before BAL procedure.

Study Protocol

In mechanically ventilated patients with clinically suspected PTE, pulmonary angiograms were performed. In patients with proven PTE, following the above angiographic criteria, a pulmonary artery catheter (Opticath; Abbott, North Chicago, IL), via an internal jugular or a subclavian vein, as well as an arterial line, were introduced. When the patients were hemodynamically stable, the first bronchoalveolar lavage (BAL) was performed. The time interval between diagnosis of PTE and the first BAL was less than 18 h (range, 3 to 18 h; mean, 7 h). The patients underwent the second BAL 10 d afterwards. Patients with evidence of lung infection such as a quantitative culture of BAL fluid specimen $\geqslant 10^4$ cfu/ml and/or a protected brush specimen $\geqslant 10^3$ cfu/ml, respectively, were excluded from the study.

Measurements

For each patient with PTE, the following parameters were recorded: age, duration of mechanical ventilation, Pa_{O_2}/FI_{O_2}, mixed venous O_2 saturation (Sv_{O_2}), pulmonary artery wedge pressure (Ppaw), cardiac index (CI), pulmonary artery pressure (Ppa), systemic artery pressure (Psa), chest radiograph, electrocardiograph, the percentage of pulmonary vessel obstruction in angiography, tidal volume (V_T), peak airway pressure (Pa_{peak}), plateau pressure ($P_{plateau}$), inspiratory flow PEEP, and auto PEEP. The compliance of the respiratory system (Crs) was computed as $Crs = V_T/P_{plateau}$ − total PEEP and the inspiratory resistance of the respiratory system (Rrs) was computed as Rrs = Peak − $P_{plateau}$/inspiratory flow. The values of blood gases and lung mechanics of the day before PTE diagnosis were used as baseline measurements.

All measurements in patients with PTE and control subjects were obtained right before the BAL procedure, during passive volume-cycled mechanical ventilation, and the flow was delivered as a square-wave.

The study was conducted according to the principles embodied in the declaration of Helsinki. The protocol was approved by the Ethics Committee of the University Hospital of Ioannina, and a written informed consent was obtained from the patients or their next of kin.

BAL Procedure

BAL was performed on the day of diagnosis of pulmonary embolism and 10 d afterwards. BAL was performed by fiberoptic bronchoscopy. Patients were ventilated with a Control Mechanical Ventilation Mode. During the BAL procedure FI_{O_2} was set at 1.0, and PEEP was removed or reduced. Patients were sedated with midazolam and paralyzed with atracurium. Topical anesthetics were not used. Heart rate, Psa, $S_{A_{O_2}}$ (by pulse oximetry), and $\bar{S}v_{O_2}$ were monitored throughout the procedure. The trachea was suctioned before introducing the bronchoscope through an adapter (swivel adapter), which allowed the maintenance of mechanical ventilation. The tip of the bronchoscope

TABLE 3

CHEST RADIOGRAPH ON THE DAY OF DIAGNOSIS OF PTE

Pulmonary parenchymal abnormality	6/7 (85%)
Pleural effusion	5/7 (71%)
Elevated diaphragm	2/7 (29%)
Decreased pulmonary vascularity	1/7 (14%)
Prominent central pulmonary artery	1/7 (14%)

was then wedged into a segmental or subsegmental bronchus of the more affected area indicated by the angiogram. Six 20-ml aliquots of sterile normal saline 37° C were infused through the working channel of the bronchoscope. The first aspirated fluid, reflecting a bronchial sample, underwent microbiologic screening, whereas the others were collected in ice-cold tubes to avoid PAF degradation because of acetylhydrolase activity. BAL was then filtered through sterile gauze and centrifuged at 500 × *g* for 15 min at 4° C to remove mucus.

Differential Centrifugations of BAL

In certain experiments, an aliquot of the 500 × *g* supernatant, after removal of the cells, was further centrifuged at 30,000 × *g*, at 4° C for 90 min (Sorvall RC-5B; Dupont, Canada). The pellet from 30,000 × *g* was suspended in a small volume of saline and was kept at −20° C until the analysis of total lipid phosphorus and total proteins. The supernatant of 30,000 × *g* was submitted to ultracentrifugation at 105,000 × *g* at 4° C for 1 h (Beckman L5-65B with SW41 rotor; Irvine, CA). The pellet and supernatant were treated as before.

