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Effect of Unilateral Pneumonectomy on the Secretory Function of Type II Pneumocyte and Compensatory Growing Pattern of the Residual Lung in Growing Rabbits

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= ABSTRACT =

At the fifth day after right lung pneumonectomy in New-Zealand white rabbits (0.8~1.1 kg B.W.), phospholipid and protein concentration in the left lung lavage fluid were measured for clarification of the effect of unilateral pneumonectomy on the secretory function of the type II pneumocytes in growing rabbits. In an attempt to evaluate the effect of unilateral pneumonectomy on the compensatory growth of the residual lung, left lung weight and left lung weight-body weight ratio and DNA concentration, RNA/DNA and total DNA content in the left lung tissue were measured in pneumonectomized and in sham operated control rabbits. The lung weight of pneumonectomized rabbit was approximately two times heavier than that of the control rabbits. DNA concentration and RNA/DNA of the lung tissue were not changed but total DNA content was increased significantly. Phospholipid concentration in the lung lavage fluid of the pneumonectomized rabbits was over two times higher than that of control rabbits. From these experimental results, It is concluded that unilateral pneumonectomy in growing rabbits might cause to increase the secretion of pulmonary surfactant from type II pneumocyte of the residual lung. The cellular hyperplasia seems to be the primary response of the compensatory growing lung in unilateral pneumonectomized growing rabbits.

Key Words: Pneumonectomy, Compensatory lung growth, Pulmonary surfactant, Phospholipid, Type II pneumocyte.

INTRODUCTION

It is well known that unilateral pneumonectomy causes a compensatory growth of the residual lung in various experimental animals (Boatman, 1977). Cellular hyperplasia has been demonstrated in the compensatory lung especially in growing animals (Watkins et al, 1985).

Brody, et al (1978), reported that the process of the compensatory growth of the lung was very similar to the maturation process of the fetal lung. During the maturation period of the fetal lung the secretion of the pulmonary surfactant from the type II pneumocytes begins at the end of the gestational period (King, 1982), whereas the secretory function of the type II pneumocytes in the process of the compensatory growth of the lung is not clearly elucidated.

Although some investigators (Buhain & Brody, 1973) suggested that there might be some changes of the secretory function of the type II pneumocyte, its function in the compensa-

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tory growing still remains a controversy.

Therefore, the present study was designed to obtain information on the function of the type II pneumocytes by quantitative analysis of the surfactant in compensatory growing lung.

METHODS

Male New-Zealand white rabbits, 0.8~1.1 kg in body weight, were used for the present study. The animals were divided into two groups: thoracotomized, sham operated control group and right lung excised pneumonectomized group.

Nine rabbits underwent pneumonectomy with pentobarbital sodium anesthesia (30 mg/kg BW, iv), and the right lung was carefully excised. The rabbit was mechanically ventilated through tracheostomy with Narco-Biosystem V5KG respirator (Narco Biosystem, Texas, USA) throughout the surgery. During artificial respiration, inspiratory intrapulmonic pressure was adjusted at 10 cm H_2O and inspiration/expiration ratio was fixed at 1 : 2. Rabbit's chest was opened by incision through the fifth intercostal space and right pneumonectomy performed after ligation of hilum. Before one day and after three days of pneumonectomy, streptomycin (0.25 g/kg BW, sid) was injected intramuscularly for prevention of infection. Eight rabbits underwent sham thoracotomy for the control.

At the 5th day after the surgery, rabbits were anesthetized with pentobarbital sodium (30 mg/kg BW, iv) and sacrificed by bleeding with cutting of carotid artery in both groups. For measuring of lung weight/body weight ratio (L/B), left lung was excised carefully from the chest cavity and trachea and main bronchus were cut away with scissors. The left lung weight-body weight ratio was calculated for the evaluation of the degree of the compensatory lung growth.

DNA and RNA concentrations of the lung tissue were determined by Schneider's method (1945), and total DNA content was calculated by multiplying the lung weight and DNA concentration. With the total DNA content, the number of nuclei was calculated by Enesco's equation (1962).

