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Short Communication

Elevated Concentrations of Leukotriene D_4 in Pulmonary Edema Fluid of Patients with the Adult Respiratory Distress Syndrome[1]

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The possible contribution of metabolites of arachidonic acid to the increased permeability of the alveolar–capillary barrier in the adult respiratory distress syndrome was examined by quantifying the pulmonary edema fluid concentrations of lipoxygenase and cyclooxygenase products. The concentration of leukotriene D_4 in pulmonary edema fluid of 10 patients with the adult respiratory distress syndrome (18.5 ± 6.8 pmol/ml; mean ± SD), assessed by specific radioimmunoassay after isolation of the mediator, was significantly higher ($P < 0.001$) than that of five patients with cardiogenic pulmonary edema (4.4 ± 1.1 pmol/ml). The concentrations of leukotrienes B_4 and C_4, prostaglandin E_2, and thromboxane B_2 in edema fluid were not significantly different in the adult respiratory distress syndrome patients than in the other subjects with pulmonary edema. The edema fluid concentration of leukotriene D_4 correlated with the ratio of edema fluid to plasma concentrations of albumin ($r = 0.64$). Leukotriene D_4 thus may contribute to the permeability defect which allows an accumulation of protein-rich alveolar fluid in the adult respiratory distress syndrome.

KEY WORDS: Leukotrienes; adult respiratory distress syndrome; pulmonary edema.

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INTRODUCTION

The protein-rich pulmonary edema of the adult respiratory distress syndrome (ARDS) is attributable principally to an increase in permeability of the alveolar–capillary barrier (1–3). The many etiologies of ARDS suggest that altered permeability is a common cellular response of the alveolar epithelium and pulmonary capillary endothelium to diverse extrinsic and endogenous challenges. Although the results of studies of patients with ARDS and animal models of ARDS imply an involvement of polymorphonuclear (PMN) leukocytes (4, 5), intravascular coagulation with platelet aggregation (6, 7), and fluid-phase mediators, such as complement (8, 9) and prostaglandins (10, 11), no primary causative relationship has been established definitively. The present finding of an elevated concentration of leukotriene D_4 (LTD_4) in pulmonary edema fluid of patients with ARDS relative to that found in cardiogenic edema fluid suggests a contribution to the permeability abnormalities of ARDS.

MATERIALS AND METHODS

Selection and Evaluation of Patients

Each of the 19 patients studied (Table I) had acute pulmonary edema that was severe enough to require endotracheal intubation and mechanical ventilation. Clinical and laboratory data, as well as systemic and pulmonary hemodynamic values,

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Table I. Characteristics of Patients with Pulmonary Edema[a]

Case no.	Age/sex	Diagnosis	PAW (mm Hg)	AL (EF)/AL (P)	TP (EF)/TP (P)	Clinical outcome
			Cardiogenic pulmonary edema			
1	31/F	CHF, renal failure	28	0.50	0.43	Survived
2	64/F	CHF, CPR	30	0.41	0.34	Died
3	55/F	CHF, renal failure	33	0.50	0.65	Survived
4	18/M	CHF, nephrosis	—[b]	0.36	0.34	Survived
5	57/M	CHF, AMI	—[b]	0.67	0.56	Survived
				0.49 ± 0.11[c]	0.46 ± 0.14[c]	
			ARDS			
6	42/F	Leukemia, sepsis	14	1.12	1.03	Died
7	70/F	Gastric aspiration	—[d]	1.12	0.90	Died
8	30/F	Renal failure, transfusion	10	0.99	0.97	Survived
9	35/M	GI bleed, sepsis	10	0.69	0.58	Died
10	23/M	Methanol overdose	—[d]	0.73	0.61	Survived
11	30/F	Acetaminophen overdose	—[d]	0.84	0.71	Survived
12	70/F	Vascular surgery, sepsis	12	0.85	0.77	Died
13	42/F	Cirrhosis, sepsis	6	0.85	0.79	Died
14	15/F	Leukemia, sepsis	7	—	—	Died
15	52/F	Alcoholism, pancreatitis	8	—	—	Survived
				0.90 ± 0.16[c]	0.80 ± 0.16[c]	

