EFFECT OF EXPERIMENTAL MATERNAL PNEUMONIA ON SURFACTANT ACTIVITY AND ULTRASTRUCTURE OF TYPE II ALVEOLOCYTES IN THE FETAL AND NEONATAL LUNGS

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UDC 616.24-053.1-076.4-02:616.24-002-055.25]-092.9

KEY WORDS: surfactant; type II alveolocytes; pneumonia

Much attention is currently being paid to problems connected with the study of the mechanisms delaying maturation of the fetal lungs in the intrauterine period, one result of which, according to a number of authorities, is the development of neonatal respiratory distress syndrome [6], in the pathogenesis of which a leading role is played by immaturity of the lung surfactant (LS) system. Among the factors inhibiting maturation of the fetal lungs and of the LS system are extragenital maternal pathology [5] and also intrauterine fetal hypoxia [1-4], arising in various pathological states of the mother during pregnancy.

The aim of this investigation was to study the state of surface activity of LS and the ultrastructure of the type II alveolocytes of the fetal and neonatal lungs, responsible for LS production, during experimental maternal pneumonia.

EXPERIMENTAL METHOD

The maximal and minimal surface tension (ST) of the surface-active fraction of animal lung extracts was determined by means of Wilhelmy's scales. The content of total lipids and phospholipids of LS was determined by biochemical methods. The lungs of 14 live-born and five stillborn guinea pigs (19 observations altogether), born to eight mothers, and divided into four groups, were subjected to histological and electron-microscopic investigation. Group 1 consisted of eight animals: five stillborn and three live born, to three mothers on the 64th-65th days of pregnancy, into which sterile threads were inserted transtracheally under open ether anesthesia on the 58th-60th days of pregnancy. Group 2 consisted of five live-born guinea pigs, born to two mothers, into which the sterile threads were inserted transtracheally before the beginning of pregnancy.

To rule out any possible effect of brief ether anesthesia in the mother on the state of the LS system of the fetus, the lungs of two live-born guinea pigs, born to the same

TABLE 1. ST, Concentrations of Lipids and Phospholipids, and Their Qualitative Composition in Surface-Active Fraction of Lung Extracts from Guinea Pigs Born to Mothers with Experimental Pneumonia (M ± m)

Group of animals	ST_{min}, mN/m	IS	Total lipids, g/liter	Phospholipids		
				total, mmoles/liter	phosphatidylcholine, %	phosphatidylethanolamine, %
1 ($n = 8$)	21,2±1,1	0,8±0,04	1,06±0,03	0,025±0,006	19,0±4,2	37,5±2,9
2 ($n = 5$)	23,4±0,8	0,9±0,04	1,02±0,01	0,019±0,004	14,9±3,9	21,8±2,8
3 ($n = 2$)	16,4±1,1	1,1±0,07	1,05±0,01	0,055±0,012	36,4±2,1	15,2±3,3
4 ($n = 4$)	17,8±0,8	1,0±0,06	1,03±0,05	0,063±0,006	27,3±6,4	25,6±4,6

Legend. 1. ST_{min}) minimal ST, IS) index of stability. 2. Significance of differences (p) given in text after Table 1. 3. Data on concentrations of cholesterol, neutral lipids, and various phospholipid fractions identified by thin-layer chromatography are not given in this Table.

Department of Pathological Anatomy, Crimean Medical Institute, Simferopol'. (Presented by Academician of the Academy of Medical Sciences of the USSR, D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 6, pp. 753-756, June, 1989. Original article submitted October 21, 1988.

