

Case Report

Pulmonary Surfactant Protein B Deficiency: A Case Report

Verna E. Nelson, BS, RRT, Steven C. Raithel, BS, CCP, George F. Glenn, BA, BS, CCP

St. Louis University School of Allied Health Professions, St. Louis Children's Hospital, St. Louis, Missouri

Keywords: congenital alveolar proteinosis, surfactant protein B deficiency, extracorporeal membrane oxygenation, exogenous surfactant replacement

ABSTRACT

The first reported case of pulmonary alveolar proteinosis (PAP) was documented in 1958. Since then, more cases have become evident of this unusual disease. PAP is a disease characterized by positive periodic acid-Schiff, diastase-resistant granular eosinophilic material within the alveoli. Individuals with PAP have impaired gas exchange and progressive hypoxemia. In congenital PAP, newborn infants show severe and progressive pulmonary failure at birth which leads to death. In one family, a male sibling was born with severe respiratory distress syndrome and diagnosed with congenital alveolar proteinosis (CAP) by open lung biopsy. His female sibling died at one month of age nineteen years earlier with severe respiratory disease. Her autopsy slides were consistent with CAP. This case sibling followed the same clinical course as his brother and sister diagnosed with CAP by family history. Surfactant protein 'B' deficiency was identified as the cause of the congenital alveolar proteinosis. Aggressive therapy was initiated in the case sibling with exogenous surfactant replacements, extracorporeal membrane oxygenation (ECMO) as a bridge to transplant, lung lavage, and maximum ventilator settings via conventional mechanical ventilation. The child died on ECMO day 29 after 696 hours of support.

Address correspondence to:
Verna E. Nelson, BS
St. Louis University Health Sciences Center
Surgery Department, Perfusion Division
3635 Vista Avenue at Grand Blvd.
St. Louis, Missouri 63110-0250

INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is characterized by histopathic findings of positive periodic acid-Schiff, diastase-resistant granular eosinophilic material within the alveoli (1-3). This accumulation of an insoluble amorphous proteinaceous material is due to an overproduction of surface active material containing phospholipids and lipoproteins (4).

Individuals with PAP have impaired gas exchange and progressive hypoxemia (4). Since the first reported case of pulmonary alveolar proteinosis (1), more cases have become evident of this unusual disease (2,4). In PAP of a congenital nature (congenital alveolar proteinosis [CAP]) newborn infants show severe and progressive pulmonary failure at birth, which inevitably leads to death (5). Several cases of CAP have been reported and only recently a familial surfactant protein 'B' (SP-B) deficiency has been identified as one cause. SP-B deficiency is an extremely rare type of CAP of unknown incidence (2,5). At St. Louis Children's Hospital two siblings were previously reported with inherited SP-B deficiency diagnosed through clinical course and autopsy slides of one sibling and open lung biopsy in the second (3). Their SP-B deficiency was caused by an absence of messenger ribonucleic acid (mRNA) due to a pretranslational mechanism (5). Our case represents the third child of this family with probable SP-B deficiency diagnosed by analysis of amniocentesis fluid. The patient developed severe respiratory distress at birth. Exogenous surfactant replacements were administered immediately at birth and continued daily. Extracorporeal membrane oxygenation (ECMO) was utilized to bridge the patient to transplantation for a lung or heart-lung transplant (6-8). Despite early identification of this common disease, aggressive therapy could not prevent death. This case warrants reporting due to the uniqueness of this cause of respiratory failure and the therapy undertaken for the majority of the child's life.

MATERIALS AND METHODS

Two types of exogenous replacement surfactants were used. Survanta^a is an intratracheal suspension of pulmonary surfactant proteins SP-B and SP-C derived from natural bovine lung extract. It also contains 25 mg/ml phospholipids. Infasurf^b is an exogenous surfactant replacement currently under investigation. Infasurf contains SP-B and SP-C and is made from calf lung extract; it contains 35 mg/ml phospholipids.

The ECMO circuit used was similar to those previously described (9-12). Cannulation was via the right carotid artery and right internal jugular vein with #10 French and #12 French Biomedicus^c cannulas, respectively. The circuit prime consisted of 150 ml lactated Ringers solution, 50 ml THAM, 3000 units

heparin, 5 mEq sodium bicarbonate, 200 ml packed red blood cells, 300 mg calcium chloride, and 50 ml 25% albumin.

Immunoblotting uses the total protein of the lung tissue that undergoes electrophoresis. Antiserum against SP-A, SP-B and SP-C causes an immunoreaction to occur, identifying the proteins. RNA blotting uses RNA isolated from frozen lung tissue. Radiolabeled human complementary DNA (cDNA) probes were applied for SP-A, SP-B and SP-C, hybridized, washed, and exposed to x-ray film. A signal upon reprobing the sample indicates the presence of mRNA (5).