[a]CHF, congestive heart failure; ARDS, adult respiratory distress syndrome; CPR, cardiopulmonary resuscitation; AL, albumin; TP, total protein; EF, pulmonary edema fluid; P, plasma; PAW, pulmonary arterial wedge pressure.
[b]The central venous pressure of both patient 4 and patient 5 was elevated to greater than 15 mm Hg and echocardiography demonstrated left ventricular dysfunction.
[c]Mean ± SD.
[d]The central venous pressure and left ventricular function were normal.

were recorded at the time of intubation. ARDS was defined operationally by an arterial to alveolar oxygen partial pressure ratio of ≤0.20, the appearance of diffuse bilateral infiltrates on chest roentgenogram, acute respiratory failure severe enough to require mechanical ventilation, a pulmonary arterial wedge pressure equal to or less than 15 mm Hg, and the absence of congestive heart failure, pleural effusions, atelectasis, or pneumonia (12–14). Cardiogenic pulmonary edema was defined on the basis of an unequivocal history and signs of left ventricular failure and either a pulmonary arterial wedge pressure greater than 25 mm Hg or an elevated central venous pressure with evidence of decreased left ventricular function by a two-dimensional echocardiogram. Within 12 hr after endotracheal intubation, edema fluid samples of greater than 3 ml were suctioned gently with a soft plastic catheter from frothing edema fluid in the endotracheal tube into a Lukens trap and 100 U of heparin was added to each sample. Heparinized plasma and edema fluid samples were centrifuged at 6000*g* for 10 min. Albumin concentrations were measured by the bromcresol green dye-binding technique (15) and total protein concentrations by the Biuret method (16) in both the edema fluid and the plasma samples.

Experimental Materials and Methods

[14,15-^{3}H(*N*)]Leukotriene C_4 (34.0 Ci/mmol), [14,15-^{3}H(*N*)]leukotriene D_4 (36.0 Ci/mmol), [1-^{14}C]prostaglandin E_2 (25–50 mCi/mmol), [5,6,8,9,11,12,14,15-^{3}H(*N*)]thromboxane B_2 (100–160 Ci/mmol) (New England Nuclear, Inc., Boston), [5,6,8,9,11,12,14,15-^{3}H(*N*)]leukotriene B_4 (180–221 Ci/mmol) (Amersham Corp., Arlington Heights, IL), Sep-Pak columns (Waters Associates, Milford, MA), and kits for radioimmunoassays of prostaglandin E_2 and thromboxane B_2 (Seragen, Inc., Boston) were obtained from the suppliers noted. Synthetic leukotrienes B_4, C_4, D_4, and E_4 were provided generously by Dr. J. Rokach of Merck Frosst Laboratories, Dorval, Canada.

Pulmonary edema fluids of 0.1–0.5 ml were acidified to pH 3.5 with 0.1 *M* acetic acid and applied to Sep-Pak columns, the columns were washed with 2 ml of distilled water, 2 ml of 0.001 *M* acetic acid, and 2 ml of methanol:0.001 *M* acetic acid (1:4, v:v),

and the leukotrienes and other eicosanoids were eluted in 2 ml of methanol. One-half of each of the eluates was dried *in vacuo* and redissolved for reverse-phase high-performance liquid chromatography (HPLC) on a 4.6 mm × 25-cm column of 10 μm octadecylsilane (Ultrasil, Altex Division of SmithKline Beckman, Inc., Berkeley, CA) that was developed isocratically with methanol:0.03 g phosphoric acid per 100 ml distilled water (pH 3.5 with ammonium hydroxide) (70:30, v:v) at a flow rate of 1 ml/min in a dual metered-pump system (Beckman Instruments, Inc., San Jose, CA) (17). The elution times of LTC_4, LTB_4, and LTD_4, as assessed optically, were 14.1–15.6, 19.3–20.7, and 22.4–23.8 min, respectively (range, $N = 8$).