To evaluate the secretory function of type II peneumocyte, phospholipid and protein content in pulmonary lavage fluid were determined. The excised lung was washed ten times with 30 ml of normal saline through the trachea with a polyethylene tube connected to a 50 ml capacity syringe. Approximately 20 ml of the obtained lung lavage fluid was centrifuged at 3000 rpm for 10 minutes, and the supernatant was used for determination of phospholipid and protein.

For determination of phospholipid, pulmonary lavage fluid was mixed with chloroform: methanol (v/v, 2: 1, Merck Co) solution and phospholipid was extracted according to Folch et al (1957). By Beveridge & Johnson's method (1949) phospholipid phophorus was determined and phospholipid content was calculated according to Corbet et al (1983). The protein content was determined by Lowry's method and bovine serum albumin (Sigma Co) was used as standard.

Statistical analysis: All experimental data were tested by the unpaired two-tailed Student's t-test. A p value of less than 0.05 was considered significant. Values are given as mean±S.E.

RESULTS

Table 1 shows the values obtained from 9 right pneumonectomized and 8 control rabbits. The left lung weight (mean±S.E.) at the 5th day in the pneumonectomized rabbits was 4.27±0.507 gm. It was over two times heavier than that of the control rabbits ($p<0.001$). The left lung weight-body weight ratio was also higer than that of the control ($p<0.001$).

DNA concentration and the RNA/DNA were not changed significantly but the total

Table 1. Changes in various parameters after right lung pneumonectomy

	Control (n=8)	Pneumonectomy (n=9)
Body weight(kg)	0.89±0.026	1.10±0.042
Left lung weight(gm)	1.96±0.107	4.27±0.507*
L/B×10^{-3}	2.20±0.103	4.23±0.577*
DNA(mg/gm wet tissue)	3.34±0.163	3.01±0.189
RNA(mg/gm wet tissue)	2.99±0.143	3.34±0.118
RNA/DNA	0.91±0.031	1.11±0.077
Total DNA(mg/lung)	6.44±0.261	11.25±2.117*

Values are given as Mean±S.E; n indicates number of cases;
*$P<0.001$, control vs pneumonectomy; L/S×10^{-3}; lung weight/body weight ×10^{-3}.

Table 2. Phospholipid and protein in left lung lavage fluid in control and right lung pneumonectomized rabbits

	Control (n=8)	Pneumonectomy (n=9)
Phospholipid (umol/gm wet lung)	22.50±1.962	53.97±4.590*
Protein (mg/gm wet lung)	3.5 ±0.19	2. 4±0.17*

Values are given as mean±S.E.; n indicates number of cases;
$P<0.001$, control vs pneumonectomy

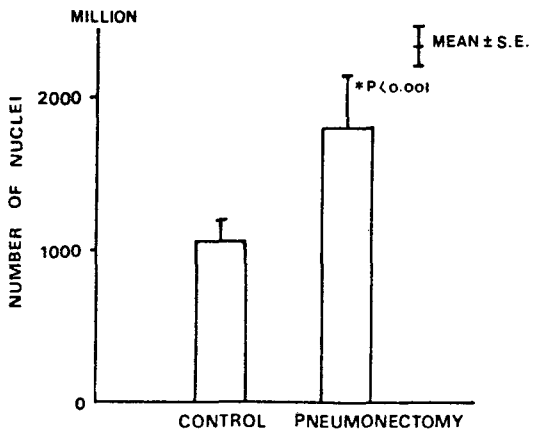

Fig. 1. A comparison of number of nuclei in young control and pneumonectomized rabbit left lung. There is a significant increase of number of nuclei in the pneumonectomized lung in comparison with control rabbit left lung.

content of DNA in the residual lung tissue was increased significantly ($p<0.001$) by the pneumonectomy. It signifies that cellular hyperplasia is the primary response of the compensatory growth of the residual lung after pneumonectomy in growing rabbits.

