CHANGES IN STATE OF THE SURFACTANT IN EXPERIMENTAL PNEUMONIA

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The state of the lung surfactant in rabbits at different stages of development of experimental pneumonia (3-60 days) was compared with the dynamics of oxidoreductases in the alveolar epithelium and cells of the inflammatory focus of infiltration. In the initial stage (3-7 days) of activation of cell metabolism there was a brief increase in the intensity of surfactant lipid synthesis, accompanied by relative inhibition of phospholipid synthesis. Later, development of degenerative changes and sclerosis of the parenchyma was accompanied by inhibition of synthesis of all components of the surfactant. The surface activity of the surfactant became stabilized at a low level.

KEY WORDS: *pneumonia; surfactant; phospholipids; pneumocytes; degeneration.*

Various pathological processes are accompanied by a disturbance of the state of the surface-active substance of the lungs — the surfactant. The possibility cannot be ruled out that these disturbances are a pathogenetic factor in the subsequent development of the disease. This is particularly likely in inflammatory lung diseases accompanied by marked degeneration of lung tissue [2], by atelectasis, and by disturbance of ventilation and perfusion. Data on the state of the surfactant system in the presence of inflammatory changes in the lung are few in number and contradictory in nature [1, 4, 7-9].

The object of this investigation was to study changes in the surfactant at various stages of development of inflammation.

EXPERIMENTAL METHOD

The state of the surfactant was studied in 25 rabbits at different stages of development of experimental pneumonia. The lungs of 10 healthy rabbits served as the control. Inflammation was produced by injection of sterile sawdust into the trachea [3]. Fragments of lungs not containing foci of suppuration or necrosis were investigated. The surface-active fraction was isolated by differential centrifugation in a density gradient [6]. The surface tension (ST) of the isolated fraction was measured on Wilhelmy—Langmuir scales in the modification of Nesterov et al. [5]. The coefficient of stability

$$\overline{S} = \frac{2(ST_{max} - ST_{min})}{ST_{max} + ST_{min}}$$

was calculated. The biochemical investigation of the surface-active fraction included determination of the content of total lipids, phospholipids, cholesterol, and total protein. Thin-layer chromatography of the lipids of the surface-active fraction (the consolidated layer of LS 5/40 silica gel, obtained from "Lachema") also was carried out. Sections through the lungs were stained by histological survey methods (hematoxylin—eosin, picrofuchsin) and by histochemical methods to study the activity of lactate, succinate, and malate dehydrogenases, and the concentrations of NAD and NADP (nitro-BT), DNP, RNP, and protein (mercuric chloride and bromphenol blue), and also glycogen.

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TABLE 1. Changes in Surfactant Characteristics Depending on Stage of Development of Inflammation of the Lungs in Rabbits (M ± m)

Index	Control ($n=10$)	Time after beginning of development of inflammation of lungs, days				
		3 ($n=6$)	7 ($n=5$)	14 ($n=5$)	30 ($n=3$)	60 ($n=6$)
ST_{min}, dynes/cm	5,1±0,34	6,70±3,45	13,30±1,21	13,20±1,54	12,60±1,79	14,60±1,19
P		>0,1	<0,001	<0,001	<0,02	<0,001
S	1,47±0,05	1,38±0,17	0,98±0,21	0,99±0,08	0,96±0,06	0,70±0,07
P		>0,05	<0,001	<0,001	<0,001	<0,001
TL, mg/g	2,52±0,31	6,25±1,41	5,60±1,76	3,75±0,78	1,13±0,01	1,07±0,48
P		<0,02	<0,05	0,05<P<0,1	<0,001	<0,01
PL, mg/g	1,89±0,19	4,10±1,29	3,00±0,82	1,54±0,36	0,59±0,06	0,43±0,20
P		0,05<P<0,1	>0,1	>0,5	<0,01	<0,001
PL/TL, %	71,5±2,17	64,70±11,20	58,60±11,70	47,40±5,04	53,10±9,00	34,10±11,20
P		>0,5	>0,1	<0,001	<0,01	<0,05
CH, mg/g	0,40±0,01	0,20±0,01	0,12±0,02	Traces	Traces	Traces
P		<0,02	<0,01			
TP, mg/g	2,83±0,22	7,10±0,34	6,00±0,45	4,10±0,10	1,10±0,02	1,00±0,01
P		<0,001	<0,001			<0,01

Legend. TL) Total lipids, PL) phospholipids, CH) cholesterol, TP) total proteins.

