Lipid Peroxidation and Pulmonary Hyaline Membranes of the Newborn:

A Histochemical, Fluorescent Microscopic and Ultrastructural Study

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ABSTRACT

Representative portions of lung from 17 newborn infants with hyaline membrane disease were studied. The consistent findings in the hyaline membranes of Schiff's positivity requiring periodic acid pretreatment, their autofluorescence between 350 and 400 nm, and the granular ultrastructure of the membrane matrix provided morphologic evidence for lipid peroxidation's having occurred in association with the genesis of pulmonary hyaline membranes of the newborn.

Introduction

Necrosis of alveolar pneumocytes and respiratory bronchiolar epithelium is well established as one of the primary processes contributing to the formation of hyaline membranes in the newborn.3,4,10,11,12,15 Because oxygen toxicity has long been regarded as a factor in hyaline membrane disease and in view of the fact that the methyl ester of linoleic hydroperoxide can produce necrosis of pneumocytes under experimental conditions,1 the possibility has been considered that lipid peroxidation plays an important role in the formation of such hyaline membranes.

The purpose of this report is to provide histochemical, fluorescent microscopic, and ultrastructural evidence for lipid peroxidation having occurred in association with the formation of pulmonary hyaline membranes of the newborn.

Materials and Methods

The 17 newborn patients used in this study had gestational ages estimated to range from 24 to 38 weeks. Eighty-two
percent (14/17) of the patients were premature by weight (< 2500 g) with the smallest being 970 g. The postnatal survival times were recorded in 14 cases and ranged from 2.5 to 72 hours, with a mean of 27 hours. All patients had a necropsy diagnosis of hyaline membrane disease of the newborn. No cases with associated bacterial pneumonitis were included in this study.

Histochemical studies on paraffin sections of lung included periodic acid Schiff (PAS) with and without diastase, Schiff's reagent without periodic acid pretreatment, Fraser-Lendrum and Putt's stains for fibrin, and Southgate's mucicarmine and alcian blue for mucin.

Fluorescent microscopy employing epi-illumination and combinations of exciter filters and dichroic beam splitting mirrors in an attempt to approximate the excitation maxima of fluorescent elements was carried out on deparaffin sections of lung mounted in phosphate buffered saline. The degree of autofluorescence and any changes in same affected by varying the combinations of exciter filters and dichroic beam splitting mirrors was graded on a scale of 0 to 4+ agreed upon by two observers. A case of Dubin-Johnson syndrome containing abundant lipofuscin pigment was used as a positive control for fluorescent peroxidation products. Non-necrotic appearing alveolar pneumocytes and respiratory bronchiolar epithelium served as an internal control for any positive histochemical reactions or autofluorescence in the hyaline membranes that might be interpreted as peculiar to the necrosis of such cells during the formation of the membranes.

Frozen sections of formalin fixed lung tissue from two of the cases were stained with PAS with and without chloroform/methanol extraction for glycolipids. In addition, formalin-fixed tissue from these two cases was post-fixed in buffered paraformaldehyde/glutaraldehyde followed by osmium and processed for electron microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined in a Siemens 101 electron microscope.

Results

Although the intensity of the histochemical reaction varied among and within the cases, all 17 (100%) showed positive staining of membranes with PAS (figures 1 and 2). This PAS positivity was diastase resistant, and the Schiff's positivity required periodic acid pretreatment (figure 3). Chloroform/methanol extraction failed to eliminate the diffuse PAS positivity of the membranes in frozen sections. Mucin did not account for the PAS positivity given the fact that the membranes did not stain with mucicarmine or alcian blue. Parallel staining of sections from eight of the PAS positive cases revealed linear fibrin strands or aggregates in only two (25%) using the Fraser-Lendrum and Putt's techniques (figure 4).

Autofluorescence of the membranes of varying degrees (figure 5) was noted in seven out of seven (100%) from this same series of cases, thereby matching the relative frequency of the PAS reactions. The excitation maxima of the membrane fluorescence paralleled that of fluorescent erythrocytes and was estimated between 350 and 400 nm, using the combination of exciter filters and dichroic mirrors outlined in table 1. Similar PAS positivity and autofluorescence were not evident in apparently viable (non-necrotic) alveolar pneumocytes and respiratory bronchiolar epithelium in these same sections.