Biochemical Parameters in BAL

Total proteins and albumin were measured using the methods of Lowry and colleagues (16) and Doumas and Biggs (17) in the 500 × *g* supernatant.

Total lipids were extracted from the 500 × *g* supernatant using the method of Bligh and Dyer (18) and separated into classes with thin-layer chromatography, after two successive developments on K-6 thin-layer plates (Whatman, Clifton, NJ) at the same direction, using the following solvent systems: (*1*) chloroform-petroleum ether-methanol-acetic acid (50:30:15:10, vol/vol) to the top; (*2*) chloroform-methanol-water-acetic acid (65:35:5:10, vol/vol) to the top. Phospholipids were visualized under an ultraviolet lamp, after spraying with 2-(*p*-toluidinyl)-naphthylene-6-sulfonic acid. Then they were scraped off the plate and measured as described below.

Total phospholipids and individual phospholipid classes after thin-layer chromatography separation were determined from their lipid phosphorus content after perchloric acid digestion, according to Bartlett (19).

PAF was purified from the lipid extract of BAL fluid with thin-layer chromatography, using chloroform-methanol-water (65:35:7, vol/vol). The area between authentic sphingomyelin and lyso-phosphatidylcholine, where PAF migrates, was scraped off the plate, extracted (18), and tested for biologic activity. PAF determination was based on the aggregation of washed rabbit platelets pretreated with CP/CPK, an ADP scavenger, and acetylsalicylic-lysine, a cyclooxygenase inhibitor, using the method of Bossant and colleagues (20). The standard curve was assessed using the hexadecyl analog of PAF. Detection limit under our experimental conditions was 40 pg PAF/9 ml BAL fluid. Low temperatures were maintained throughout the BAL treatment to avoid PAF degradation because of PAF-acetylhydrolase hydrolysis.

PAF-AcH activity was determined in BAL fluids using the method of Tselepis and colleagues (21), after trichloroacetic acid precipitation. The specificity of the activity was tested in the presence of EDTA and bromophenacylbromide and in competition with 0.5×10^{-4} M 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

Statistical Analysis

Data are reported as mean ± SD. Statistical comparisons between groups were performed using analysis of variance (ANOVA) for repeated measurements (software package statistica 4.3). The significance of differences between values was determined with Bonferroni

TABLE 4

BLOOD GAS DETERMINATIONS, HEMODYNAMICS, AND LUNG MECHANICS ON THE DAY OF PTE DIAGNOSIS

Patient No.	$F_{I_{O_2}}$	$Pa_{O_2}/F_{I_{O_2}}$	Pa_{CO_2}	pH	$\overline{Pa}$	$\overline{Ppa}$	Ppw	$S\bar{v}_{O_2}$	CI	Crs*	Rrs*	PVO (%)
1	0.6	135	35	7.38	80	30	8	55	2.2	51	10	30
2	0.6	155	33	7.39	85	32	10	62	2.3	66	10	35
3	0.5	140	38	7.36	90	34	12	71	4.1	70	11	25
4	0.6	115	42	7.35	75	26	8	66	2.5	50	15	30
5	1.0	75	35	7.33	90	33	6	60	2.8	44	18	45
6	0.4	235	39	7.37	92	24	12	69	4.0	52	14	20
7	0.5	180	31	7.40	82	37	14	73	3.2	64	9	40
Mean		148	36	7.37	85	31	10	65	3.0	57	12	32
SD		50	4	0.02	6	5	2.8	6.4	0.8	9.8	3.3	9

Definition of abbreviations: $\overline{Pa}$ = mean artery pressure; $\overline{Ppa}$ = mean pulmonary artery pressure; Ppw = pulmonary wedge pressure; $S\bar{v}_{O_2}$ = mixed venous O_2 saturation; CI = cardiac index; PVO = pulmonary vessel obstruction. For other definitions, *see* Table 1.

* Crs and Rrs expressed in $cm^3/cm\ H_2O$ and $cm\ H_2O/L$, respectively.