CASE REPORT

A full-term 4.2 kg male infant with a familial history of SP-B deficiency was born at an outlying hospital. A lecithin/sphingomyelin (L/S) ratio done prior to birth revealed <0.6 ratio and a negative phosphatidyl glycerol (PG). Because of the significant history the patient was intubated immediately after birth and given two doses of Survanta at 4 ml/kg endotracheally. The patient was then transported to St. Louis Children's Hospital. Initial Apgar scores were 7 and 8 at one and five minutes. Chest x-ray demonstrated ground glass markings consistent with respiratory distress syndrome (RDS). Echocardiogram revealed a small patent ductus arteriosus with left to right shunting. Pulmonary function tests showed severely reduced functional residual capacity and lung compliance. Amniotic fluid, lung lavage fluid and gastric aspiration samples obtained at birth showed no detectable SP-B, and an increased amount of SP-C. The patient continued to deteriorate despite maximum mechanical ventilation support and increasing Survanta infusions to 6 ml/kg. Bronchial alveolar lavages were performed daily. Within five days the patient required exogenous surfactant replacement with Survanta at dose rates of 8 ml every two hours. Since the patient was not responding to this therapy, Survanta was replaced with Infasurf, an experimental exogenous surfactant replacement, at 1 ml/kg every 12 hours. Steroid therapy with dexamethasone was also initiated. Multiple episodes of arterial desaturations continued and progressive deterioration was noted. The patient was evaluated for lung or heart-lung transplantation and placed on a transplant list. ECMO was started on the 25th day of life as bridge to transplantation. Bleeding from the cannulation site was a complication of systemic heparinization during ECMO. Episodes of arterial desaturation continued despite ECMO support at 120-126 ml/kg total flow. Infasurf therapy was continued while on ECMO. On day 20 of ECMO noticeable seizure activity was seen when the patient was not paralyzed. Phenobarbital and phenytoin were administered and the pancuronium discontinued to assess neurological status. Despite therapeutic levels of phenobarbital, clonic movements of the left upper extremity continued. A neurological sonogram showed bilateral cortical atrophy, bilateral extra-axial fluid collections and increased echogenicity above the third ventricle. Blood cultures on day 28 of ECMO were positive for enterococcus and coagulase negative staphylococcus. Due to poorly controlled seizure activity, which

a Ross Laboratories, Columbus, Ohio 43216

b Ony, Incorporated, Amherst, New York 14228

c Biomedicus, Eden Prairie, Minnesota 55344

potentially could worsen with cyclosporin administration, the patient no longer met criteria for organ transplantation. Therefore, the decision was made by the family and physicians to discontinue ECMO. The patient was weaned from ECMO on day 29 following 696 hours of support, and expired within a few hours from removal of ECMO. Autopsy results showed global pulmonary atelectasis, fresh and organizing cranial subarachnoid hemorrhages, focal hemorrhage of the right kidney, and an enlarged liver and spleen.

DISCUSSION

A major cause of morbidity and mortality in premature infants is RDS (9,13-16). Surfactant deficiency contributes to RDS (15). Surfactant maintains alveolar stability by reducing surface tension at the air-liquid interface (5,15). Surfactant is made up of phospholipids, primarily phosphatidylcholine (lecithin), and small amounts of protein and carbohydrates (13,17). When surfactant is secreted from the lamellar bodies of alveolar type II cells it expands into a complicated lattice-like structure called tubular myelin. The spreading of this tubular myelin stabilizes the alveolus by lowering the surface tension (18). Phospholipids are accepted as the main components that reduce surface tension (14,15,17,19). However, surfactant proteins are also identified as contributing to surface tension reduction (19). The four surfactant proteins are identified as SP-A, SP-B, SP-C and SP-D (17). SP-A is the most abundant and is a high molecular weight hydrophilic glycoprotein important in surfactant metabolism and host defense (5). It binds with phospholipid to form tubular myelin (20). SP-D is a recently identified protein similar to SP-A (17). SP-B and SP-C are low molecular weight hydrophobic proteins. SP-B is involved in two processes with the phospholipids. First, it increases phospholipid uptake by alveolar type II cells which helps bind the tubular myelin-like structure to the lipid mixture containing SP-A. Secondly, along with SP-C, it increases spreading of this phospholipid mixture on the alveolus air-liquid interface (16).