Radioimmunoassays for leukotrienes were performed as described with rabbit antisera of a high specificity for LTC_4 and LTB_4 (17, 18) and a rabbit antiserum which recognizes each of the C-6 peptide leukotrienes, using [^{3}H]LTD_4 and synthetic LTC_4 or LTD_4 as the competing ligand (Drs. E. Hayes and A. Rosenthal, Merck Laboratories, Inc., Rahway, NJ) (19). Each value was corrected for the recovery of radiolabeled compound.

RESULTS

Characteristics of Patients with Pulmonary Edema

The five patients considered to have cardiogenic pulmonary edema (Table I), based on either elevated pulmonary arterial wedge pressure (patients 1–3) or elevated central venous pressure and left ventricular dysfunction (patients 4 and 5), had ratios of pulmonary edema fluid to plasma concentrations of both albumin and total protein (Table I) which were in the range described by other investigators for high-pressure pulmonary edema (3). For the 10 patients with ARDS, the pulmonary artery wedge pressure (patients 6, 8, 9, 12–15) or central venous pressure was normal at the time of collection of pulmonary edema fluid and there was no evidence of heart failure. The ratios of the edema fluid to plasma concentrations of albumin and total protein in eight of the ARDS patients were significantly higher than those of the cardiogenic pulmonary edema patients ($P < 0.05$) (Table I) and were in the range reported for patients with increased lung vascular permeability (20).

Clinical and hemodynamic evaluations of the four patients with pulmonary edema of indeterminate etiology indicated that all appeared to have both a moderate elevation in pulmonary vascular pressure and an element of increased pulmonary vascular permeability. The ratios of pulmonary edema fluid to plasma concentrations of 0.69 ± 0.15 (mean ± SD) for albumin and 0.62 ± 0.19 for total protein were midway between the values for patients in the cardiogenic and ARDS groups (Table I).

Four of five patients with cardiogenic pulmonary edema survived, while 6 of 10 patients with ARDS died, of whom 5 had gram-negative bacterial sepsis and evidence of multisystem failure.

Concentrations of Leukotrienes and Other Eicosanoids in Pulmonary Edema Fluids

The leukotrienes in replicate samples of pulmonary edema fluids were resolved by HPLC and quantified by the LTB_4 radioimmunoassay and the C-6 peptide radioimmunoassay for LTC_4, LTD_4, and LTE_4. The concentrations of LTD_4 in edema fluids of ARDS patients (18.5 ± 6.8 pmol/ml, mean ± SD) were elevated significantly relative to control values (4.4 ± 1.1 pmol/ml), whereas no significant differences were established for LTB_4 or LTC_4 (Fig. 1), and LTE_4 was not detectable in any of the samples. The edema fluid concentrations of LTC_4, as determined by a monospecific radioimmunoassay of Sep-Pak eluates prior to HPLC, also were not significantly different for the ARDS (5.4 ± 2.3 pmol/ml) and control (3.7 ± 2.1 pmol/ml) patients. Although the concentrations of PGE_2 and TxB_2 in the edema fluids of some ARDS patients were elevated strikingly, the respective means for the group of ARDS patients evaluated were not significantly higher than for the control patients with cardiogenic pulmonary edema (Fig. 1). In order to rule out substantial conversions of the leukotrienes in edema fluid *in vitro* during processing, duplicate 0.1-ml portions of fluid from three patients with ARDS were incubated for 15 min at room temperature with 0.1 μCi of [^{3}H]LTC_4 or [^{3}H]LTD_4 and 10 pmol of the respective unlabeled leukotriene and subjected to Sep-Pak extraction and HPLC. The mean recoveries of the radioactivity of LTC_4 and LTD_4 were 67 and 82%, respectively. The conversion of LTC_4 to LTD_4 was less than 2% and that of LTD_4 to LTE_4 ranged from 6 to 13%.