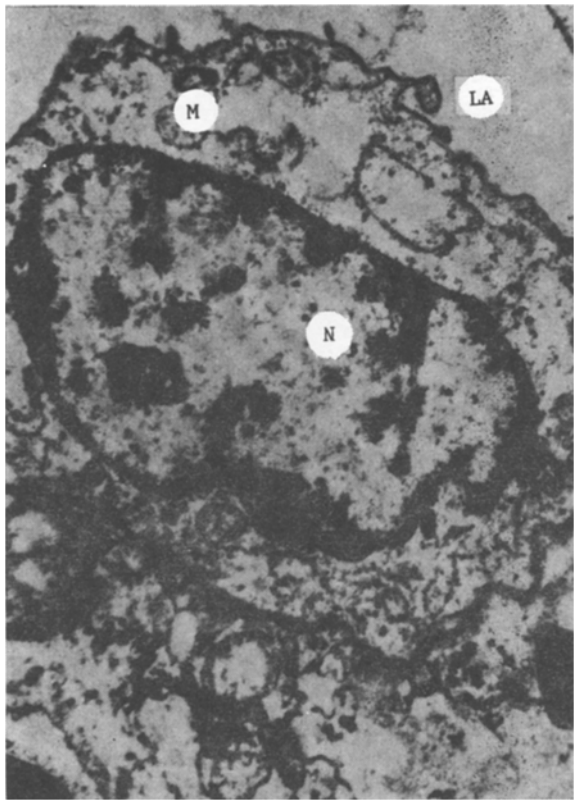

Fig. 1. Edema of type I alveolocyte. Here and in Figs. 2-3: N) nucleus, M) mitochondria, LA) lumen of alveolus. 18,000×.

mother, anesthetized with open ether for 15 min on the 58th-60th days of pregnancy, were studied. These animals constituted group 3. Group 4 included four live-born guinea pigs, born on the 65th-69th days of pregnancy, to two healthy mothers. All the live-born guinea pigs were decapitated under thiopental sodium anesthesia 2-4 h after birth. Pieces of tissue for electron microscopy were prepared by the usual methods. To identify morphological changes in the lungs and the state of surface active properties of LS, five mothers whose young were used in the observations of groups 1 and 2 also were decapitated on the day of giving birth or next day.

EXPERIMENTAL RESULTS

On microscopic investigation signs of catarrhal bronchitis and bronchioloitis, or interstitial and intraalveolar edema, and of serous and, in some places, serofibrinous pneumonia were found in the lungs of three mothers undergoing the operation on the 58th-60th days of pregnancy. On microscopic investigation, against the background of small foci of serous and serofibrinous pneumonia, marked changes in the bronchi were observed in the lungs of two of the mothers undergoing operation before pregnancy, and manifested as panbronchities or as discrete foci of bronchiectasis. Atelectases and dystelectases, combined with areas of emphysema and pneumofibrosis, were identified. The last condition was observed mainly in the form of carnification and peribronchial fibrosis.

The results of a study of the surface-active properties of LS from the fetuses are given in Table 1. In all eight guinea pigs of group 1 the surface-active properties of LS were inhibited, as shown by a significant ($p < 0.05$) increase in ST_{min} of the surface-active fraction of the lung extracts (21.2 ± 1.1 mN/m compared with 17.8 ± 0.8 mN/m in the control, accompanied by reduction of Clements' index of stability from 1.0 ± 0.06 to 0.8 ± 0.04 ($p < 0.02$). This increase in ST_{min} was evidently the result of a sharp fall of the phospholipid level in the composition of LS from 0.063 ± 0.006 mmole/liter in the control to 0.025 ± 0.006 mmole/liter ($p < 0.001$). It is an important fact that the phospholipids of LS of the experimental animals contained only 19.0 ± 4.2% of phosphatidylcholine, which is the phospholipid with the greatest surface activity, whereas in the control group, it accounted for

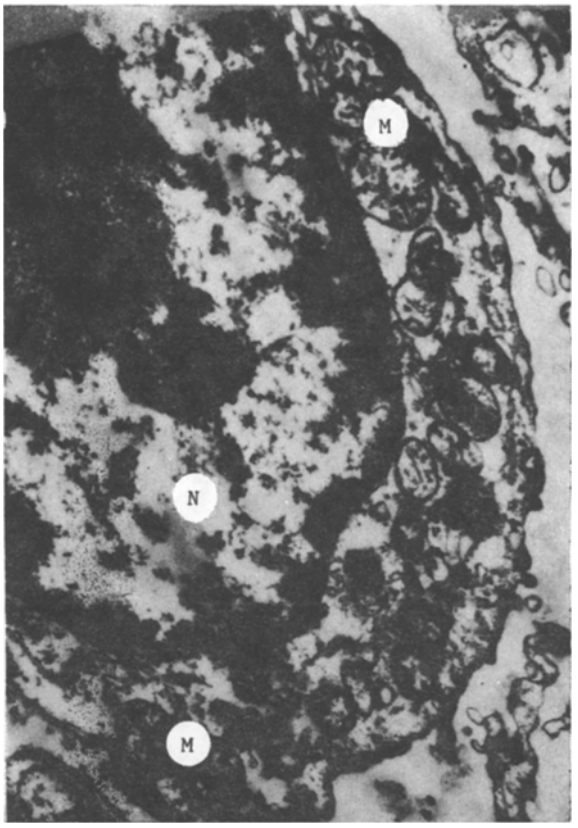

Fig. 2. Matrix of mitochondria of type II alveolocyte translucent, cristae shortened and partly disoriented. 26,000×.

