Proteins and Phospholipids in BAL from Patients with Hydrostatic Pulmonary Edema

GEORGE NAKOS, JOHN PNEUMATIKOS, IRAKLIS TSANGARIS, CONSTANTINOS TELLIS, and MARILENA LEKKA

Intensive Care Unit, University Hospital of Ioannina, Chemistry Department, University of Ioannina, Ioannina, Greece

The purpose of the present study is twofold: to evaluate alterations in total phospholipid content and individual phospholipid classes of the surfactant, and to detect markers of inflammatory reaction in bronchoalveolar lavage (BAL) from patients with hydrostatic pulmonary edema (HPE). Mechanically ventilated patients with HPE (Group 1) were compared with mechanically ventilated patients without cardiopulmonary disease (Group 2), considered as the control group. Group 3, including patients with high-permeability pulmonary edema, was used for further comparison. BAL was obtained and immediately cooled at 4° C. Total proteins, albumin, and platelet-activating factor–acetylhydrolase (PAF-AcH) were measured. Total lipids were extracted and analyzed after thin-layer chromatographic separation. PAF was determined with bioassay. Total BAL proteins and albumin were found significantly higher in patients with HPE compared with control, but were lower compared with adult respiratory distress syndrome (ARDS). PAF was elevated in patients with HPE and ARDS, whereas in the control group it was actually in nondetectable levels. PAF was significantly higher in ARDS than in HPE patients. BAL neutrophils concentration was higher in HPE compared with control, but lower compared with ARDS. There was an inverse correlation between PAF-AcH and PAF. Quantitative reduction of total BAL phospholipids (PL) and qualitative deficiency was observed in both patients with HPE and ARDS. The findings of this study suggest that there is evidence of inflammation in the airspaces of patients with HPE.
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Hydrostatic pulmonary edema (HPE) is associated with increased hydrostatic pressure gradient. If pressure increases sufficiently to force liquid flux to exceed lymphatic clearance, liquid accumulates with eventual alveolar flooding.

Recently, there is accumulative evidence that patients with advanced congestive heart failure express elevated circulating levels of inflammatory cytokines such as tumor necrosis factor (TNFα) and leukotrienes (LTs) (1–3). Although the clinical significance of these substances is unclear, it is apparent that they could play a role in pathogenesis of congestive heart failure and HPE in terms of modulating the structure and function of lung and heart. It is known that proinflammatory mediators induce heart dysfunction and pulmonary edema which are hallmarks of HPE. However, the distinction between hydrostatic effects and proinflammatory-associated effects is a complex issue. Furthermore, high concentrations of β-thromboglobulin-like antigen and neutrophil activation peptide-2 were found in pulmonary edema fluid from patients with congestive heart failure. Neutrophil activation peptide-2 is chemotactic for neutrophils and can be generated by the cleavage of β-thromboglobulin-like antigens with cathepsin G. These data suggest that attention must be paid to the possible role of inflammation in the pathogenesis of pulmonary edema in patients with HPE (4).

Platelet-activating factor (PAF), a potent phospholipid mediator in inflammatory and allergic reactions, is known to be implicated in acute, but also in long-term pulmonary effects. It causes bronchoconstriction, increased vascular permeability, and recruitment of inflammatory cells, especially eosinophils, neutrophils, and platelets in the lungs. Recent studies strongly suggest that PAF is also an important mediator in immunoregulatory responses affecting cytokines (5, 6). Its metabolic inactivation involves cleavage of the acetyl group from alkylacetylglycerophosphocholine by a specific PAF-acetylhydrolase (PAF-AcH). The enzyme has no effect on phosphatidylcholine with long acyl chain at the second position and does not require calcium ions as typical phospholipase A_2 does. Its active form has been found in plasma associated with low-density lipoproteins (7, 8). PAF could cause or enhance pulmonary edema via pulmonary vasoconstriction and increased vascular permeability (7, 9, 10). In addition, PAF possibly directly or via proinfammatory cytokines inactivates surfactant and decreases surfactant synthesis (11).