SP-B is secreted mainly in the lungs and is regulated at different stages in the development of the fetus. It is first detected at 32 weeks gestation and then sharply increases in amount (5,14). The analysis of SP-B, along with L/S ratios and PG, can be used to predict potential pulmonary disease at birth (14). The L/S ratio uses amniotic fluid to test for the evidence of surfactants secreted from the lungs of the fetus. A comparison is made between the surfactants lecithin and sphingomyelin. An increase in lecithin above sphingomyelin accurately predicts fetal lung maturity. A mature L/S ratio is considered greater than 2. This indicates, with a probability of nearly 100% on an uncomplicated vaginal delivery, that an infant will not develop RDS (15). PG is a phospholipid abundant in amniotic fluid. It is secreted by the lungs of the fetus. PG is increased with advancing gestational age suggesting maturity of the lungs (14). Some infants show respiratory symptoms similar to RDS with a mature L/S ratio but an absence of PG. This patient presented with an immature L/S ratio

of 0.6 and an absence of both PG and SP-B. All are essential for surfactant function and metabolism (5). The importance of SP-B was shown by Robertson (21) when he used an antibody to SP-B to block its function. The result was severe structural and functional pulmonary abnormalities (21). Noguee, et al, stated that lacking SP-B is neither due to pulmonary immaturity nor secondary to therapeutic measures (5). In CAP where SP-B is deficient, tubular myelin is also absent. In comparison, PAP in adults and older infants is thought to be a nonspecific response to alveolar injury, secondary to viral, pyogenic or pneumocystic carinii pneumonia. Additionally, thymic aplasia is often associated with the adult form of PAP (1,2), while SP-B and tubular myelin are present (5,22).

Aggressive medical treatment was immediately instituted in this patient and it has been reported in other studies (4). None have proven effective. This case demonstrated early identification of deficiency and a treatment protocol instituted immediately at birth. Survanta can lower surface tension to less than 8 dynes/cm *in vitro*. The other exogenous surfactant replacement used, Infasurf, is under investigation. Infasurf can lower surface tension to less than 3 dynes/cm within five minutes post administration. Both can improve lung compliance, pulmonary mechanics, and blood gas values. Infasurf was employed eight days after birth since the patient was continuing to deteriorate (23,24). Weaver, et al, have shown that significant improvement in pulmonary function resulted following exogenous surfactant replacement in infants with RDS (17). The patient in this case report showed little or no improvement.

In adults with PAP and children with non-congenital PAP, pulmonary lavage offers some benefit and may reverse symptoms (1,3). Whole lung lavage while on ECMO has been reported in adults and children (4). This is currently the most effective treatment of PAP of a non-congenital origin (4).

Open lung biopsy, a definitive diagnostic technique for PAP and CAP, was deferred on our case patient for two reasons. First, this case patient's brother had previously undergone biopsy at a time when SP-B should have been present. Results of the biopsy using the protein immunoblotting and RNA blotting techniques proved SP-B deficiency (5). Immunoblotting is used to identify the various surfactant proteins. RNA blotting is used to identify the mechanism underlying SP-B deficiency. Second, biopsy was deferred because of increased risk of hemorrhage due to ECMO consideration as a bridge to transplant. Testing of alveolar epithelial cells and intraalveolar proteinaceous material from both siblings of the case patient proved SP-B deficient as well (5). This paralleled the results from the case patient's lung lavages, gastric fluid and amniotic fluid samples. The cause of CAP in the first two siblings was identified by Noguee as an inherited SP-B deficiency and absence of its mRNA due to a pretranslational mechanism (5). This confirms an inborn error of surfactant metabolism.

ECMO has been used as a treatment for newborn infants with RDS when conventional therapy did not work (10). ECMO allows the lungs to rest and recover (10) and may be used to

sustain life for days or weeks (9). ECMO was previously employed and failed in five patients with CAP by our group and others (4,5). The brother of our patient was on ECMO for 14 days without improvement. Termination of ECMO in our patient was due to irreversible neurological damage and seizures.

At present, the various treatments do not change the course of CAP. Familial SP-B deficiency is a type of CAP with a poor prognosis similar to other CAP disease entities (2,4,5). Since none of the reported existing treatments have produced survivors, their efficacy is questionable. Two therapies left untested for CAP—transplantation and gene therapy—need to be examined (5).

