# Effect of histamine blockers on histamine induced decrease in lecithin levels in BAL (Broncho alveolar lavage) fluid in rats.

By:
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Supported by:
**U.S.M. Short term research grant**

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USM J/P-06

Semua laporan kemajuan dan laporan akhir yang dikemukakan kepada Bahagian Penyelidikan dan Pembangunan perlu terlebih dahulu disampaikan untuk penelitian dan perakuan Jawatankuasa Penyelidikan di pusat pengajian

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## Laporan Akhir Projek Penyelidikan Jangka Pendek

1) **Nama Penyelidik:** Dr.G. Janardhana Rao

**Nama Penyelidik-Penyelidik Lain *(Jika berkaitan)*** :

2) **Pusat Pengajian/Pusat/Unit** : PPSP, Department of Physiology

3) **Tajuk Projek:** Effect of histamine blockers on histamine induced decrease in lecithin levels in BAL (Broncho alveolar lavage) fluid in rats.

4) (a) **Penemuan Projek/Abstrak**

***(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).***


## Summary

Lecithin,a major surface active substance of surfactant system of lung was estimated in bronchoalveolar lavage (BAL) fluid in four groups of healthy adult male albino rats. Rats from group I were not administered any drug and acted as control. Group II were administered histamine diphosphate.Group III were given $H_1$ blocker (Pyrilamine maleate) followed by histamine diphosphate. Group IV received $H_2$ blocker (Ranitidine hydrochloride) followed by histamine diphosphate. Lecithin content of BAL fluid in control group was compared with that in the other three groups. A significant decrease in lecithin content was observed in the rats that received either histamine diphosphate or $H_1$ blocker followed by histamine diphosphate. However, compared to control rats no significant difference in lecithin content was seen in the rats that received $H_2$ blocker followed by histamine diphosphate. The results clearly indicate that the decrease in surface active lecithin content in BAL fluid following administration of histamine diphosphate was unaffected by prior administration of $H_1$ blocker, but was blocked by prior administration of $H_2$ blocker. It was concluded that histamine induced decrease in lecithin content of BAL fluid is mediated through $H_2$ receptors. Since the predominant source of intraalveolar lecithin is secretion of Type II cells of alveolar epithelium, it is possible that Type II cells have $H_2$ receptors, stimulation of which resulted in decreased intraalveolar lecithin.

(b) **Senaraikan Kata Kunci yang digunakan di dalam abstrak:**

| **Bahasa Malaysia** | **Bahasa Inggeris** |
|---|---|
| ..... | Lecithina, bronchoalveolar lavage, |
| ..... | Histamine, histamine blocker |
| ..... | ..... |
| ..... | ..... |
| ..... | ..... |
| ..... | ..... |
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5) **Output Dan Faedah Projek**

(a) Penerbitan *(termasuk laporan/kertas seminar)*
***(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).***

N/A
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(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten:
*(Jika ada dan jika perlu, sila gunakan kertas berasingan)*

N/A

(c) Latihan Gunatenaga Manusia

*i) Pelajar Siswazah* .....

*ii) Pelajar Prasiswazah:* .....

*iii) Lain-Lain* : .....

6. **Peralatan Yang Telah Dibeli:**

No new instruments.....

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**UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI**

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PROF MADYA (DR) MAFAUZY MOHAMED
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Universiti Sains Malaysia
16150 Kubang Kerian
Kelantan

T/TANGAN PENGERUSI
J/K PENYELIDIKAN
PUSAT PENGAJIAN

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# Summary

Lecithin,a major surface active substance of surfactant system of lung was estimated in bronchoalveolar lavage (BAL) fluid in four groups of healthy adult male albino rats. Rats from group I were not administered any drug and acted as control. Group II were administered histamine diphosphate.Group III were given $H_1$ blocker (Pyrilamine maleate) followed by histamine diphosphate. Group IV received $H_2$ blocker (Ranitidine hydrochloride) followed by histamine diphosphate. Lecithin content of BAL fluid in control group was compared with that in the other three groups. A significant decrease in lecithin content was observed in the rats that received either histamine diphosphate or $H_1$ blocker followed by histamine diphosphate. However, compared to control rats no significant difference in lecithin content was seen in the rats that received $H_2$ blocker followed by histamine diphosphate. The results clearly indicate that the decrease in surface active lecithin content in BAL fluid following administration of histamine diphosphate was unaffected by prior administration of $H_1$ blocker, but was blocked by prior administration of $H_2$ blocker. It was concluded that histamine induced decrease in lecithin content of BAL fluid is mediated through $H_2$ receptors. Since the predominant source of intraalveolar lecithin is secretion of Type II cells of alveolar epithelium, it is possible that Type II cells have $H_2$ receptors, stimulation of which resulted in decreased intraalveolar lecithin.