EXPERIMENTAL RESULTS

The dynamics of the changes in the indices reflecting the state of the surfactant is illustrated in Table 1. In the early stages of development of inflammation (third to seventh days) the content of total lipids, phospholipids, and protein in the surface-active fraction increased. Processes of cellular proliferation and infiltration were detected at these times. They were accompanied by intensification of metabolism in the pneumocytes, phagocytes, and lymphocytes of the epithelium and endothelium, as demonstrated by an increase in their DNP, RNP, and protein content and by the increase in their oxidoreductase activity. The phospholipid concentration increased less than that of total lipids, so that there was a decrease in the coefficient of stability and an increase in ST_{min}. Later (14–30 days) the surface activity of the isolated fraction still remained low. The absolute content of total lipids, phospholipids, and protein, and also the ratio of phospholipids to total lipids, fell below the control level. Morphologically, this period was characterized by progression of infiltration and the development of degenerative processes (abscess formation, bronchiectasis). Progressive degenerative changes were found histochemically in the alveolar cells and in the epithelium and endothelium, as shown by a decrease in their content of DNP, RNP, protein, reactive SH groups, and glycogen and in their oxidoreductase activity.

Toward the end of the investigation the surface activity fell more slowly, but the tendency for it to decrease remained.

The maximal decrease in the index of stability by 48%, in the content of phospholipids, total lipids, and protein (by 58, 78, and 66%, respectively), and in the phospholipids/total lipids ratio (by 53%) was observed after 2 months, when sclerotic canges began to develop in the lungs. Massive infiltration and destruction of phagocytes were accompanied by a decrease in their RNP and protein content and by a further decrease in oxidoreductase activity in the alveolar cells and bronchial epithelium.

Thin-layer chromatography showed that the phospholipids of the surfactant at all stages of the experiment were represented chiefly by lecithin with $R_f = 0.35$ and sphingomyelin with $R_f = 0.60$.

The development of inflammation of the bronchopulmonary tissue in rabbits thus takes place in two stages. In stage 1 activation of metabolism is observed in the alveolar cells (an increase in the protein and RNP content and in oxidoreductase activity). This corresponds to increased synthesis of the lipid component of the surfactant, but to relative inhibition of phospholipid synthesis, with a consequent decrease in surface activity in the early period. In stage II, one of degeneration and destruction, the intensity of metabolism falls, as demonstrated by a reduction in the synthesis of surfactant lipids. Despite the profound disturbances of metabolism, the physicochemical characteristics of the surface-active substance becomes stabilized in the late stage of the experiments, although at a lower level than at the beginning of inflammation. This points to definite compensation of the process as it moves into the chronic stage. The pattern observed could reflect specific regulation of surfactant synthesis determined by the functional requirements of the organ.

The results confirm the view that a low level of surface activity of the surfactant may have a role in the pathogenesis of pneumonia.

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INHIBITORS OF TRYPSIN-LIKE PROTEOLYTIC ENZYMES AS SUBSTANCES PREVENTING THE DEVELOPMENT OF SECONDARY NECROSIS IN BURN WOUNDS

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A biochemical, histological, and histochemical study was made of the effect of contrykal, an inhibitor of proteolytic enzymes, on the healing of experimental burn wounds in rats. Healing of burn wounds (flame burns covering 20% of the body surface) in animals untreated with contrykal was found to be accompanied by the development of secondary necrosis, by a marked inflammatory reaction, and by increased activity of certain proteases and peptidases. Administration of contrykal prevented the development of secondary necrosis, which is evidently associated with a reduction in the activity of the tissue proteolytic enzymes.

KEY WORDS: *contrykal; proteases; necrosis; healing of burn wounds.*

A distinguishing feature of the burn wound is its gradual deepening through the formation of secondary necrosis. The mechanism of development of secondary necrosis is linked with a disturbance of the circulation in the region of the burn, hypoxia, the inflammatory reaction, and destruction of the tissues beneath the primary necrotic zone. Destruction of the underlying tissues may be caused by proteolytic enzymes. Besides evidence of a decrease in their activity in the zone of burns [4, 5, 8], there are also indications of subsequent activation of proteases [7] in leukocytes [9] and in the wound discharge; the increase in proteolytic activity in the wound exudate is usually accompanied by autolysis of the grafts and their rejection [12]. These circumstances have led some workers to use inhibitors of proteolytic enzymes in the treatment of burns [2, 6]. To undertake a detailed study of this

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