Pulmonary surfactant is a material with good surface properties that lines lungs and its physiologic role is to lower surface tension and protect alveoli from collapsing, especially at the end of expiration. It is composed mainly of phospholipids (90%, wt/wt) and specific surfactant proteins (10%, wt/wt). Many lung disorders are associated with surfactant deficiency, qualitative and/or quantitative. Surfactant is recovered in bronchoalveolar lavage (BAL).

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Correspondence and requests for reprints should be addressed to George Nakos, M.D., F.C.C.P., Intensive Care Unit, University Hospital of Ioannina, University Street, 45500 Ioannina, Greece.

TABLE 1

DEMOGRAPHIC CHARACTERISTICS OF PATIENTS WITH HPE

Patient No.	Age (yr)	Sex	Disease
1	72	M	Ischemic heart disease
2	47	F	Cardiomyopathy
3	47	M	Ischemic heart disease
4	73	M	Ischemic heart disease
5	70	M	Ischemic heart disease
6	75	M	Hypertensive cardiovascular disease
7	59	M	Ischemic heart disease
8	71	M	Ischemic heart disease
9	68	F	Ischemic heart disease
Mean	63		
SD	12		

TABLE 3

CLINICAL CHARACTERISTICS OF CONTROL SUBJECTS

Patient No.	Age/Sex (yr)	Disease	IMV (days)	Pa_{O_2}/FI_{O_2}
1	75/F	Neuromuscular	3	340
2	28/M	Cranial trauma	8	420
3	45/M	Cranial trauma	2	378
4	34/M	Spinal trauma	7	360
5	55/M	Neuromuscular	2	410
6	50/M	Cranial trauma	5	380
Mean	48		4.50	381.33
SD	16.6		2.59	29.98

Definition of abbreviation: IMV = interval from initiation of mechanical ventilation to BAL procedure.

The purpose of the present study is twofold: to evaluate alterations in total phospholipid content and individual phospholipid classes of the surfactant, and to detect markers of inflammatory reaction in bronchoalveolar lavage (BAL) from patients with HPE.

METHODS

Patient Selection

Nine mechanically ventilated patients, 7 men and 2 women, 47 to 75 yr of age (mean age: 63 ± 12 yr) with acute HPE (Group 1) were eligible for this study (Table 1).

Criteria for diagnosis of pulmonary edema were as follows: sudden, recent onset of severe dyspnea, bilateral rales, chest radiographic findings consistent with pulmonary edema without a history suggesting aspiration or infection (12). The diagnosis of HPE was confirmed by right heart catheterization. The cause of HPE and clinical data are shown in Tables 1 and 2.

Indications for intubation and mechanical ventilation were: left ventricular failure refractory to medical support, hypotension and/or severe hypoxemia, respiratory muscle fatigue (rise of Pa_{CO_2}, rapid and shallow breathing, exhaustion). Patients with acute myocardial infarction, severe hypoxemia (ratio of arterial oxygen pressure to fraction of inspired oxygen [Pa_{O_2}/FI_{O_2}] < 100), and hemodynamic instability 24 h after initiation of mechanical ventilation were excluded.

Six mechanically ventilated patients, 5 men and 1 woman, 28 to 75 yr of age (mean age 48 ± 16.6 yr) without cardiopulmonary disease were used as the normal control group (Group 2). Diagnosis, the cause of mechanical ventilation, and clinical characteristics of the control group patients are shown in Table 3.

In addition, six patients, 4 men and 2 women, with average age 61 ± 15 yr (range 35 to 75 yr) with adult respiratory distress syndrome (ARDS) in early stage (Group 3), were included as a further comparison with HPE (Table 4).

Standard criteria for diagnosis of ARDS were: (*1*) acute hypoxemic respiratory failure requiring mechanical ventilation; (*2*) diffuse bilateral alveolar infiltrates on the chest roentgenogram; (*3*) refractory hypoxemia (Pa_{O_2}/FI_{O_2} < 200 regardless of positive end-expiratory pressure [PEEP] level); (*4*) pulmonary artery wedge pressure < 18 mm H_2O or no clinical evidence for left atrial hypertension, and (*5*) appropriate clinical setting or risk factor for the development of ARDS (13).