TABLE 5

BIOCHEMICAL CHARACTERISTICS IN BAL FROM PATIENTS WITH PULMONARY EMBOLISM AND IN CONTROL SUBJECTS*

Parameters	Control A	Control B	Pulmonary Embolism First Day	Pulmonary Embolism Tenth Day
Total protein, μg/ml	109 ± 29	195 ± 37†	1,750 ± 877.4†	645 ± 96†‡
Albumin, μg/ml	42 ± 14	54 ± 16	639 ± 238†‡	209 ± 47†‡
PAF, pg/9 ml BAL	0	0	769 ± 325†	81 ± 18†‡
PAF-AcH, nmmol PAF/ml BAL/min	0.006 ± 0.006	0.007 ± 0.006	0.28 ± 0.06†	0.02 ± 0.01‡
Total phospholipids (PL), μg P/ml BAL	2.9 ± 0.8	2.3 ± 0.7	4.3 ± 0.72†	2.05 ± 0.57†‡
Phosphatidylcholine (PC), % of total PL	70 ± 3.5	62 ± 7.3	47 ± 4.0†	49 ± 5.2†
Phosphatidylglycerol (PG), % of total PL	9.3 ± 1.9	10.2 ± 2	5.8 ± 1.3†	6.9 ± 2.0
Sphingomyelin (SM), % of total PL	4.6 ± 0.5	6.4 ± 0.9	15.8 ± 1.86†	11.5 ± 1.6†‡
Phosphatidylethanolamine (PE), % of total PL	4.8 ± 0.7	5.1 ± 0.8	7.3 ± 1.7	5.5 ± 1.1
Phosphaditylserine (PS), % of total PL	5.1 ± 0.5	4.4 ± 0.8	6.2 ± 1.4	7.3 ± 1.29
Phosphatidylinositol (PI), % of total PL	4.9 ± 0.6	6 ± 1	8.1 ± 0.78†	7.6 ± 1.0†
Lysophosphatidylcholine, % of total PL	—	—	1.9 ± 0.56†	—

* Values are means ± SD.

† Denotes statistically significant difference compared with at least one of the controls.

‡ Denotes statistically significant difference between first and tenth day.

§ Denotes statistically significant difference between two controls.

correction for multiple comparisons. Correlation between values was analyzed by linear regression, using a standard software package (Statview 512+). The level of significance was defined as a p value of less than 0.05.

RESULTS

Patient Data

The basic chest radiographic findings, hemodynamics, respiratory system mechanics, as well as blood gas determinations of patients with PTE are shown in Tables 3 and 4. After PTE, a significant decrease in Pa_{O_2}/FI_{O_2} and respiratory system compliance as well as an increase in airflow resistance were observed ($p < 0.01$).

BAL Fluid

The volume of BAL fluids recovered from the 120 ml instilled saline ranged from 40 to 70%, without differences between the evaluated groups.

Protein Content

Total proteins in BAL fluids from the patients in Control B were higher than those in patients in Control A ($p < 0.05$), but there was no statistical difference in albumin.

Total proteins and albumin in the 500 × *g* supernatant BAL fluid were higher on the first as well as on the tenth day of PTE compared with those in the control subjects ($p < 0.001$ and $p < 0.05$, respectively). A reduction on the above levels was observed on the tenth compared with the first day of PTE ($p < 0.05$) (Table 5).

Surfactant Phospholipids

PTE resulted in an increase in the total phospholipid content of the 500 × *g* supernatant BAL fluid on the first day and a decrease on the tenth day after the diagnosis of PTE compared with that in the control subjects ($p < 0.05$). Moreover, the difference of total phospholipid levels between the two successive BALs was statistically significant (< 0.05) (Table 5). Finally, the percent total phospholipid content in the pellet of 30,000 × *g*, which represents the surfactant fraction with good surface properties, was significantly reduced in comparison with that in the control group ($p < 0.05$). The absolute value of total phospholipid in the pellet of 30,000 × *g* was significantly lower on the tenth day of PTE compared with that on the first day and with that in the control groups as well, but there was no statistical difference between the first day and the control groups (Table 6).