Pulmonary edema fluids from the four patients in the indeterminate category were analyzed in parallel with the index populations. The concentrations of LTC_4 determined after Sep-Pak extraction were 4.2 ± 1.2 pmol/ml, and those of LTC_4, LTD_4, and

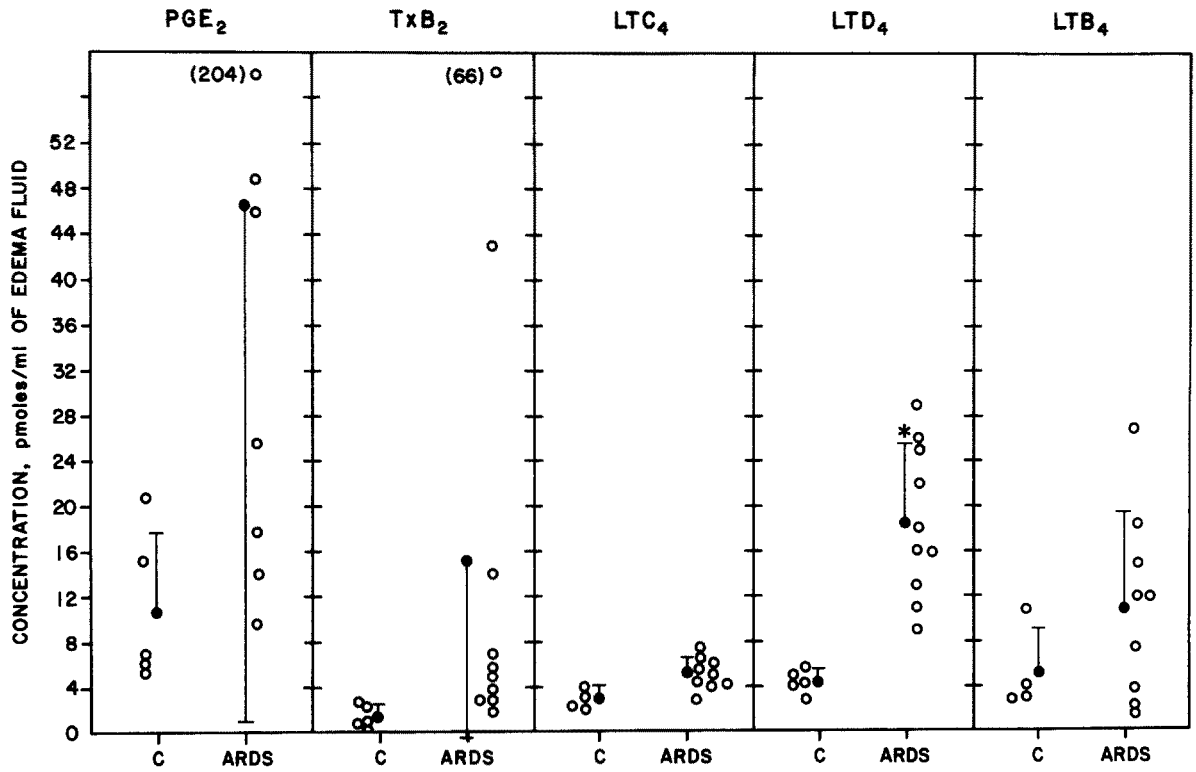

Fig. 1. Concentrations of arachidonic acid metabolites in airway edema fluid. Each frame shows the individual values (○) for each patient with cardiogenic pulmonary edema (C) or ARDS, as well as the group mean (●) and SD. Mucoid fluids suctioned from two patients (LTD_4 = 18 and 16 pmol/ml) and free edema fluid from the others were used for analysis. The quantities of LTB_4 and LTC_4 recovered from HPLC and of PGE_2 from Sep-Pak columns were insufficient for analyses in a few of the patients. All values are derived from radioimmunoassays and have been corrected for chromatographic recovery. The significance of the difference between the mean value for ARDS and that for C patients was determined by a standard two-sample *t* test for unpaired populations with unequal variance and is shown by the asterisk ($P < 0.001$).