REFERENCES

1. Coleman M, Dehner LP, Sibley RK, Burke BA, L'Heureux PR, Thompson TR. Pulmonary alveolar proteinosis: An uncommon cause of chronic neonatal respiratory distress. *Ame Rev Dis.* 1980;121:583-586.
2. Schumacher RE, Marrogi AJ, Heidelberger KP. Pulmonary alveolar proteinosis in a newborn. *Pediatr Pulmonol.* 1989;7:178-182.
3. Knight DP, Knight JA. Pulmonary alveolar proteinosis in the newborn. *Arch Pathol Lab Med.* 1985;109:529-531.
4. Moulton SL, Krous HF, Merritt TA, Odell RM, Gangitano E, Cornish JD. Congenital pulmonary alveolar proteinosis: Failure of treatment with extracorporeal life support. *J Pediatr.* 1992;120:297-302.
5. Nogee LM, deMello DE, Dehner LP, Colten HR. Deficiency of pulmonary surfactant protein B in congenital alveolar proteinosis. *N Engl J Med.* 1993;326:406-410.
6. Egan TM, Saunders NR, Murphy E, Cooper JD. Successful extracorporeal membrane oxygenation for twenty-four days in a patient who underwent two lung transplants for paraquat poisoning: A case report. *Proc Am Acad Cardiovasc Perf.* 1983; 4:154-158.
7. Delius RE, Zwischenberger JB, Cilley R, et al. Prolonged extracorporeal life support of pediatric and adolescent cardiac transplant patients. *Ann Thorac Surg.* 1990;50:791-795.
8. Galantowicz ME, Stolar CJ. Extracorporeal membrane oxygenation for perioperative support in pediatric heart transplantation. *J Thorac Cardiovasc Surg.* 1991;102:148-152.
9. Bartlett RH, Gazzaniga AB, Huxtable RF, Schippers HC, O'Connor MJ, Jefferies MR. Extracorporeal circulation (ECMO) in neonatal respiratory failure. *J Thorac Cardiovasc Surg.* 1977;74:826-833.
10. Bartlett RH, Toomasian J, Roloff D, Gazzaniga AB, Corwin AG, Rucker R. Extracorporeal membrane oxygenation (ECMO) in neonatal respiratory failure: 100 cases. *Ann Surg.* 1986;204:236-245.
11. Bartlett RH, Gazzaniga AB, Jeffries R. Extracorporeal membrane oxygenation (ECMO) cardiopulmonary support in infancy. *Trans Am Soc Artif Intern Organs.* 1976;22:80-88.
12. Weber TR, Connors RH, Tracy TF, Bailey PV, Stephens C, Keenan W. Prognostic determinants in extracorporeal membrane oxygenation for respiratory failure in newborns. *Ann Thorac Surg.* 1990;50:720-723.
13. Sarin VK, Gupta S, Leung TK, et al. Biophysical and biological activity of a synthetic 8.7-kDa hydrophobic pulmonary surfactant protein SP-B. *Proc Natl Acad Sci.* 1990;87:2633-2637.
14. Pryhuber GS, Hull WM, Fink I, McMahan MJ, Whitsett JA. Ontogeny of surfactant proteins A and B in human amniotic fluid as indices of fetal lung maturity. *Pediatr Res.* 1991;30:597-605.
15. Farrell PM, Avery ME. Hyaline membrane disease. *Am Rev Respir Dis.* 1975;111:657-688.
16. Wikenheiser KA, Wert SE, Wispè JR, et al. Distinct effects of oxygen on surfactant protein B expression in bronchiolar and alveolar epithelium. *Am J Physiol.* 1992;262:L32-39.
17. Weaver TE, Whitsett JA. Function and regulation of expression of pulmonary surfactant-associated proteins. *Biochem J.* 1991;273:249-264.
18. Hawgood S, Clements JA. Pulmonary surfactant and its apoproteins. *J Clin Invest.* 1990;86:1-6.
19. Kobayashi T, Nitta K, Takahashi R, Kurashima K, Robertson B, Suzuki Y. Activity of pulmonary surfactant after blocking the associated proteins SP-A and SP-B. *J Appl Physiol.* 1991;71:530-536.
20. Whitsett JA, Hull WM, Ohning B, Ross G, Weaver TE. Immunologic identification of a pulmonary surfactant-associated protein of molecular weight = 6000 daltons. *Pediatr Res.* 1986;20:744-749.
21. Roberston B, Kobayashi T, Ganzuka M, Grossman G, Li W, Suzuki Y. Experimental neonatal respiratory failure induced by a monoclonal antibody to the hydrophobic surfactant-associated protein SP-B. *Pediatr Res.* 1991;30:2390243.
22. Hook GE, Gilmore LB, Talley FA. Dissolution and reassembly of tubular myelin-like multilamellated structures from the lungs of patients with pulmonary alveolar proteinosis. *Lab Invest.* 1986;55:194-208.
23. Whitsett JA, hull WA, Luse S. Failure to detect surfactant protein-specific antibodies in sera of premature infants treated with Survanta, a modified bovine surfactant. *Pediatrics.* 1991;87:505-510.
24. Whitsett JA, Ohning BL, Ross G, et al. Hydrophobic surfactant-associated protein in whole lung surfactant and its importance for biophysical activity in lung surfactant extracts used for replacement therapy. *Pediatr Res.* 1986;20:460-467.