Alterations in surfactant phospholipid classes were also observed: the percentages of phosphatidylcholine and phosphatidylglycerol decreased ($p < 0.05$ and $p < 0.01$, respectively), whereas sphingomyelin, phosphatidylinositol, and lyso-phosphatidylcholine increased on the first day of PTE compared with control subjects ($p < 0.001$, $p < 0.05$, and $p < 0.001$, respectively). Phosphatidylcholine decreased and sphingomyelin and phosphatidylinositol increased on the tenth day of PTE compared with the control group. The statistically significant differences between the first and the tenth day of PTE concerning the phospholipid classes were observed only in the level of sphingomyelin and lyso-phosphatidylcholine ($p < 0.05$ and $p < 0.01$) (Table 5).

There were no significant differences between the two control groups as concerns total and individual phospholipids.

PAF and PAF-AcH Content

PAF in BAL fluid was detected in high levels on the first and in lower levels on the tenth day of PTE ($p < 0.001$ and $p < 0.05$, respectively). PAF was not detectable in any patient in the control groups (Table 5).

There was a direct correlation between levels of PAF and the ratio of Pa_{O_2}/FI_{O_2} ($r = 0.75$, $p = 0.05$) (Figure 1). No correlation was found between other clinical data and biochemical parameters.

PAF-AcH was significantly increased on the first day of PTE ($p < 0.001$). Acetylhydrolase activity did not explicitly require calcium ions and was not affected by the presence of 10 mM EDTA. It was not inactivated by *p*-bromophenacyl bromide, an inhibitor of typical phospholipase A_2 and was not influenced by the presence of excess of phosphatidylcholine

TABLE 6

DIFFERENTIAL CENTRIFUGATIONS OF BAL FLUID*

	Pellet 30,000 × *g*	Pellet 105,000 × *g*	Supernatant 105,000 × *g*
Proteins			
1st day of PTE			
(% of total)	10.0 ± 2.5	3.0 ± 1.5	87 ± 3.5
Absolute values	175 ± 26	21 ± 12	1,439 ± 183
10th day of PTE			
(% of total)	12.5 ± 2.9	2.0 ± 0.9	85 ± 6.8
Absolute values	85 ± 24	14 ± 9	550 ± 81
Control			
(% of total)	20.2 ± 2.4	4.0 ± 1.0	76 ± 5
Absolute values	18 ± 8	6 ± 2	90 ± 18
Lipids			
1st day of PTE			
(% of total)	47.0 ± 7.3†	8.0 ± 4.0	43 ± 3.9
Absolute values	2.1 ± 0.6†	0.3 ± 0.1	1.8 ± 0.7
10th day of PTE			
(% of total)	51.0 ± 6.4†	7.0 ± 3.0	41 ± 7
Absolute values	1.1 ± 0.3†‡	0.2 ± 0.1	0.9 ± 0.2
Control			
(% of total)	68.0 ± 6.0	5.0 ± 2	26 ± 8
Absolute values	2.0 ± 0.5	0.15 ± 0.07	0.7 ± 0.2

* Values are means ± SD.
† Denotes statistically significant difference in lipids compared with control.
‡ Denotes statistically significant difference between 1st and 10th days of PTE.

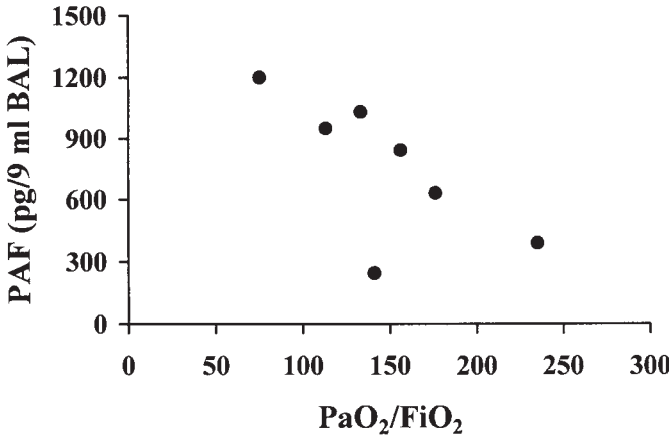

Figure 1. Correlation between Pa_{O_2}/FI_{O_2} ratio and PAF level in BAL fluids from patients with PTE ($r = 0.75$, $p \sim 0.05$).