The protocol was approved by the ethics committee of the University Hospital of Ioannina and the patients or the next of kin gave an informed consent to the study.

Study Protocol

Patients following the clinical criteria of HPE diagnosis with indication for intubation were intubated, and mechanical support of breathing was initiated. A pulmonary catheter (Opticath; Abbott, Chicago, IL), via an internal jugular or subclavian vein, as well as an arterial line were introduced. When the patients were hemodynamically stable and blood oxygenation was acceptable Pa_{O_2} > 60 mm Hg, $FI_{O_2} \leq 0.5$), BAL was performed. The time interval between intubation and BAL was less than 24 h (range 12 to 24 h).

Measurements. For each patient with HPE, the following parameters were recorded: age, duration of mechanical ventilation, Pa_{O_2}/FI_{O_2}, mixed venous O_2 saturation (Sv_{O_2}), PEEP, compliance of respiratory system (Crs), pulmonary arterial wedge pressure (Pwp), cardiac index (CI), pulmonary arterial pressure (Ppa), and systemic arterial pressure (AP). Capillary pressure (Pcap) was calculated by the formula: Pcap = Pwp + 0.4 (mPpa − Pwp), where mPpa is mean pulmonary arterial pressure.

TABLE 2

CLINICAL CHARACTERISTICS OF PATIENTS WITH HPE

Patient No.	Chest X-ray Score	MV (days)	IMV (h)	Crs (ml/cm H_2O)	mAP (mm Hg)	mPAP (mm Hg)	mPcap (mm Hg)	mPwp (mm Hg)	CI (L/min/m^2)	Po_2/FI_{O_2}	Sv_{O_2} (%)	PEEP (cm H_2O)	Outcome
1	24	3	18	52	104	32	29	27	2.1	145	62	10	S
2	2/4-P	2	24	48	11.6	37	30.4	26	2.4	170	54	8	S
3	4/4-P	4	12	36	85	30	27	25	1.9	134	50	7	S
4	2/4	2	24	55	87	40	32.8	28	2	159	48	11	S
5	4/4-P	6	12	31	80	37	28.6	23	2.1	128	50	8	D
6	4/4	3	24	38	75	35	30.8	28	2.3	190	51	7	S
7	4/4	3	18	44	83	37	30.4	26	2.1	155	46	10	S
8	4/4-P	5	18	35	73	39	30	24	2.1	140	45	10	S
9	4/4-P	5	18	32	70	35	29	25	1.8	140	44	9	S
Mean		3.7	18.7	41.2	78.0	35.8	29.8	25.8	2.1	151.2	50.0	8.9	1/9
SD		1.4	4.7	8.8	6.4	3.2	1.6	1.7	0.2	19.5	5.5	1.4	

Definition of abbreviations: MV = mechanical ventilation; IMV = interval from initiation of mechanical ventilation to BAL procedure; Crs = compliance of respiratory system; mAP, mPAP, mPcap, mPwp = mean arterial, pulmonary, capillary, wedge pressure, respectively; P = pleuritis.

TABLE 4

CLINICAL CHARACTERISTICS OF THE ARDS PATIENTS

Patient No.	Age/Sex	Diagnosis	ALI Score	MV (*days*)	IMV	Po_2/Fio_2	PEEP	Crs	Chest X-ray Score	Outcome
1	70/M	Pneumonia	2.75	12	7	156	7	35	4/4	S
2	75/M	Peritonitis	3	11	2	98	9	28	4/4	S
3	35/F	Pneumonia	3	13	6	92	8	23	4/4	S
4	46/F	Viral pneumonia	3.25	7	2	90	9	18	4/4	D
5	71/M	Multiple trauma	3.5	8	2	88	10	18	4/4	D
6	67/M	Multiple trauma	3.5	16	3	86	10	19	4/4	D
Mean	61		3.1	11.2	3.7	101.7	8.8	23.5		
SD	15		0.3	3.0	2.2	26.9	1.2	6.8		

Definition of abbreviations: ALI = acute lung injury; MV = mechanical ventilation; IMV = interval from initiation of mechanical ventilation to BAL procedure; D = death; S = survival.