27.3 ± 6.4%. It must be emphasized that LS of the experimental animals contained significantly more (37.5 ± 2.9%) phosphatidylethanolamine, with low surface activity, than in the control (25.6 ± 4.6%; $p < 0.05$).

Electron-microscopic investigation of the lungs of the animals of this group showed the main changes to have taken place in cells of the alveolar epithelium, mainly the type II alveolocytes. Just as in the type I cells (Fig. 1), they showed evidence of edema and hyperhydration. Although on the whole the type II alveolocytes in the animals of this group had all the characteristic features of these cells, changes indicating immaturity of the type II alveolocytes were found in most of them (Fig. 2). A characteristic feature of these cells is the presence of hypertrophied tubules of the rough endoplasmic reticulum, profiles of which were clearly visible in the type II alveolocytes. The number of osmiophilic lamellar bodies (OLB) in the cells varied within wide limits: from 6-8 to 12-14 per cell. The mature, well developed OLB, which in the control observations were round structures, consisting of bimembranous osmiophilic lamellae tightly twisted into a coil, were rarely seen. In the overwhelming majority of cases OLB were present as transitional forms from multivesicular bodies to OLB (Fig. 3).

These data are evidence that reduction of the surface-active properties of LS in the fetuses of group 1 was evidently the result of immaturity of a high proportion of the type II alveolocytes responsible for production of LS, possibly due to exposure to hypoxia and toxic factors.

Analysis of the data in Table 1 shows that inhibition of the surface-active properties of LS also took place in the surface-active fraction isolated from lung extracts of guinea pigs born to mothers undergoing the operation before the beginning of pregnancy. This was shown by the significant ($p < 0.001$) increase in ST_{min}, compared with the control, to 23.4 ± 0.8 mN/m and the sharp decline in the phospholipid content of LS to 0.019 ± 0.004 mmole/liter ($p < 0.001$). The phosphatidylcholine content in the phospholipids under these circumstances fell to 14.9 ± 3.9%, or 0.004 mmole/liter, whereas in the control its fraction amounted to 27.3 ± 6.4%, or 0.017 mmole/liter ($p < 0.05$).

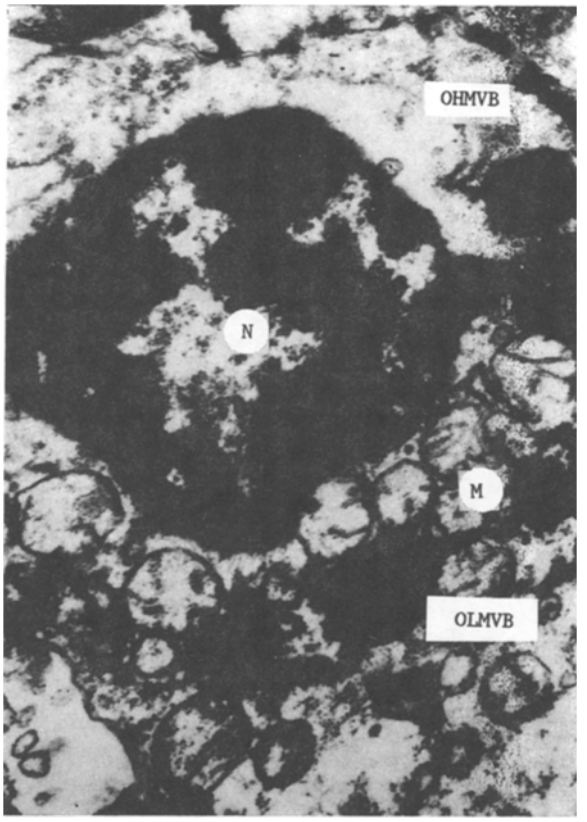

Fig. 3. Cytoplasm of type II alveolocyte contains immature OLB: osmiophilic homogeneous multivesicular bodies (OHMVB) and osmiophilic lamellar multivesicular bodies (OLMVB).