# Introduction

The surface tension forces of alveolar lining were demonstrated as early as 1929 by Von Neergard.However,a problem oriented approach to this phenomenon started with the discovery of increased surface tension forces leading to collapse of lung and respiratory distress in hyaline membrane disease by Avery and Mead in 1959 (1). The presence of surfactant in normal lungs to act against the surface tension forces was demonstrated by Clements and Pattle (2,3). Later, it's biochemical composition in various species including human beings was explored (4,5,6).

Pulmonary surfactant is a mixture of lipids, proteins and carbohydrates. About 90 % of pulmonary surfactant is lipids. A major portion of lipids is in the form of dipalmitoyl phosphotidyl choline, the other phospholipids like phosphotidyl glycine, phosphotidyl glycerol and phosphotidyl ethanolamine being in minor concentrations. The phospholipids form a surface active film lining the alveoli where as proteins help in stabilizing the film.

Pulmonary surfactant is produced by Type II cells of alveolar epithelium. Lamellar bodies containing whorls of phospholipids are formed in these cells which are secreted as tubes of lipid, the tubular myelin in to the alveoli. The tubular myelin in turn forms the phospholipid film. As the lamellar bodies are formed in Type II cells, the surfactant associated proteins are also incorporated in these structures. Further, there is a continuous secretion of surfactant into alveoli and uptake of surfactant from alveoli by Type II cells maintaing homeostasis of this surface active material in the alveoli.

The surface active property of pulmonary surfactant also acts against the tendency towards pulmonary edema (7). In the absence of surfactant, the combination of surface tension forces of alveoli and hydrostatic forces of pulmonary capillary can excced the oncotic pressure in pulmonary capillary resulting in pulmonary edema. Pulmonary surfactant was also shown to assist macrophages in their scavanger function(8). It was also shown to have immuno suppressive property (9).

The innervation to Type II cells being sparse, the regulation of surfactant was found to be mainly hormonal. Detailed reports on the effect of various hormones and other circulating chemicals on pulmonary surfactant system have been published .Since the importance of pulmonary surfactant in maintaining pulmonary patency was first observed in respiratory distress syndrome in newborn, the research was focussed on the development of pulmonary surfactant system during fetal life. Thyroxine and cortisol were shown to be essential in the develpoment of fetal pulmonary surfactant system (10). Ante partum glucocorticoid administration to mothers to prevent respiratory distress syndrome in babies was advocated prior to the advent of exogenous administration of surfactant preparations to newborn with hyaline membrane disease(11,12).

The author and Co-workers studied the effect of bronchodilators, anti-diuretics, hydrocortisone and histamine on pulmonary surfactant system in adults.Administration of terbutaline and aminophylline led to decreased surface tension of alveolar extracts in adult rats(13).Frusemide also had similar effect(14).Administration of either hydrocortisone or histamine resulted in decreased lecithin levels in bronchoalveolar lavage fluid in adult rats(15,16).The present study was designed to identify the type of histamine receptor through which the effect of histamine is probably mediated.

# Materials and methods

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

Broncho-alveolar lavage (BAL) is a standard procedure to assay various components of pulmonary surfactant system(17). Lecithin is the major surface active phospholipid of pulmonary surfactant system. Thus, assay of lecithin in BAL fluid was employed in the present study to identify the type of histamine receptor responsible for histamine induced decrease in lecithin levels in BAL fluid.

**Broncho alveolar lavage:**

The rats were given pentobarbitone sodium intraperitoneally at a dose of 40 mg/kg.The anaesthetised animals 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 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 till a volume of about 15 ml was extracted for each animal.

Lungs which had abnormal appearence,as for example,haemorhagic spots or patches were not subjected to lavage. Samples from lungs which showed leakage of fluid during lavage,and lavage fluid which was contaminated with blood or not clear was not used for estimation of lecithin. Further,samples collected with less than 70% of retrieval of instilled saline were also not used for determination of lecithin content.

**Assay of lecithin:**

Assay of lecithin was performed by enzymatic method using chemicals and protocol supplied by Boehringer-Mannheim.