BAL Procedure

BAL was performed by fiberoptic bronchoscopy. Patients were ventilated with a volume control mechanical ventilation model (Model 900C; Siemens, Sweden). During the BAL procedure, Fio_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, AP, arterial oxygen saturation by pulse oximetry, and mixed Svo_2 were monitored throughout the procedure. Trachea was suctioned before introducing the bronchoscope through an adapter (swivel adapter) which allows the maintenance of mechanical ventilation. The tip of the bronchoscope was then wedged in a segmental or subsegmental bronchus of the right middle lobe or the lingula. Six aliquots of 20-ml 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, while the others were collected in ice-cold tubes to avoid PAF degradation due to AcH activity. BAL was then filtered through sterile gauze and centrifuged at 500 × *g* for 15 min at 4° C to remove mucus and cells respectively.

Biochemical Parameters in BAL

Total protein and albumin were measured according to the methods of Lowry and coworkers (14) and Doumas and Biggs (15).

Total lipids were extracted according to Bligh and Dyer (16) and separated into classes after two successive developments of K-6 thin-layer plates (Whatman) in the same direction, using the following solvent systems: (A) chloroform–petroleum ether–methanol–acetic acid (50:30:15:10, vol/vol) to the top, and (B) chloroform–methanol–water–acetic acid (65:35:5:10, vol/vol) to the top.

Total phospholipids and individual phospholipid classes (after thin-layer chromatography separation) were measured according to Bartlett (17).

PAF was determined in the lipid extract of BAL, after thin-layer chromatography purification with chloroform–methanol–water (65:35:7, vol/vol), from the aggregation caused on aspirinated washed rabbit platelets pretreated with creatine phosphate–creatine phosphokinase (CP/CPK), according to Bossant and coworkers (18). Standard curve was assessed using the hexadecyl analog of PAF. Detection limit under our experimental conditions was 4 pg PAF/ml BAL. Low temperatures were maintained throughout the procedure to avoid PAF degradation.

AcH activity was assessed in BAL fluids as well as in sera, according to Tselepis and coworkers (19), after trichloracetic acid (TCA) precipitation. The activity was also tested in the presence of Ca^{2+}, EDTA, bromophenacylbromide, and 0.5×10^{-4} M 1,2-dipalmitoyl-sn-glycero-3-phosphocholine to assess its specificity.

BAL Cells

BAL cell differential counts were performed by counting at least 300 cells in cytocentrifuge preparations stained with hematoxylin–eosin.

Statistical Analysis

Data are reported as mean ± SD. Statistical comparisons between groups were performed using analysis of variance (ANOVA) (Statistica 4.3 software package). Correlations between values were analyzed by linear regression, using a standard software package (Statview 512+). The level of significance was defined as $p < 0.05$.

RESULTS

BAL Fluid

The volumes of BAL fluid recovered from the 120 ml saline instilled ranged from 25 (21%) to 64 ml (53%), mean volume 39 ml (33%).

Protein Content

Total BAL protein was found significantly increased ($p < 0.001$) in patients with HPE (mean value 427 ± 181 μg/ml) compared with our control group (mean value 125 ± 48 μg/ml), but was significantly ($p < 0.001$) lower in comparison to ARDS patients (mean value 1,286 ± 333 μg/ml). Albumin was significantly higher in HPE (106 ± 31 μg/ml, $p < 0.001$) and ARDS (328 ± 65 μg/ml, $p < 0.001$) compared with our normal control group (26 ± 11 μg/ml). Albumin was significantly lower in HPE in comparison to ARDS patients ($p < 0.001$) (Table 5).

BAL Cell Differential Count

Alveolar macrophages were found significantly decreased and neutrophils increased in BAL from patients with HPE and ARDS compared with the control group ($p < 0.01$). BAL neutrophils in ARDS were significantly higher ($p < 0.01$) in comparison with HPE patients (Table 5). The total number of BAL cells was higher in HPE and ARDS patients compared with the control group, [$(38.5 \pm 33) \times 10^4$, $(53 \pm 40) \times 10^4$, and $(9.5 \pm 7) \times 10^3$ in HPE, ARDS, and control, respectively]. The difference between HPE and ARDS patients was not statistically significant.