Figure 1 showes a significant increase of number of nuclei in the pneumonectomized rabbit left lung in comparison with that of control rabbit left lung ($p<0.001$).

The phospholipid content (mean±S.E.) in lung lavage fluid was 53.97±4.590 μmol/gm wet tissue in pneumonectomized rabbits. The content was over two times greater than that of the control groups ($p<0.001$). On the other hand, the protein content in the lavage fluid in pneumonectomized rabbit was lower compared with that of the control rabbits (Table 2).

DISCUSSION

A number of observations on the compensatory growth of residual lung after unilateral pneumonectomy have been reported (Nattie et al, 1974; Inselman et al, 1977). The compensatory growth response is different according to age, sex and species of experimental animals (Smith et al, 1980). The cellular responses durging compensatory growth of the lung can generally be inferred by measuring lung weight, lung weight-body weight ratio, and by determination of DNA concentration, RNA/DNA and total DNA content in the lung tissue (Bennet et al 1985). In the present study, the left lung weight and lung weight-body weight ratio of pneumonectomized rabbits were over two times higher than those of the sham-operated control rabbits. DNA concentration and RNA/DNA were not changed, whereas total DNA content was increased significantly at the fifth day after surgery. These experimental results suggest that the cellular hyperplasia is the primary response of the compensatory growing lung in growing rabbits. The present observations are in agreement with those of Buhain & Brody (1973).

Even if little is known on the secretory function of the type II pneumocyte in compensatory growing lung after unilateral pneumonectomy, Lee, et al (1980), reported that there was a tendency of phospholipid concentration to increase, and a significant increase of the total phospholipid content of the lavage fluid at the third day after pneumonectomy in adult rabbits. Many investigators (Anderson et al, 1981; Thurlbeck et al, 1981; Lee & Lee, 1984) have insisted that steroid stimulates the secretory function of type II pneumocyte and inhibits cellular hyperplasia in compensatory growing lung.

So it could be thought that a cellular hyperplasia is not a sole cause of increased phospholipid secretion from type II pneumocyte.

In the present study in which growing rabbits were used, the phospholipid concentration in the lung lavage fluid of the pneumonectomized rabbits was over two times higher than that of sham-operated control rabbits. It is thought to be very important finding that total increment of the phospholipid in the pulmonary lavage fluid of pneumonectomized rabbits was over four times greater than that of control rabbits. Contrary to the increase of the phospholipid, the protein was decreased significantly in pulmonary lavage fluid after pneumonectomy. Jobe et al (1983), reported that increased secretion of the pulmonary surfactant was followed by the decreased content of protein in pulmonary lavage fluid, which was the similar result in the present study. Considering all these experimental results, it can be concluded that unilatral pneumonectomy causes an increase in the secretion of pulmonary surfactant from the type II pneumocyte of the residual lung in growing rabbits.

In regard to the cellular event during the compensatory growth of the lung, it has been well known that the type II pneumoncytes proliferate markedly in early period of the compensatory growing after unilateral pneumonectomy (Lee, et al, 1980). Although the secretory and/or synthetic activity of the individual type II pneumocyte were not studied in the present study, the increased amount of phospholipid of the pulmonary lavage fluid seems, on the basis of other reports, to be the consequence of the increased number of type II pneumocytes of the compensatory growing lung. According to Smith, et al (1980), pneumonectomized rabbit serum stimulated DNA synthesis of cultured type II pneumocytes, which was a result of the effect of a humoral factor in pneumonectomy. In this regard, it was thought that a humoral factor in pneumonectomyized serum might be related to the compensatory growth of the lung and/or the secretory function of the type II pneumocyte after pneumonectomy.

In summary, a greater content of surfactant phospholipid was observed in the pulmonary lavage fluid of unilateral pneumonectomized

rabbits than that of the sham-operated control rabbits. These experimental results suggest that the production of surfactant can be stimulated by pneumonectomy in growing rabbits.

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