Changes in Fatty Acids in Phospholipids of the Bronchoalveolar Fluid in Bacterial Pneumonia and in Adult Respiratory Distress Syndrome

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Fatty acids of the phospholipid fraction of bronchoalveolar lavage fluid from patients with bacterial pneumonia or with the adult respiratory distress syndrome were chromatographed and the patterns compared with those for a control group. In the control group, palmitic acid (16:0) was the predominant fatty acid, accounting for 58.0% (SD 8.25%) of the total fatty acid, a proportion significantly higher (p <0.001) than in the distress-syndrome group (42.1%, SD 4.88%) or the acute pneumonia group (32.1%, SD 1.73%). There was a greater proportion of oleic acid (18:1) in the disease groups; thus the ratio of palmitic to oleic acid was useful in distinguishing these three groups. No patient with a palmitic/oleic acid ratio >2.45 had evidence of parenchymal inflammation. Of those with a ratio <1.3, 89% had acute bacterial pneumonia.

Mammalian lung metabolizes phospholipid (1, 2). Bronchoalveolar lavage (BAL), a method of sampling lower-airway secretions for biochemical analysis, has been particularly used in the adult respiratory distress syndrome (ARDS), where defective phospholipid metabolism leads to abnormal surfactant stability (3, 4). Although there is no evidence that surfactant deficiency plays a primary role in the development of ARDS, ample evidence indicates that surfactant loss exacerbates the pathophysiological processes (5). The fatty acid composition of the phospholipid in BAL fluid has been evaluated in patients with ARDS, and specific changes have been reported in humans and experimental animals (6, 7).

Primary pulmonary infections can lead to secondary ARDS. In addition, primary ARDS can be—and often is—complicated by secondary infections. We undertook to examine the fatty acid composition of phospholipid in BAL fluid from patients with primary ARDS and primary bacterial pneumonias, and to compare it with that in individuals without active inflammatory pulmonary disease.

Materials and Methods

Patients. We studied three groups of patients:

(a) The control group consisted of 13 patients (11 men), ages 22 to 72 years (mean ± SD = 49 ± 14.2 years). These patients were undergoing bronchoscopy for local lung lesions. Seven patients in this group were smokers and five had a clinical history compatible with the diagnosis of chronic bronchitis, although none had acute infection at the time of study.

(b) The pneumonia group consisted of 10 patients (eight men), ages 51 to 70 years (61 ± 6.1). The diagnosis of acute pneumonia was based on the presence of fever, leukocytosis, purulent sputum, a localized area of infiltrate visible in chest roentgenograms, and a response to therapy with antibiotics. Eight of the patients in this group were smokers and seven had a clinical history of chronic bronchitis. Bronchoscopy was performed during the first three days of illness.

(c) The ARDS group consisted of 12 patients (seven men), ages 18 to 60 years (40 ± 12.0). The diagnosis of ARDS was based on a compatible clinical history, presence of rales, an arterial pO2 of less than 60 mmHg (<8 kPa) on an inspired oxygen concentration of more than 50%, diffuse bilateral alveolar infiltrates on chest roentgenogram, and the absence of cardiac decompensation as demonstrated by means of right-heart catheterization (5). All patients in this group smoked, but none gave a clinical history of chronic bronchitis.

This study was approved by the Human Research Committee of the University of Cincinnati and written informed consent was obtained from each patient before participation.

Lavage techniques. Premedication consisted of 0.4 to 0.8 mg of atropine and a narcotic, both given intramuscularly. Xylocaine was administered locally during the procedure. The bronchoscope was advanced to the area to be lavaged (in the control group, this was the side opposite the lesion) and wedged in a segmental bronchus. One hundred milliliters of isotonic saline was introduced, then withdrawn under negative pressure. Approximately 30% of the lavage fluid was recovered. In three patients with ARDS the sample was obtained from a no. 5 French polyethylene catheter inserted through an endotracheal tube. We injected 35 mL of isotonic saline through the catheter and 30 s later withdrew 5–10 mL of BAL fluid for analysis.

The lavage fluid was centrifuged (180 x g, 5 min) to sediment cellular material. Measured aliquots of the supernatant fluid were stored at −20 °C for subsequent analysis.

Fatty acid analysis. Dinonadecanoyl phosphatidylcholine (Supelco, Bellefonte, PA) was used as an internal standard throughout the study. The lipid fraction in BAL was extracted by the method of Folch et al. (8), dried at 65 °C under nitrogen, redisolved in chloroform, and applied to a column formed by placing, on a plug of glass wool in the neck of a 9-in. Pasteur pipette, 0.8 g of Hiflosil, medium grade, 60–120 mesh (Applied Sciences Laboratories, State College, PA), that had been activated by heating for 1 h at 110 °C. The column was washed with 5 mL of chloroform, followed by 2 mL of methanol; the phospholipids were eluted with another 7 mL of methanol, which should elute all phospholipids (9). The sample was dried and the fatty acids were released and analyzed by the method of MacGee and Allen (10).

The fatty acids were analyzed in a Model 2600 gas chromatograph (Bendix Corp., Ronceverte, WV) or a Model 5790A (Hewlett Packard, Avondale, PA) gas chromatograph, with a 15.2 × 0.8 cm (3 mm i.d.) glass column packed with pretreated 10% Silar-10 C on 100–120 mesh Gas Chrom Q (Applied Sciences). A 2-μL aliquot of sample, interposed between 1-μL portions of methyl acetate, was injected into.

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the chromatograph. The injection port temperature was held at 280 °C; the column temperature, initially 150 °C, was increased by 2 °C/min to 220 °C; the carrier was nitrogen, at a flow rate of 38 mL/min; and flame ionization detectors were used. Peak areas were measured with an electronic digital integrator (Autolab System I, Spectra-Physics, Santa Clara, CA; or a Hewlett-Packard Model 3370A).