PAF Content and PAF-AcH Activity

PAF was elevated in BAL from patients with HPE (196 ± 83 pg/9 ml BAL) as well as in patients with ARDS (406 ± 96 pg/9 ml) compared with the control group, in 4 of 6 samples of which PAF was in nondetectable concentrations, and in the other two it was 36 pg/9 ml ($p < 0.01$, $p < 0.001$, respectively) (Table 5). The difference between PAF concentrations in patients with HPE and ARDS was also statistically significant ($p < 0.001$).

AcH activity, catalyzing the cleavage of the acetyl group from PAF, in the absence of calcium ions, was observed in BAL fluid. The activity was not abolished by *p*-bromophenacyl bromide, an inhibitor of typical phospholipase A_2 and was not influenced by the presence of phosphatidylcholine. It was therefore characterized as PAF-specific. PAF-AcH was elevated in BAL from patients with HPE (0.063 ± 0.068 nmol PAF/ml/min) as well as in patients with ARDS (0.074 ± 0.045 nmol PAF/ml/min) compared with the control group (0.009 ± 0.01 nmol PAF/ml/min), $p < 0.01$. There was an inverse correlation ($r = 0.68$, $p < 0.05$) between PAF and PAF-AcH (Figure 1). In one case where PAF and PAF-AcH were both in high concentrations in BAL, PAF-AcH was found high in the serum as well (31.4 nmol PAF/ml/min).

TABLE 5

BIOCHEMICAL CHARACTERISTICS IN BAL FROM PATIENTS WITH HPE IN COMPARISON TO CONTROL SUBJECTS AND ARDS PATIENTS

Parameters	HPE (mean ± SD)	ARDS (mean ± SD)	Control (mean ± SD)	
Total protein, μg/ml	427 ± 181	1,286 ± 333	125 ± 48	*#+
Albumin, μg/ml	106 ± 31	328 ± 65	26 ± 11	*#+
Differential cell count				
Alveolar macrophages	70 ± 5	52 ± 7	86 ± 3	*#+
Neutrophils	22 ± 5	40 ± 8	3 ± 1	*#+
Lymphocytes	6 ± 1	7 ± 3	11 ± 3	–
Eosinophils	1 ± 0.3	2 ± 0.3	0	–
PAF, pg/9 ml BAL	196 ± 83	406 ± 96	36	*#+
PAF-AcH, nmol PAF/ml BAL/min	0.063 ± 0.068	0.074 ± 0.045	0.009 ± 0.001	*+
Total phospholipids, μg P/ml BAL	1.6 ± 0.32	1.4 ± 0.3	3.1 ± 0.9	*+
Phosphatidylcholine, % of total PL	45.0 ± 5.0	43.0 ± 11	68.3 ± 7	*+
Phosphatidylglycerol, % of total PL	7.8 ± 1.4	6.8 ± 2.4	8.4 ± 2.9	–
Sphingomyelin, % of total PL	11.4 ± 3.3	11 ± 4.1	4.9 ± 2.4	*+
Phosphatidylethanolamine, % of total PL	8.6 ± 1.8	11 ± 3.9	3.4 ± 0.5	*+
Phosphatidylserine, % of total PL	5.0 ± 1.3	9.2 ± 5.5	4.5 ± 1.3	#+
Phosphatidylinositol, % of total PL	5.6 ± 1.3	7.2 ± 2.6	3.9 ± 0.7	*+
Lyso-phosphatidylcholine, % of total PL		3 ± 1	–	#+

Definition of abbreviations: S = statistically significant. Asterisk (*) denotes statistically significant difference between HPE and control values. # denotes statistically significant difference between HPE and ARDS. + denotes statistically significant difference between ARDS and control values.