(0.5×10^{-4} M). It was therefore differentiated from typical phospholipase A_2 and was characterized as PAF-specific.

Cells

There were no significant differences in total cell count recovery in BAL fluid between the control and the PTE samples. The percentage of macrophages decreased, whereas the percentage of neutrophils increased on the first day of PTE ($p < 0.05$ and $p < 0.001$, respectively). On the tenth day, the cells showed a tendency to return to the preembolic state (Table 7).

DISCUSSION

The results from the present study demonstrate that pulmonary embolism is associated with an increase in protein concentration, qualitative and quantitative alterations in surfactant, and an increase of inflammatory markers such as PAF and neutrophils as determined in BAL fluid.

Patient Data

As shown in Table 4, after PTE, Crs and Pa_{O_2}/FI_{O_2} decreased, whereas Rrs and Ppa increased. Atelectasis, which is associated with surfactant deficiency, could justify the drop in Crs. Regarding the changes in Pa_{O_2}/FI_{O_2}, Rrs and Ppa could be etiologically related to the high levels of PAF in BAL fluid.

Protein Content

The increase in proteins can be attributed either to an increase in the alveolar fluid volume recovery in BAL because of an increase in the lavaged area or to increased alveolar protein concentration. This distinction, though, is not feasible because of the absence of reliable alveolar fluid volume markers (22). However, the increase in protein levels in the BAL fluid of patients with PTE was a consistent finding, most probably signifying that protein concentration is high because of an increase in alveolar-capillary permeability. In other words, embolism probably induces high permeability lung edema. The development of postembolic pulmonary edema has been shown to play a role in ventilation/perfusion inequality, which is a notable abnormality during the course of PTE. The cause(s) of the pulmonary edema could be an increase alveolar-capillary permeability or a rise in pulmonary capillary pressure, or both (23, 24). Johnson and Malik (25), using a model of pulmonary embolism, found that small glass beads 200 μm in diameter induced high permeability pulmonary edema, whereas larger beads (500 μm) increased the hydrostatic pressure. This suggests that microembolization more often causes tissue damage than the occlusion of large pulmonary vessels. However, even the large thromboemboli can eventually give rise to microembolization of the more distal vessels.

The release of mediators such as PAF that affect endothelial permeability may play a significant role in the pathogenesis of high permeability pulmonary edema. On the other hand, surfactant abnormalities may also contribute to hydrostatic pulmonary edema formation, giving rise to an increased transmural hydrostatic force, possibly by decreasing perimicrovascular pressure (26, 27). Whether lung permeability increases with surfactant dysfunction is controversial (28, 29).

Surfactant Phospholipids

The increase in total phospholipids at the onset of PTE is the main finding in this study. Whether this increase is caused by an enhanced production, an increase in secretion, by a defect in reuptake of phospholipids by type II alveolar cells, or by a certain amount of phospholipids originating from damaged cell membrane has to be determined. Hyperventilation, which is a characteristic feature for PTE, could promote phospholipid production by acetylcholine and/or beta-adrenergic-mediated mechanisms (30, 31).

Differential centrifugations of BAL were applied for the isolation of surfactant structures with different physicochemical properties. Large aggregates, obtained in the pellet of $30{,}000 \times g$, consists of lamellar bodies as well as of tubular myelin. Small aggregates, containing light phospholipid vesicles were retained in the pellet of $105{,}000 \times g$. Large aggregates can reduce surface tension to very low values, whereas small aggregates exhibit poor surface activity (32). Our results show a reduced percentage of phospholipids in the pellet of $30{,}000 \times g$ in patients with PTE compared with those in the control group. It is also worth noting that the increase in absolute value of phospholipids on the first day of PTE was mainly due to an increase of phospholipids isolated in supernatant of $105{,}000 \times g$. These are consistent with a functional deficiency in the surfactant of the patients with PTE.