LTB_4 after HPLC purification were 4.4 ± 1.9, 6.1 ± 2.3, and 3.4 ± 1.0 pmol/ml, respectively, which were not significantly different from control values. When the control patients and those in the indeterminate category were considered as one group, the concentrations of LTD_4 in edema fluid (5.2 ± 1.8 pmol/ml) were significantly lower ($P < 0.001$) than in the patients with ARDS, and there was no overlap of the values for the two groups.

DISCUSSION

The application of sensitive radioimmunoassays to analyses of leukotrienes isolated chromatographically from pulmonary edema fluid thus revealed a significant elevation in the concentration of the vasoactive and contractile factor LTD_4 in ARDS, as contrasted with cardiogenic pulmonary edema (Fig. 1). The pulmonary edema fluid concentrations of LTB_4, PGE_2, and TxB_2 were elevated in some patients with ARDS, but the group mean values were not significantly higher than those of patients with cardiogenic pulmonary edema. The present findings do not indicate the cellular source of the LTD_4 in pulmonary edema fluid. However, the results of previous studies of the generation of leukotrienes by fragments of human lung *in vitro* suggest that LTD_4 is generated principally by peripheral parenchymal tissue (21), whereas tracheal and other proximal airway tissues produce LTB_4 and 15-lipoxygenase products but no detectable LTD_4 or LTC_4 (22). No substantial interconversions of the peptidoleukotrienes were detected in edema fluid *in vitro*. The relative insensitivity of the immunoassays for LTE_4, however, does not permit definitive statements about the concentrations of this mediator in edema fluid.

A significant correlation of the concentration of LTD_4 in pulmonary edema fluid with the ratio of edema fluid to plasma concentration of albumin (r =

0.64) (Table I) suggests a contribution of LTD_4 to the altered alveolar–capillary permeability characteristic of ARDS. The results of some studies in animal models (23, 24) imply that LTD_4 alone at concentrations found in the edema fluid of ARDS could have a critical role in increasing vascular permeability.

The pulmonary edema fluid concentration of LTD_4 also did not appear to be a prognostic indicator of survival in this small group of patients with ARDS of diverse etiologies, as neither the ratios of albumin and total protein concentrations (Table I) nor the pulmonary edema fluid concentrations of LTD_4 were significantly higher in the patients with ARDS who died (17.7 ± 5.6 pmol/ml, mean ± SD, $N = 6$) than in those who survived (19.7 ± 9.0 pmol/ml; $N = 4$). An assessment of the time course of appearance of LTD_4 in relation to the development of the permeability defect in ARDS would serve to elucidate a pathogenetic or prognostic role for this mediator in patients at risk for the development of acute lung injury.