Surfactant Phospholipids

A quantitative reduction of total BAL phospholipids (PL), as well as a qualitative deficiency was observed in patients with HPE and ARDS compared with the control group. Total PL, expressed in μg lipid phosphorus (μg P) per ml BAL, were: 1.6 ± 0.3, 1.4 ± 0.3, and 3.1 ± 0.9 μg P/ml in HPE, ARDS patients, and control group, respectively. The differences between patients and control group were statistically significant ($p < 0.001$). There was no statistical difference between HPE and ARDS patients. The percentage of phosphatidylcholine (PC) of total PL was reduced in patients with HPE and ARDS compared with the control group, mean values being 45 ± 5%, 43 ± 11%, and 68.3 ± 7% respectively ($p < 0.001$). There was no statistical difference between HPE and ARDS patients. The proportion of sphingomyelin (SM) (mean values 11.4 ± 3.3%, 11 ± 4%, and 4.9 ± 2.4% in patients with HPE, ARDS, and control, respectively), phosphatidylethanolamine (PE) (mean values 8.6 ± 1.8%, 11 ± 3.9%, and 3.4 ± 0.5% in patients with HPE, ARDS, and control, respectively) and phosphatidylinositol (PI) (mean values 5.6 ± 1.3%, 7.2 ± 2.6%, and 3.9 ± 0.7% in patients with HPE, ARDS, and control, respectively) were higher in patients with HPE and ARDS compared with the control group ($p < 0.01$, $p < 0.001$, and $p < 0.05$, respectively). The differences between HPE and ARDS patients were not significant.

There was no difference in the percentage of phosphatidylglycerol (PG) (mean values 7.8 ± 1.4%, 6.8 ± 2.4%, and 8.4 ± 2.9% in patients with HPE, ARDS, and control, respectively. Phosphatidylserine (PS), though, was higher in ARDS patients (9.2 ± 5.5%) compared with HPE (5 ± 1.3%), and with the control group subjects (4.5 ± 1.3%) ($p < 0.05$). Lyso-phosphatidylcholine (Lyso-PC) was also detected in high concentrations (mean value 3 ± 1.0%) ($p < 0.001$) in BAL from ARDS. In contrast, lyso-PC was not detected in patients with HPE nor in the control group (Table 5).

DISCUSSION

Our results show that BAL protein, PAF, and neutrophils were significantly elevated in patients with HPE compared with control, but lower than in patients with ARDS. PAF-AcH activity was inversely correlated to the PAF concentrations. Quantitative and qualitative alterations in surfactant were also found in both groups of patients.

Protein Content

The fluid of HPE containing a small amount of proteins has the properties of a transudate. The ratio of protein concentration in the alveolar fluid to that in plasma is less than 0.65 (20–22). Because of the dilution, such a ratio can not however be used for BAL fluid. Alternatively, endogenous indicators such as urea, inulin, and methylene blue have been used to allow calculation of the alveolar fluid volume, but all these methods lack precision (23, 24). In this study total BAL protein and albumin from patients with HPE were compared with those of a mechanically ventilated control group on the one hand, and with a high-permeability pulmonary edema group of patients on the other. BAL protein concentrations in patients with HPE were higher in comparison to the control group, but significantly lower when compared with the ARDS patients. The increased protein observed in BAL can be attributed either to an increase of the alveolar fluid volume, or to increased alveolar protein concentration, or both; this distinction though is not feasible because of the absence of reliable alveolar fluid volume markers. Nevertheless, the elevated BAL protein in patients with HPE is expected.

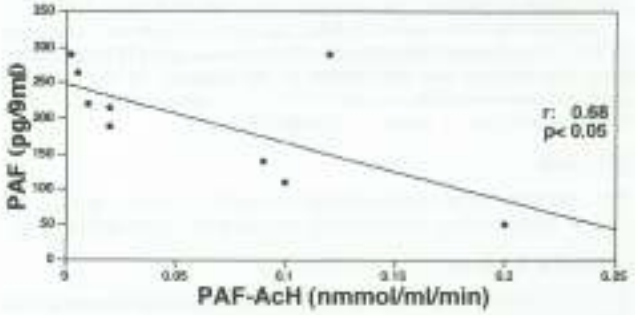

Figure 1. Relationship between PAF and PAF-AcH in patients with HPE. A significant inverse correlation was found ($r = 0.68$, $p < 0.05$).

HPE is associated with a bulk movement of protein into the airspaces secondary to high interstitial pressure which results in a flooding of the airspaces with interstitial fluid.

