

# Effect of Histamine on Lecithin Content in Broncho-Alveolar Lavage Fluid of Rat

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Pulmonary surfactant prevents collapsing tendency of alveoli.<sup>1</sup> It also keeps alveoli dry by preventing the tendency towards development of pulmonary edema.<sup>2</sup> The innervation to type II cells being sparse, the regulation of surfactant secretion appears to be mainly humoral. The effect of sympathomimetics, parasympathomimetics and other chemical mediators like substance P on surfactant system of lung has been reported.<sup>3-5</sup> Histamine is implicated as a mediator in allergic pulmonary diseases such as bronchial asthma and pulmonary aspergillosis. This study was designed to observe the effect of histamine on lecithin content in broncho-alveolar lavage fluid of rats.

## MATERIALS AND METHODS

Healthy adult male albino rats of Wistar strain weighing between 200-220 g were used for the study. The animals were maintained in cages with free access to air, food and water.

**SUMMARY** Deficiency of surfactant in alveoli leads to increased resistance to breathing. Histamine is a mediator in allergic respiratory diseases. Though the bronchoconstrictor effect of histamine is well recognised, histamine may have additional actions that contribute to pathogenesis in these diseases. The present study aimed to observe the effect of histamine on lecithin, a major component of alveolar surfactant. Lecithin content in broncho-alveolar lavage (BAL) fluid of healthy adult male rats was estimated by enzymatic method using Boehringer-Mannheim kits. Lecithin content in these control animals was compared with that in three groups of healthy adult male rats following subcutaneous administration of 0.06 mg of histamine diphosphate at 10 minutes, 30 minutes and 60 minutes intervals, respectively. A significant reduction in lecithin levels in BAL fluid was observed up to one hour after administration of histamine. The results indicate a possible additional action of histamine in the pathogenesis of allergic respiratory diseases.

Lecithin is the major surface active phospholipid of pulmonary surfactant system.<sup>6</sup> Broncho-alveolar lavage (BAL) is a standard procedure to assay various components of pulmonary surfactant system.<sup>7</sup> Thus, assay of lecithin in BAL fluid was employed in the present study to observe the effect of histamine on alveolar lecithin levels in adult rats.

## Broncho-alveolar lavage

BAL was performed as described in our previous studies.<sup>8</sup> The

control group of animals were given pentobarbitone sodium intraperitoneally at a dose of 40 mg/kg. The anaesthetised rats were incised from xiphisternum to chin. The thorax was opened and lungs along with trachea were isolated. The trachea was cannulated and alveoli were rinsed with normal saline via

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the airway. Each time 10 ml of normal saline was introduced via the trachea, and, the fluid was retained in the lungs for one minute. Then, it was rinsed back and forth, and aspirated. The procedure was repeated and a volume of about 15 ml was extracted from each animal.

Lungs which had any abnormal appearance such as haemorrhagic spots or patches were excluded from the study. Lungs were also excluded if they showed leakage during lavage or if the lavage fluid obtained was contaminated with blood or extraneous matter. A total of eight samples which had none of the above defects were taken as control samples for assay of lecithin.

To observe the effect of his-

tamine on alveolar lecithin levels, BAL was performed in three groups of healthy adult rats (8 in each group) following subcutaneous administration of 0.06 mg of histamine diphosphate (Sigma Chemical Company) at 10 minutes, 30 minutes and 60 minutes intervals, respectively. The dose of histamine administered did not produce any change in blood pressure or respiration in preliminary experiments. BAL was performed as in control group and the fluid was used for assay of lecithin.