The significance of differences among various groups was calculated by the Mann–Whitney U-test for differences between independent samples (11). Sensitivity and specificity were calculated in the usual manner (12).

Results

Our initial studies of the fatty acid composition of phospholipids in BAL fluid showed that four fatty acids predominated: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2). We therefore report the relative amounts of only these fatty acids.

Two representative chromatograms are shown in Figure 1. Figure 1A, the pattern from a control subject, shows that the dominant peak is palmitic acid. Figure 1B, the pattern for a patient with acute pneumonia, shows a decreased peak for palmitic acid and increased oleic acid peak.

Figure 2 shows that the proportion of palmitic acid in the control group is significantly greater (p <0.002) than in the two diseased groups. The proportion of palmitic acid in the ARDS group also differed significantly from that for patients with pneumonia. Although the proportions of both stearic acid and linoleic acid were lower in subjects without disease (p <0.02, Figure 2), the pattern was the same with respect to relative amounts of these fatty acids in samples from patients with ARDS or pneumonia. The proportion of oleic acid differed significantly (p <0.002) among the three groups.

The ability to differentiate among ARDS, pneumonia, and normal subjects was enhanced by considering the ratio of palmitic to oleic acid. Figure 3 shows this ratio to be significantly different (p <0.002) between the ARDS and pneumonia groups, 1.9 (SD 0.36) and 1.0 (SD 0.27), respectively. In addition, the ratio for both of the groups differed significantly from that for the control subjects (3.4, SD 1.10): p <0.002.

Discussion

Our results show that the relative amounts of the fatty acids in the phospholipid fraction of BAL fluid are significantly altered in acute bacterial pneumonia and ARDS. The relative decrease in the concentration of palmitic acid (16:0) was most profound in pneumonia, but was also marked in the ARDS group. We also observed an inverse relationship between concentrations of palmitic acid and oleic acid in both groups of diseased patients: in the pneumonia group, which showed the lowest relative concentration of palmitic acid, the relative concentration of oleic acid is highest, while the reverse is true of the control group. The utility of the C16:0/C18:1 ratio in differentiating the two disease states from each other and from the control group can be seen in Figure 3. Using a cutoff value of 2.45 for this ratio, we identified 11 of 13 control subjects (sensitivity 85%; specificity 100%). Using a cutoff value of less than 1.3, we identified eight of 10 patients with pneumonia (sensitivity 80%; specificity 96%). Eleven of 12 subjects with ARDS had ratios of 1.3 to 2.5 (sensitivity 92%; specificity 83%).

In this study, we looked at the relative proportions of fatty acids in all the phospholipids retrieved from the lung. The phospholipid in the lung in the largest quantity is phosphatidylcholine. A decrease in the relative amount of disaturated phosphatidylcholine in the BAL fluid of patients with ARDS and in experimental models of this syndrome has been reported (6, 7). Palmitic acid is the major fatty acid component of the disaturated phosphatidylcholine fraction, and less of it is present in ARDS (7). The exact pathophysiological mechanisms responsible for loss of surfactant in ARDS are not known, but the possibilities include removal of surfactant by edema fluid (13), ventilation with high tidal volumes (14), effect of high inspired oxygen concentrations (15), changes in pulmonary blood flow (16), and systemic hypotension (17). Regardless of the underlying mechanisms involved, surfactant deficiency aggravates the pathophysiological processes and the preceding functional derangements in the lungs.

![Fig. 1. Chromatographic patterns of fatty acids: (A) from a subject with a pulmonary nodule and no evidence of infection; (B) from a patient with acute bacterial pneumonia](image-url)
The relationship of pulmonary infections to ARDS has not yet been examined with respect to surfactant analysis. Such studies would seem to be relevant, because not only can infection lead to ARDS but also ARDS is often complicated by secondary infection. Here we have shown that pneumonia is associated with changes in fatty acids in BAL fluid that are in the same direction as, but clearly distinguishable from, those in ARDS. Others have shown that the surface tension of fluid extracted from lung tissue and washings is abnormal in pneumonia (18, 19). Further, preliminary studies from our laboratory indicate that the concentrations of these fatty acids return to normal during successful treatment of pneumonia.

Mink et al. (20) recently studied lung mechanics in lobar pneumonia in dogs. Because the decrease in lung compliance in pneumonia was significantly greater than could be attributed to the decrease in lung volume in liquid-filled alveoli, they speculated that the surfactant was perhaps altered, leading to development of atelectasis. Sutnick and Soloff (19) found abnormal surface-tension properties of washings from the pneumonia-affected area of the lung in four fatal cases of pneumonia. Similar results were obtained in examination of the lung tissue involved in bronchopneumonia (18). The mechanisms responsible for alterations in fatty acid during acute pneumonia are not known, but we suggest that some of the mechanisms described for ARDS could also be operative in pneumonias.

Contamination of BAL fluid with serum is possible but unlikely, because the proportions of linoleic acid (18:2), a major fatty acid in serum, changed little in BAL fluid from our patients, as compared with changes in palmitic and oleic acid. Are products of neutrophils and bacteria responsible for changes in fatty acid in pneumonia? A causative agent was usually not identified in our cases of pneumonia; however, all responded appropriately to antibiotics. Alterations in the cellular milieu, especially in Type II pneumocytes, should also be considered. Smoking (21) and age may cause changes in lung phospholipids; our three groups were similar in age and smoking history.

We conclude that analysis of BAL fluids for fatty acids has potential clinical usefulness as a diagnostic adjunct in the diagnosis of ARDS and in distinguishing it from acute pneumonia.

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References