Reabsorption of excess fluid begins from the time the airspace becomes flooded (25). Current evidence indicates that the process of alveolar fluid reabsorption depends on sodium uptake by channels on the apical membrane of alveolar type II cells, with subsequent active transport of sodium to the basolateral interstitial space by the Na^+-K^+ ATPase pump (26). The alveolar protein concentration increases in proportion to the removal of the liquid volume from the airspace. It is important also to emphasize that the reabsorption of fluid presupposes, at least in some degree, functional and intact type II alveolar cells (20). Therefore, an increase of protein concentration due to removal of alveolar fluid must be considered, provided that alveolar fluid is removed faster than the alveolar protein.

The movement of protein from the vascular into the interstitial and airspaces could be increased when the capillaries are exposed to high transmural pressure. Stress failure in pulmonary capillaries has been described with transmural pressure $\geq$ 40 mm Hg (27). High microvascular pressure could lead to modifications of alveolar epithelium (27, 28) as well as to disruptions of both epithelial and endothelial cell layers (28, 29). These epithelial and endothelial disruptions are reverted rapidly when the pressure is subsequently reduced (30). Nicolaysen and colleagues (31) also showed that in 3 of 6 lungs the increase in hydraulic conductivity was fully reversible when the pressure was lowered. However, in the other three lungs the filtration rate remained elevated after the pressure had been reduced. They concluded that some capillary wall change must be involved. In the study by Rippe and associates (32), no difference from the control group in capillary filtration coefficient was found when the left atrial pressure was < 30 cm H_2O. However, with left atrial pressure > 55 cm H_2O, the filtration coefficient was ≥ 1.5 times that of the controls. The authors concluded that these results are consistent with either the stretching of existing pores or the opening of a few large pores. In our patients with HPE the capillary pressure was calculated as high as 30 mm Hg. Because the wall stress of the capillaries under these conditions is considered high, the movement of protein from the vascular to the alveolar space could be due to stress failure (28) and this could explain, at least partially, the presence of proteins in the alveolar space. BAL proteins and especially albumin from patients with HPE were lower than those from the ARDS patients, as is expected in high-permeability pulmonary edema (22, 33), where in addition, the alveolar–capillary changes are not so rapidly reversible as in HPE.

Our findings are consistent with those of Doyle and coworkers (34) who showed that surfactant protein SP-A was significantly elevated in patients with acute HPE compared with control subjects, but was lower than that in patients with ARDS. They concluded that the increased leakage of SP-A into the bloodstream reflects the change in alveolar–capillary permeability and that serum SP-A is a marker of alveolar–capillary membrane injury. It should be mentioned, though, that the increase of SP-A movement to the serum does not prove alveolar or capillary injury leading to increase of alveolar–capillary permeability but may simply reflect the widening of epithelial and endothelial pores.

There is an abundance of literature documenting that remote tissue hypoxia results in pulmonary microvascular injury with increased capillary–alveolar membrane permeability (35). Our patients with HPE did not undergo a sustained cardiopulmonary arrest. Therefore, ischemia/reperfusion syndrome from remote tissue hypoxia is unlikely to result in pulmonary microvascular injury.

Mechanical ventilation as well as BAL procedure may result in lung injury even in intact lungs (23, 36). The factors predisposing for lung damage during mechanical ventilation are: high inspiratory pressure or volume, long duration of mechanical ventilation, and repetitive closing and opening of terminal airways (27). PEEP may prevent lung injury from mechanical ventilation eliminating the opening and closing of terminal airways, thereby decreasing shear stress (38, 39). Our patients with HPE were lavaged soon after initiation of mechanical ventilation. Ventilation with relatively low tidal volume, airway pressure, and PEEP were applied. Therefore, ventilator-induced lung injury does not seem very likely.