REFERENCES

1. Brigham KL, Woolverton WC, Blake LH, Staub NC: Increased sheep lung vascular permeability caused by Pseudomonas bacteremia. J Clin Invest 54:792–804, 1974
2. Anderson RR, Holliday RL, Driedger AA, Lefcoe M, Reid B, Sibbald WJ: Documentation of pulmonary capillary permeability in the adult respiratory distress syndrome accompanying human sepsis. Am Rev Resp Dis 119:869–877, 1979
3. Fein A, Grossman RF, Jones JG, Overland E, Pitts L, Murray JF, Staub NC: The value of edema fluid protein measurement in patients with pulmonary edema. Am J Med 67:32–39, 1979
4. Heflin AC, Brigham KL: Prevention by granulocyte depletion of increased vascular permeability of sheep lungs following endotoxemia. J Clin Invest 68:1253–1260, 1981
5. Zimmerman GA, Renzetti AD, Hill HR: Functional and metabolic activity of granulocytes from patients with the adult respiratory distress syndrome. Am Rev Resp Dis 127:290–300, 1983
6. Saldeen T: The microembolism syndrome. Microv Res 11:227–259, 1976
7. Bone RC, Frances PB, Pierce AK: Intravascular coagulation associated with the adult respiratory distress syndrome. Am J Med 61:585–589, 1976
8. Till GD, Johnson KJ, Kunkel R, Ward PJ: Intravascular activation of complement and acute lung injury. J Clin Invest 69:1126–1135, 1982
9. Hammerschmidt DE, White JG, Craddock PR, Jacobs HS: Corticosteroids inhibit complement-induced granulocyte aggregation. J Clin Invest 63:789–796, 1979
10. Voelkel NF, Simpson J, Worthen S, Reeves JT, Henson PM, Murphy RC: Platelet-activating factor causes pulmonary vasoconstriction and edema via platelet-independent leukotriene formation. *In* Advances in Prostaglandin, Thromboxane, and Leukotriene Research, Vol 12, B Samuelsson, R Paoletti, P Ramwell (eds). New York, Raven Press, 1983, pp 179–183
11. Dahlen S, Bjork J, Hedqvist P: Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: In vivo effects with relevance to the acute inflammatory response. Proc Natl Acad Sci USA 78:3887–3891, 1981
12. Weinberg PF, Matthay MA, Webster RO, Roskos KV, Goldstein IM, Murray JF: Relationship between complement activation and acute lung injury in patients with sepsis syndrome. Am Rev Resp Dis 130 (in press) 1984
13. Pepe PE, Potkin RT, Holtman D, Hudson LD, Carrico CJ: Clinical predictions of the adult respiratory distress syndrome. Am J Surg 144:124–130, 1982
14. Fowler AA, Hamman RF, Good JT, Benson KN, Baird M, Eberle DJ, Petty TL, Hyers TM: Adult respiratory distress syndrome: Risk with common predispositions. Ann Intern Med 98:593–597, 1983
15. Rodkey FL: Direct spectrophotometric determination of albumin in human serum. Clin Chem 11:478–487, 1965
16. Cannon DC, Olitzky I, Inkpen JA: Proteins. *In* Clinical Chemistry, Principles and Techniques, RJ Henry, DC Cannon, J Winkteman (eds). New York, Harper & Row, 1974, pp 405–502
17. Goetzl EJ, Phillips MJ, Gold WM: Stimulus specificity of the generation of leukotrienes by dog mastocytoma cells. J Exp Med 158:731–737, 1983
18. Lindgren JA, Hammarstrom S, Goetzl EJ: A sensitive and specific radioimmunoassay for leukotriene C_4. FEBS Lett 152:83–88, 1983
19. Hayes E, Lombardo DL, Girard Y, Maycock AL, Rokach J, Rosenthal AS, Young RN, Egan RW, Zweerink HJ: Measuring leukotrienes of slow reacting substance of anaphylaxis: Development of a specific radioimmunoassay. J Immunol 131:429–433, 1983
20. Carlson RW, Schaeffer RC, Carpio M, Weil MH: Edema fluid and coagulation changes during fulminant pulmonary edema. Chest 79:43–49, 1981
21. Lewis RA, Austen KF, Drazen JM, Clark DA, Marfat A, Corey EJ: Slow reacting substance of anaphylaxis: Identification of leukotrienes C-1 and D from human and rat sources. Proc Natl Acad Sci USA 77:3710–3715, 1980
22. Holtzman MJ, Aizawa H, Nadel JA, Goetzl EJ: Selective generation of leukotriene B_4 by tracheal epithelial cells from dogs. Biochem Biophys Res Commun 114:1071–1076, 1983
23. Hammarstrom S, Lundberg JM, Xiao-Ying H, Dahlen SK: Action and metabolism of circulating leukotriene C. J Allergy Clin Immunol (in press), 1984
24. Saga T, Yoshii K, Said SI: Protection by FPL 55712 or indomethacin of leukotriene D_4-induced pulmonary edema and airway constriction in guinea pig lungs. Am Rev Resp Dis 129:333(A), 1984