At the electron microscopic level, the membrane matrix was noted to have a granular appearance (figure 6) in some regions.

Discussion

PAS positivity and autofluorescence are two constant features of pulmonary
hyaline membranes in the newborn in our study. The list of substances showing histochemical PAS positivity includes glycogen, fibrin, mucin, glycolipids, glycoproteins and lipid peroxidation products. Based on the histochemical findings reported in this paper, all can be excluded with the exception of glycoproteins and lipid peroxidation products. Furthermore, in the context of their concomitant autofluorescent properties estimated between 350 to 400 nm, the interpretation is favored by us that the PAS positivity of the membranes is consequent to lipid peroxidation. The basis for such an interpretation has established factual support. The process of lipid peroxidation results in the formation of malonaldehyde which can react with protein-linked amino acids to produce 1-amino-3-iminopropene derivatives. These derivatives are capable of fluorescing at excitation maxima between 360 and 380 nm and theoretically of being converted to protein-linked aldehydes under acid conditions. These acid conditions might be produced by periodic acid treatment, thereby accounting for both the autofluorescence and Schiff's positivity of the hyaline membranes. This sequence is schematically illustrated in figure 7. Other evidence to support a role for lipid peroxidation in the pathogenesis of hyaline membrane formation includes: (1) the presence of granular osmiophilic foci within the membrane matrix, which is a known ultrastructural appearance of peroxidation products; and (2) the ability of radiation to produce both malonaldehyde, thereby mimicking lipid peroxidation, and pulmonary hyaline membranes.

The elements essential for lipid peroxidation include molecular oxygen and polyunsaturated fatty acids (PUFA). The primary anatomic distribution of hyaline membranes along aerated (non-atelectatic) respiratory bronchioles, alveolar ducts, and alveoli would be consistent with the need for the former. The source of PUFA could be from cellular structural lipid or from endogenously mobilized stores of linoleic and linolenic acid. Although the latter possibility is favored by the present authors, based on histochemical studies of accumulated lipid in membranes, companion biochemical studies under consideration are necessary before a definitive statement can be made. However, regardless of the source of the PUFA, the subsequent auto-oxidation of structural lipids by toxic levels of fatty acid hydroperoxide, peroxide radicals, and other peroxidation products could theoretically lead to necrosis of alveolar pneumocytes and respiratory bronchiolar epithelium and hyaline membrane formation.

Although it seems reasonable to attempt to link the surfactant deficiency and concomitant progressive atelectasis of hyaline membrane disease of the newborn at least in part to peroxidation induced injury of type II pneumocytes, there is no direct evidence from this or other studies to support such at this time. From a therapeutic standpoint, reducing the potential for lipid peroxidation may be desirable and could probably be achieved.
FIGURE 1. Low power view reveals periodic acid Schiff's (PAS) positive (red) hyaline membranes lining aerated pulmonary spaces (PAS with diastase pretreatment x 100).

FIGURE 2. High power view of portion of PAS positive hyaline membrane (arrow left) illustrating typical staining pattern and tinctorial quality. Compare with brighter staining reaction of mucin in bronchiolar gland on right (PAS with diastase pretreatment x 1000).

FIGURE 3. Portions of hyaline membrane in left hand frame show absence of reaction with Schiff's reagent alone in contrast to positive (red) reaction following periodic acid pretreatment illustrated on right (x 400).

FIGURE 4. Medium (x 400) and high (x 1000) power views illustrate focal nature of histochemical positivity (red staining) for fibrin in the hyaline membranes (Fraser-Lendrum).

FIGURE 5. Autofluorescence of hyaline membranes contrasts with low fluorescence of interstitial tissue (x 1000).
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**Figure 6.** Granular, osmiophilic nature of hyaline membrane matrix as appreciated ultrastructurally (x 85,300).

**Figure 7.** Schematic proposal of events in lipid peroxidation leading to the consistent periodic acid Schiff's positivity and autofluorescence of the pulmonary hyaline membranes.
by early institution in high risk infants of a combination of “antilipolytic therapy,” such as hyperalimentation or hypertonic glucose and insulin, vitamin E supplementation, and moderation in the administration of oxygen therapy.

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References