#### Assay of lecithin

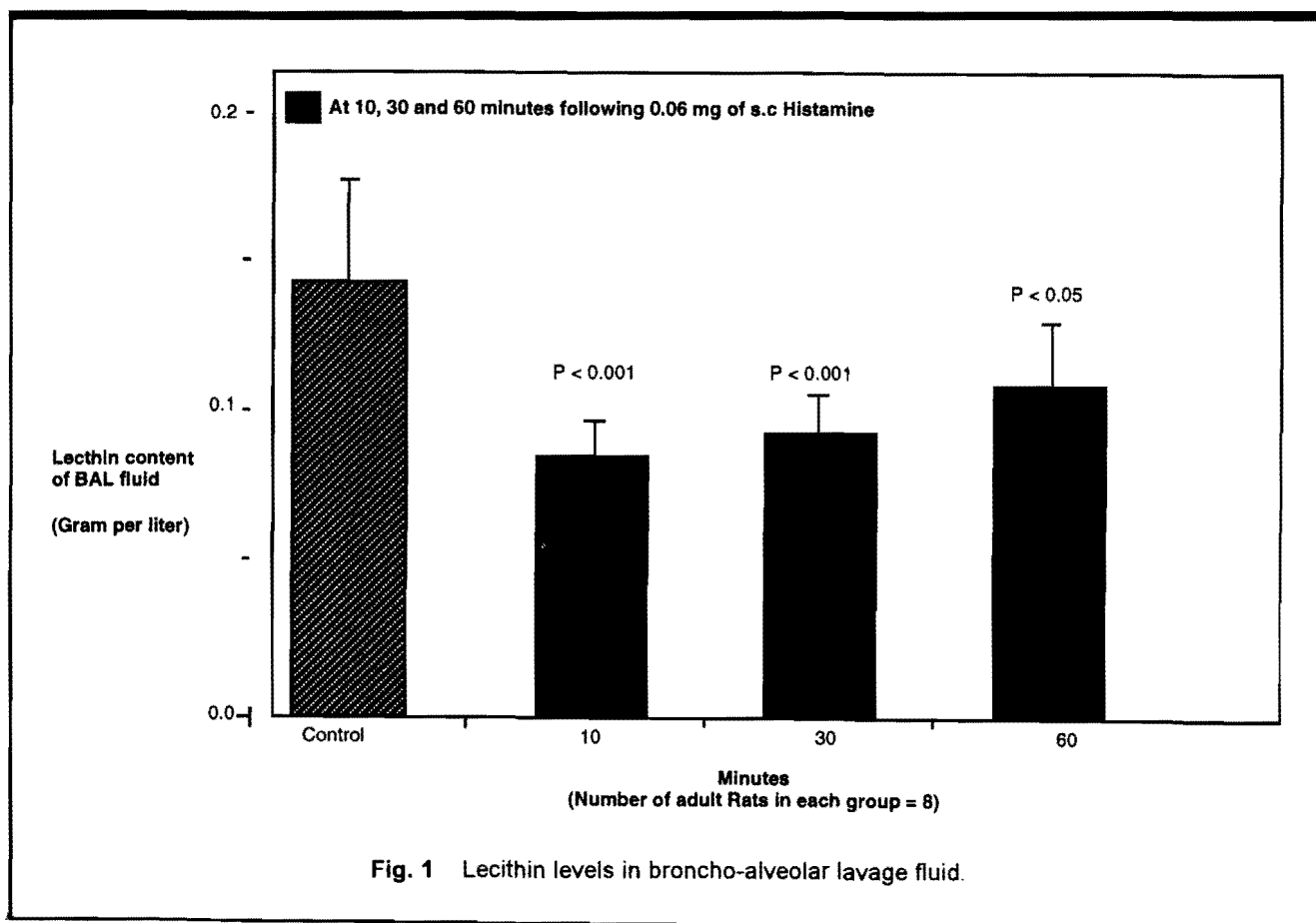
Assay of lecithin was performed by enzymatic method using Boehringer-Mannheim kits according to the manufacturer's instructions.

#### Statistical analysis

Statistical analysis of the results obtained was done using Student's unpaired *t*-test. Lecithin levels in the BAL fluid of each of the experimental groups were compared with those values obtained in control group. If the *p* value was 0.05 or less, it was considered as significant difference in the values between the groups that were compared.

## RESULTS

The volume (mean  $\pm$  SD) of BAL fluid retrieved from the control group was  $13.375 \pm 0.517$  ml. BAL fluid (mean  $\pm$  SD) retrieved from the experimental groups at 10, 30 and 60 minutes following s.c. hista-



mine were  $13.25 \pm 0.463$ ,  $13.5 \pm 0.534$  and  $13.125 \pm 0.353$  ml, respectively. The volume retrieved was about 70% in each group. Compared with control group, there was no significant difference in volume retrieved from any of the experimental groups.

The lecithin content (mean  $\pm$  SD) per liter of BAL fluid from the control group was  $0.145 \pm 0.032$  g. The lecithin content (mean  $\pm$  SD) per liter of BAL fluid from the experimental groups at 10, 30 and 60 minutes following s.c. histamine were  $0.088 \pm 0.008$ ,  $0.096 \pm 0.008$  and  $0.112 \pm 0.020$  g, respectively (Fig. 1). Compared with the control group, there was a significant difference in lecithin content of BAL fluid at 10 minutes, 30 minutes and 60 minutes following s.c. histamine. The *p* values were less than 0.001, 0.001 and 0.05, respectively at 10 minutes, 30 minutes and 60 minutes.