In brief, it consists of hydrolysis of lecithin to phosphorylcholine. Phosphorylcholine is then hydrolysed to choline. Phosphorylation of choline in presence of ATP yields phosphorylcholine and ADP. Reconversion of ADP to ATP yields pyruvate.Reduction of pyruvate to lactate by oxidation of NADH gives lactate and NAD. The oxidised NADH is equivalent to lecithin and is estimated by spectrophotometry.

In brief, the steps are:

1. Hydrolysis of lecithin

   Lechitin was hydrolyzed by the enzyme phospholipasec and alkaline phosphatase at ph 8.0.

   $$\text{Lecithin} + H_2O \xrightarrow{\text{phospholopase c}} 1,2 \text{ diglyceride} + \text{phosphorylcholine}$$

   phosphorylcholine is hydrolyzed by alkaline phosphatase to choline and inorganic phosphate.

   $$\text{Phosphorylcholine} + H_2O \xrightarrow{\text{alkaline phosphatase}} \text{choline} + PO_4^{3-}$$

2. Determination of the hydrolyzed lecithin.

   After inactivation of the alkaline phosphatase by heating the solution in a boiling water bath, choline was phosphorylated in the presence of ATP to phosphorylcholine by enzyme choline kinase

   $$\text{Choline} + \text{A.T.P} \xrightarrow{\text{choline Kinase}} \text{phosphorylcholine} + \text{ADP}.$$

   The ADP formed was reconverted by pyruvate kinase with phosphoenol pyruvate in to ATP with the formation of pyruvate.

   $$\text{ADP} + \text{Phosphoenolpyruvate} \xrightarrow{\text{pyruvate kinase}} \text{ATP} + \text{pyruvate}$$

   In the presence of lactate dehydrogenase (LDH), pyruvate was reduced to lactate by reduced Nicotinamide - adenine dinucleotide (NADH) with oxidation of NADH to NAD.

   $$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD} +$$

   The amount of NADH oxidised in the above reaction is stoichiometric with the amount of lecithin . NADH is determined by means of it's absorbance at 340 nm.

**Experimental protocol:**

The animals were divided into 4 groups of 8 each.

Rats from group I were not administered any drug and lecithin content in BAL fluid in this group of animals acted as control.

Rats from group II were adminstered 0.06 mg per animal of histamine diphosphate (sigma chemical company) subcutaneously. The dose of histamine administered did not produce any untoward effects in the pilot experiments. After 10 minutes of administration of histamine, BAL was performed and lecithin content was estimated.

Group III were given 0.25 mg per animal of pyrilamine maleate (sigma chemical company), a $H_1$ blocker, intraperitoneally. After 45 minutes, each of the animals was administered 0.06 mg of histamine diphosphate subcutaneously. Following 10 minutes of administration of histamine, lecithin content in BAL fluid was estimated.

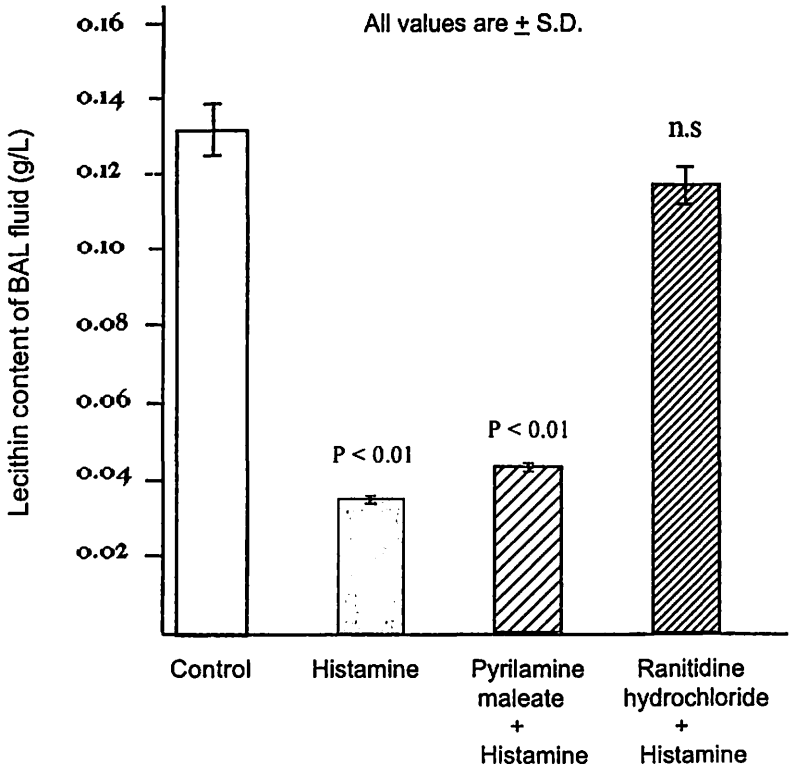
Group IV were injected 0.25 mg per animal of ranitidine hydrochloride (sigma chemical company), a $H_2$ blocker, intraperitoneally. Following 45 minutes of administration of ranitidine, each of the animals was given 0.06 mg of histamine diphosphate subcutaneously. After 10 minutes of injection of histamine, BAL was performed and lecithin content was estimated.

