PULMONARY SURFACTANTS IN PULMONARY OEDEMA INDUCED BY HEAD INJURY IN MICE

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Summary: A study has been carried out to examine the role of pulmonary surfactants in pulmonary oedema induced by head injury. Pulmonary oedema was induced by head injury in mice by the method of Mackay (5). There was no change in surface tension as well as total phospholipid concentration of the lung between control and test groups suggesting that there was no involvement of lung surfactant system in pulmonary oedema induced by head injury. Since the animals in the test group died within a few minutes, possibly there may not have been sufficient time for alteration of lung surfactant system.

Key words: pulmonary surfactants, pulmonary oedema, head injury, surface tension, phospholipid

INTRODUCTION

It has been discovered in recent years that surface tension lowering agents, known as pulmonary surfactants are present in the alveoli and counteract the surface tension forces. The patency and stability of the alveoli is maintained to a large extent by pulmonary surfactants. It is now well established that the pulmonary surfactants consist of mainly phospholipids (4). Pattle (6) stressed the idea that surface tension must also be taken into account as one of the forces influencing the distribution of the liquid between the alveoli and the capillaries. Scarpelli (7) proposed a hypothesis that factors which tend to alter lung surfactants (i.e. increased surface tension) tend to promote pulmonary oedema. Since head injury produces pulmonary oedema (5) the present study was designed to examine the role of pulmonary surfactants in pulmonary oedema induced by head injury.

MATERIALS AND METHODS

Adult albino mice of either sex (17-25 g) were divided into control and test groups. The control animals were killed by an over dose of ether. The animals of the other group were subjected to head injury to produce pulmonary oedema (5). The animals at rest had their heads fixed between two laterally placed wooden blocks one of which was fixed. A sudden static force sufficient to produce a fatal head injury was applied to the mobile block. This caused the animals in the test group to die within 1 to 3 min during which clonic convulsive movements were noticed. After the death of the animals in both the groups, they were dissected and the lungs were

removed. The lung body index (L.B.I.) which is a standard parameter for evaluating pulmonary oedema was calculated as follows :

$$\text{Lung body index (L.B.I.)} = \frac{\text{weight of lung}}{\text{body weight}} \times 100$$

Preparation of lung extract:

The extract was prepared by mincing the lung tissue finely with scissors. The minced tissue was stirred in saline (3 g of lung 50 *ml* of saline) and filtered through four layer gauze to remove coarse particles (7). The prepared lung extract was used for surface tension measurement and for total phospholipid estimation.

Surface tension measurement:

Surface tension was measured by the method of Brown *et al* (1). For this purpose, bubbles of lung extract were blown at the end of a "T" tube connected to a syringe and strain gauge pressure transducer. The formula used was $\gamma = Pr/4$, where γ is the surface tension, P is the pressure difference across the bubbles and r is the radius of the bubble. Radius of the bubble was calculated by using the formula $V = 4 \pi r^3/3$.

The following parameters were measured :

(1) Minimum surface tension (γ min.), surface tension measured when the bubble size is minimum.

(2) Maximum surface tension (γ max.), surface tension of the bubble with the largest diameter possible.

(3) Stability index ($\bar{S}$), which denotes the stability of the alveoli.

$$\bar{S} = \frac{\text{change of tension}}{\text{average tension}} = \frac{2\,(\gamma \text{ max.} - \gamma \text{ min})}{\gamma \text{ max.} + \gamma \text{ min.}}$$

Phospholipid estimation :

Total phospholipid of the lung extract was estimated by the method of Connerty *et al* (2). According to this method, proteins were precipitated by trichloroacetic acid and the precipitate containing the phospholipids, was digested with a sulphuric acid-perchloric acid reagent. The inorganic phosphorous librerated from phospholipid reacted with ammonium molybdate in the presence of the reducing agent metol giving a blue colour which was estimated colorimetrically using a red filter or at 700 *mμ*.

RESULTS

Table I summarises the results of our experiments. There was a highly significant difference in L.B.I. ($P < 0.001$) between control and test group suggesting that head injury in mice induced pulmonary oedema. There was no significant difference in r min. r max., stability index and total phospholipid content of the lungs between control and test groups ($P > 0.05$ for all the parameters) suggesting that pulmonary surfactants were not affected at all in pulmonary oedema induced by head injury.

TABLE I : Showing the role of pulmonary surfactants in pulmonary oedema induced by head injury.

Group	*Mean L.B.I. ±S.E.*	*Surface tention parameters (mean ± S.E.M.)*			*Mean Phospholipid mg P/g of wet lung ±S.E.*
		minimum dynes/cm	*maximum dynes/cm*	*stability index ±S.E.*	
Control (n = 12)	0.72 ± 0.03	17.0 ± 1.2	60.5 ± 6.6	1.12 ± 0.02	5.94 ± 0.16
Test (n = 12)	1.38 ± 0.14	17.2 ± 0.5	59.8 ± 2.3	1.10 ± 0.04	5.43 ± 0.22
	P < 0.001	P > 0.05	P > 0.05	P > 0.05	P > 0.05

It was also noted on gross examination of the lungs of the animals in the test group (where pulmonary oedema was induced by head injury) were oedematous, heavy and with haemorrhagic patches and spots. Fairly increased weight of the lungs and outpouring of frothy fluid from the cut end of the alveoli and from bronchi and trachea on manual pressure differentiated the oedematous lungs from pulmonary congestion in which there was only an increased stasis of the blood in pulmonary capillaries without much increased weight of the lungs.

DISCUSSION

Schaefer and his colleagues (8) exposed guinea pigs to 15% carbon dioxide and noted that after one hr all the animals had pulmonary oedema of mild severity, moderate atelectasis and normal surface tension properties (stability index was 1.72). After 22 hr of the exposure, the severity of atelectasis and oedema and γ min. were increased and stability index of lung extract was reduced, (stability index was 0.57). Goldenberg *et al.* (3) induced pulmonary oedema by bilateral cervical vagotomy and observed that lungs from all the animals had areas of atelectasis, local oedema and severe capillary congestion. They observed that surface tension of lung extracts was normal after one hr, abnormal in two of five animals after five hr and abnormal in three of seven after six hr.

In contrast to the above observations, we found that in the test group (where pulmonary oedema was induced by head injury) there was no change in surface tension ($P > 0.5$ for γ min., γ max and stability index.). Correspondingly there was no change in the phospholipid content of the lungs. In our experiments, all the amimals in the test group died within 1 to 3 min and pulmonary oedema was found in all the animals. Since the animals died within a short time,

possibly there may not have been sufficient time for alteration of the lung surfactant system. Schaefer *et al.* (8) and Goldenberg *et al.* (3) also noticed that after one hr of the exposure to 15% carbon dioxide and one hr after bilateral cervical vagotomy, there was no change in surfactant system, though oedema was noted. Thus the time may be an important factor in pulmonary oedema for alterating the lung surfactant system.

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