TABLE 7

ABSOLUTE NUMBER AND DIFFERENTIAL CELL COUNT IN BAL FLUID*

			PTE	
Differential Cell Count	Control A	Control B	First Day	Tenth Day
Total cell count $\times 10^3$/ml	150 ± 37	164 ± 40	161 ± 29	146 ± 42
Alveolar macrophages, %	79 ± 9	76 ± 11	58 ± 7.1†‡	71 ± 9.3†‡
Neutrophils, %	7 ± 2	11 ± 3.3	31 ± 9.5†‡	14 ± 8.0†‡
Lymphocytes, %	9 ± 4.0	8 ± 4.0	69 ± 1.5	7.8 ± 4.2

* Values are means ± SD.
† Denotes statistically significant difference compared with control groups.
‡ Denotes statistically significant difference between 1st and 10th days of PTE.

The decrease in total phospholipids later on in the course of PTE could reflect type II cell injury. Moreover, the high levels of PAF and neutrophils indicate lung parenchyma inflammation and alveolar cell damage. The pathogenesis of the inflammatory reaction in PTE is unclear. Data from a recent study support the observation that one of the mechanisms in the development of alveolar injury after PTE is the resolution of the thrombus, at least partially, and reperfusion of occluded lung tissue (33). The qualitative alterations of surfactant, mainly the decrease in PC and PG, could also be attributed to type II cell injury, which is the source of these phospholipids (34).

It is well known that atelectasis in the lung tissue may develop after embolic obstruction of the pulmonary vessel supplying this particular lung parenchyma (2, 35). It is reasonable that the qualitative deficiency of surfactant contributes in the development of atelectasis. The findings of this study, especially the decrease in the percentage of PC in the $500 \times g$ supernatant, the decrease in the percentage of $30{,}000 \times g$ pellet total phospholipids, and finally the increase in total phospholipids in the $105{,}000 \times g$ pellet strongly suggest qualitative surfactant abnormalities. Lyso-PC, produced by phospholipids subjected to phospholipase A_2 hydrolysis, can also dramatically affect surface tension, resulting in atelectasis since it has detergent properties.

PAF and Neutrophils

The marked increase of PAF and neutrophils in BAL fluid from patients with PTE presumably reflects lung tissue injury and inflammation. It seems that alveolar cell injury is not simply caused by the occlusion of supplying pulmonary arteries, but reperfusion of unperfused lung unit is indispensable, provided that lung tissue is relatively resistant to ischemic lesions because of the double blood supply (31). In a model of reperfusion injury developed in isolated guinea pig hearts, it was shown that adenosine and PAF appear to play a significant role as mediators in heart reperfusion injury (36). In both, lung and heart reperfusion injuries, neutrophils had a significant role in tissue stunning (31, 35). In our study, the levels of PAF in BAL fluids were high and inversely correlated with the Pa_{O_2}/FI_{O_2} ratio. PAF could be implicated in the pathogenesis of hypoxemia but also in pulmonary hypertension and in an increase of Rrs by different mechanisms. In particular, resulting in the generation of interstitial edema because of its ability to cause microvascular leaking (37), stimulating biosynthesis of potent inflammatory mediators (10), inducing pulmonary venous vasoconstriction increasing the pulmonary capillary pressure (18, 38), causing bronchoconstriction and vasoconstriction (39), decreasing the vascular reactivity to hypoxia (40), and by causing inflammatory reaction by neutrophils activation and chemotaxis (41).

The reduction of phosphatidylglycerol levels in BAL fluids, which is a natural anti-PAF agent (42), could enhance the action of PAF in the alveoli area.

Hydrolysis catalyzed by PAF-AcH appears to be the predominant mechanism by which PAF is inactivated. Therefore, PAF-AcH plays a major role in the potential for PAF to circulate or to function as a locally acting autacoid (43). The fact that there were high levels of PAF-AcH in BAL may suggest that PAF-AcH, at least partially, originates from plasma, crossing through the alveolar-capillary membrane because of increased permeability (44). The increased number of cells secreting PAF-AcH such as neutrophils may also play a role.

In conclusion, pulmonary gas exchange abnormalities and changes in lung mechanics after pulmonary embolism could be associated with alterations in lung surfactant and inflammation of lung tissue, expressed by an increase in PAF and neutrophils. The degree of hypoxemia is related to release of mediators such as PAF rather than to the magnitude of obstruction of pulmonary vessels.

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