With these considerations in mind it can be concluded that reduction of the surface-active properties of LS in the animals of this group was connected to a definite degree with disturbances of synthesis of the phospholipids of LS, including phosphatidylcholine.

The results of the electron-microscopic investigation of the lungs of this group of fetuses indicate that at birth of the animals maturation of the type II alveolocytes is on the whole complete. The large alveolar cells contain a complex of well-developed organelles in their cytoplasm, among which well-formed OLB can frequently be seen. Meanwhile, signs indicating that many type II alveolocytes have not yet attained complete maturity were found in a high proportion of cells.

The results are thus evidence that functional immaturity of the type II alveolocytes is present in guinea pigs born to mothers with acute and chronic experimental pneumonia. One result of the immaturity of the cells responsible for LS production is inhibition of the surface-active properties of the surface-active fraction of extracts of the animals' lungs.

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INDUCTION OF LATENT LIVER DAMAGE BY CYCLOPHOSPHAMIDE

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UDC 616.36-036.12-02:615.277.3]-07-092.9

KEY WORDS: regeneration of the liver; cyclophosphamide; mitotic activity; chromosomal aberrations

Ionizing radiation is known to induce latent damage in the intact liver, which is manifested in the course of induced proliferation in the form of certain biochemical and morphological changes, more especially by inhibition of DNA synthesis and of mitotic activity, and also by chromosomal aberrations [3, 5, 6, 9]. A similar effect is given by certain chemical substances (cytostatics). Cyclophosophamide is one of the most frequently used cytostatics. Although the mechanisms of their action differ at the molecular level, the eventual results of damage to hematopoietic and lymphatic organs after exposure to ionizing radiation and to cyclophosphamide are identical in many respects [10, 11, 13].

The aim of this investigation was to discover whether cyclophosphamide can induce latent damage in nonproliferating tissues also. For this purpose we studied the effect of preoperative administration of cyclophosphamide on some cytological indicators of damage in the rat liver regenerating after partial hepatectomy (PHE).

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar albino rats with a mean weight of 250 g. The animals were kept under standard conditions in the animal house with free access to food and water. Before PHE (two-thirds of the liver) all the animals were divided into three groups: 1) control, operation only; 2) animals receiving cyclophosphamide, 100 mg/kg intraperitoneally, 2 h before the operation; 3) rats receiving 200 mg/kg cyclophosphamide intraperitoneally 2 h before the operation.

Cyclophosphamide, the diamide ester of N,N-bis(β-chloroethyl)-N',O-propylene phosphoric acid + NaCl (East Germany), was synthesized as the nontoxic transport form of the alkyl derivative $C_7H_{15}Cl_2N_2O_2P$. Cyclophosphamide is inactive in vitro. It is activated in vitro by liver oxidases, with the formation of biologically active metabolites, possessing marked mutagenic properties. PHE was performed by the standard method [7] and the animals were studied 24, 30, and 48 h and 3, 7, and 14 days after the operation. At each time interval five animals in each group were tested. The animals were killed between 6 and 8 a.m. in order to reduce the influence of circadian rhythms. Sections were cut from the regenerating liver tissues and stained by Feulgen's method. During analysis of 50,000-60,000 cells in each group studied, all mitotic figures and chromosomal aberrations in postmetaphase were noted. These data served as a basis for calculation of the mitotic index (MI; the number of mitotic figures per 1000 cells), the ratio of the number of metaphase to the number of prophases, and the number of chromosomal aberrations as a percentage of the total number of postmetaphase figures found among all the cells examined. The statistical significance of the results were estimated by the t test.

EXPERIMENTAL RESULTS

Mitotic figures were rare in the regenerating liver of the control animals until 24 h after PHE (Fig. 1). Later MI rose sharply to reach a maximum (27.291 ± 0.310‰) at the 30th

Department of General Biology, P. J. Šafarik University, Košice, Czechoslovakia. (Presented by Academician of the Academy of Medical Sciences of the USSR, D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 6, pp. 756-758, June, 1989. Original article submitted July 8, 1988.