## DISCUSSION

A highly significant reduction in lecithin content in BAL fluid was observed within 10 minutes of administration of histamine. Subsequently, however, there was a gradual increase in lecithin content although even at one hour the lecithin levels were still significantly lower compared to that in control animals. This indicated an acute decrease in lecithin content in BAL fluid. The gradual increase observed in this study may be a reflection of a very short plasma half life of histamine.

Mechanism of decreased lecithin levels following administration of histamine can not be ascertained

from this study. Type II alveolar cells constantly secrete pulmonary surfactant into alveoli. Reuptake of this substance from the alveoli by the same cells maintains homeostasis of this surface active material in the alveoli.<sup>9, 10</sup> Thus, the administered histamine may have inhibited the secretion or stimulated the uptake or both. Pulmonary surfactant and the surfactant associated proteins are stored in lamellar bodies of Type II alveolar cells.<sup>11</sup> Some of the surfactant associated proteins inhibit secretion and some facilitate uptake.<sup>12, 13</sup> The effects observed in this study could also be due to secretion of these proteins. Further studies may help identify the mechanisms involved in the reduction of lecithin content.

Histamine was reported to cause secretion of surfactant by Type II cells *in vitro*.<sup>14</sup> However, in the present study a decrease in lecithin level was observed following histamine administration. This emphasizes the fact that *in vitro* and *in vivo* effects may be different in view of the complexity of responses in the whole animal. Decreased lecithin levels in alveoli lead to increased resistance to breathing. It is possible that such reduction in lecithin levels may be one of the factors responsible for the respiratory disability in allergic pulmonary diseases like bronchial asthma and pulmonary aspergillosis. Assay of lecithin in BAL fluid in these patients may elucidate the effect of histamine on surfactant system of lung in the adult human.

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## REFERENCES

1. Mead J. Mechanical properties of lungs. *Physiol Rev* 1961; 41: 281-330.
2. Janardhana Rao G, Ramnarayan K, Krishna Rao A, Nalini K. Experimental production of high surface tension pulmonary edema. *Indian J Pathol Microbiol* 1988; 31: 1-7.
3. Janardhana Rao G, Aroor AR, Krishna Rao A, Sreedhar S. Effect of bronchodilators on surfactant system of lung. *Indian J Med Res* 1989; 90: 266-9.
4. Massaro D, Clerck L, Massaro GD. Surfactant secretion: evidence that cholinergic stimulation of secretion is indirect. *Am J Physiol* 1982; 243 (cell physiol): C39-45.
5. Rice WR, Singleton FM. Regulation of surfactant secretion from isolated type II pneumocytes by substance P. *Biochem Biophys Acta* 1987; 889: 123-7.
6. King RJ. Pulmonary surfactant. *J Appl Physiol* 1982; 53: 1-8.
7. Pattle RE. Surface lining of lung alveoli. *Physiol Rev* 1965; 45: 48-79.
8. Janardhana Rao G, Aroor AR, Nalini K, Krishna Rao A. Effect of drugs used in pulmonary edema on surfactant system of lung. *Indian J Chest Dis & Allied Sci* 1991; 33: 59-62.
9. Geiger K, Gallagher ML, Hedley-Whyte J. Cellular distribution and clearance of aerosolized dipalmitoyl lecithin. *J Appl Physiol* 1975; 39: 759-66.
10. Jacobs A, Jobe A, Ikegami M, Jones S. Surfactant phosphatidylcholine sources, fluxes and turnover times in 3 day old, 10 day old and adult rabbits. *J Biol Chem* 1992; 257: 1805-10.
11. Coalson JJ, Winter TV, Martin HM, King RJ. Colloidal gold immunultrastructural localization of rat surfactant. *Am Rev Respir Dis* 1986; 1333: 230-7.
12. Rice WR, Ross G.F, singleton FM, DingleS, Whitsett JA. Surfactant associated protein inhibits phospholipid

- secretion from Type II cells. *J Appl Physiol* 1987; 63 : 692-8.
13. Wright JR, Wager RE, Hawgood S, Dobbs L, Clements JA. Surfactant apoprotein 26000-36000 enhances uptake of liposomes by Type II cells. *J Biol Chem* 1987; 262 : 2888-94.
14. Cheng M, Brown LAS. Histamine stimulation of surfactant secretion from rat Type II pneumocytes. *Am J Physiol* 1990; 258 (Lung cell Mol Physiol) : L 195-200.