Another cause of alteration in alveolar protein could be a local inflammatory response. Our findings of increased neutrophils and PAF in BAL suggest some degree of inflammation in lung parenchyma. Therefore, the local inflammation could increase BAL protein concentrations by two mechanisms: increase of permeability, or contamination with proteins of alveolar fluid produced by inflammatory reaction, or both. However, the neutrophils and PAF in HPE were significantly lower than in ARDS patients meaning that inflammation with any consequent change in permeability should be less important in HPE compared with ARDS. Inflammation requires activation of cells, possibly neutrophils, capable of producing inflammatory mediators. It has been shown that after alveolar autologous serum instillation there was a marked increase in neutrophil recovery (25). Serum proteins seem to be a potent neutrophil chemotactic effector (40). The cause of inflammation is not well understood, but the influx of proteins into airspaces could initiate an inflammatory response. It has also been shown that edema fluid in HPE contains the chemotactic agent, neutrophil activation peptide-2, and it could lead to progressive neutrophil activation and lung injury (4). These data are compatible with our results of increased neutrophils in BAL fluid and support the concept that additional mechanisms to the hydrostatic force lead to the development of HPE (4).

PAF Content–PAF-AcH Activity

Elevated PAF in BAL from patients with HPE suggests inflammatory reaction locally in the lung parenchyma. It does not seem possible that PAF originates from a remote tissue, because of the presence in plasma of high levels of PAF-AcH activity that catalyzes the cleavage of acetyl-group from PAF. PAF, an ether-linked phospholipid with biologic activity relevant to the pathogenesis of inflammatory disorders (41), is produced by a variety of inflammatory cells. PAF could be implicated in pulmonary edema because of its ability to cause microvascular leaking (42, 43), to stimulate biosynthesis of potent inflammatory mediators (5) as well as to induce pulmonary vasoconstriction increasing the pulmonary capillary pressure (7, 43). 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 autocoid (8). In our samples, PAF and PAF-AcH had an inverse correlation, indicating once again the local origin of PAF. In the case where PAF and PAF-AcH are both in high concentrations, at least part of the enzyme could originate from plasma and enter into the alveolar fluid because of contamination of BAL with blood. PAF-AcH activity was not affected either by the presence or absence of calcium ions, or after treatment with EDTA, or by the presence in the incubation medium of 0.5×10^{-4} M phosphatidylcholine. These features are consistent with PAF-AcH properties that differ from those of a typical phospholipase A_2 (9).

Surfactant Phospholipids

Quantitative deficiency of surfactant was found in patients with

HPE as well as in ARDS. This finding agrees with the results of Gregory and colleagues (44), who documented decreased total PL content in their group of patients with ARDS. Other laboratories have not found changes of total PL content in their ARDS patients (21, 45). These differences could reflect a different degree of type II cell injury, different experimental protocol, or the lung area that was lavaged. The qualitative alterations of surfactant in patients with HPE were similar to those observed in patients with ARDS (21, 44–46). There is not a good explanation for the few observed qualitative differences between HPE and ARDS patients. Surfactant abnormalities could be attributed to type II alveolar cell dysfunction, interaction of surfactant with airspace proteins, inactivation by inflammatory mediators, or a combination of these factors (44). Surfactant abnormalities increase surface tension, giving rise to increased transmural hydrostatic force, possibly by decreasing perimicrovascular pressure (47). Whether lung permeability increases with surfactant dysfunction is uncertain. Some investigators have found that detergent-induced surfactant dysfunction has no effect on vascular permeability, whereas others have found increased permeability (48, 49).

The surfactant alterations were similar in patients with HPE and ARDS, but the clinical course as well as the outcome were much better in HPE than in ARDS patients. According to the above observations, it could be concluded that this measured level of surfactant alterations by itself is not sufficient to cause prolonged respiratory failure.

Inflammatory Reaction

Neutrophils and PAF content were elevated in BAL from both types of pulmonary edema; however, they do not seem to have the same effect on the course of acute respiratory failure. The severity of inflammation is more intense in patients with ARDS, as indicated from the higher concentrations of neutrophils and PAF, and probably plays a major role in the pathogenesis of high-permeability pulmonary edema. On the other hand, the clinical significance of the inflammation may be minor in patients with HPE as indicated from the rapid improvement of those patients.

In summary, the findings of this study suggest that inflammation and surfactant deficiency play a role in the pathogenesis of HPE. The differences between hydrostatic and high-permeability pulmonary edema could reflect the degree and the extent of the alveolar and capillary injury.

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