The doses of histamine blockers administered were equivalent to therapeutic doses employed in adult human beings.

**Statistical analysis:**

Statistical analysis of the results obtained was done using Student's unpaired *t*-test.

# Results


All values are ± S.D.
Lecithin content of BAL fluid (g/L)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
P < 0.01
P < 0.01
n.s
Control
Histamine
Pyrilamine maleate + Histamine
Ranitidine hydrochloride + Histamine

# Discussion

A significant reduction in lecithin content in bronchoalveolar lavage (BAL) fluid was observed within 10 minutes of administration of histamine. Since lecithin is the predominant surface active substance in the pulmonary surfactant system , the finding indicates an acute effect of decrease in pulmonary surfactant activity with administration of histamine.

Mechanism of decreased lecithin levels following administration of histamine can not be ascertained from the present study. Type II alveolar cells constantly secrete pulmonary surfactant into the alveoli. Reuptake of this substance from the alveoli by the same cells maintains homeostasis of this surface active material in the alveoli(18). Thus, the administered histamine might have inhibited the secretion or stimulated the uptake or both. Some of the surfactant associated proteins inhibit secretion and some facilitate uptake(19,20). The effect observed in this study could also be due to secretion of these proteins. Further studies may help identification of the mechanism involved in the reduction of lecithin content.

Lecithin constitutes 60-70% of the surface active materials found in the pulmonary surfactant system. These surface active materials reduce surface tension forces of alveolar lining and prevent collapsing tendency. Reduction in pulmonary surfactant activity leads to increased work of breathing and respiratory distress. A significant reduction in lecithin level was observed in the present study even at a dose that did not produce any untoward effects in the animals. Histamine is a common mediator in many allergic disorders. It is possible that reduction in lecithin levels may be one of the factors responsible for the respiratory disability in allergic diseases like bronchial asthma and pulmonary aspergillosis. The present study is in experimental animals and it may not be possible to extrapolate data from experimental animals to human beings. Assay of lecithin in BAL fluid in the above patients may elucidate the effect of histamine on surfactant system of lung in adult human beings.

Decrease in lecithin content in BAL fluid following administration of histamine was unaffected by prior administration of $H_1$ blocker (pyrilamine maleate) , but was blocked by prior administration of $H_2$ blocker (ranitidine hydrochloride). This indicates that histamine induced decrease in lecithin content of BAL fluid observed in the present study was mediated through $H_2$ receptors. Since the predominant source of intraalveolar lecithin is secretion of Type II cells of alveolar epithelium, it is possible that Type II cells have $H_2$ receptors, stimulation of which resulted in decreased intraalveolar lecithin.

Cheng M and Brown LA have reported stimulation of phosphatidyl choline secretion by histamine in alveolar Type II cell cultures of adult rats which could be blocked by both $H_1$

antagonist (pyrilamine) and $H_2$ antagonist (cimetidine)(21). Gilfillan AM, Lewis AJ and Rooney SA observed stimulation of phosphotidyl choline secretion in Type II cell cultures of adult rats by thiazinamium chloride which has antihistaminic properties. They have also observed stimulation of phosphotidyl choline secretion by $H_1$ antagonists (promethazine and pyrilamine) but not by $H_2$ antagonist (cimetidine). However, they were unable to demonstrate any inhibitory effect of histamine it self in the invitro culture of Type II pneumocytes(22). In the present study conducted on adult rats, a significant reduction in intraalveolar lecithin levels was observed which could be blocked by $H_2$ antagonist (ranitidine) but not by $H_1$ antagonist (pyrilamine). The findings in the present study emphasize the fact that invitro and invivo effects may be different in view of the complexity of responses in the whole animal. Further, though the findings in various studies are not similar, it appears certain that histamine has a role in the regulation of secretion of pulmonary surfactant.

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