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KESAN HISTAMIN DAN GLUKOKOTIKOID PADA SISTEM SURFAKTAN DI PARU-PARU

PENYELIDIK

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SUMMARY

Lecithin, a major surface active substance of surfactant system of the lung was estimated in bronchoalveolar lavage (BAL) fluid in healthy adult male albino rats after administration of hydrocortisone or histamine. BAL was perfomed in three groups of animals following intravenous administration of 2.5 mg. of hydrocortisone sodium succinate at 10 minute, 30 minute and 60 minute intervals respectively. Similarly, BAL fluid was obtained in three groups of animals following subcutaneous administration of 0.06mg of histamine diphosphate at 10 minute, 30 minute and 60 minute intervals. Lecithin content in BAL fluid was estimated by enzymatic method using Boehringer-Mannheim Kits. The results obtained from these animals were compared with those from control animals who received neither hydrocortisone nor histamine. A highly significant reduction in lecithin levels indicating a decrease in surfactant activity was observed within 10 minutes after administration of hydrocortisone or histamine. However, a gradual increase in lecithin levels was observed by one hour in both cases indicating an acute decrease in pulmonary surfactant activity. The effect observed might be due to a sudden inhibition of secretion of pulmonary surfactant by Type II cells or a sudden increase in uptake by Type II cells or both. Further studies are needed to explore the mechanisms involved. Since there is paucity of information on the effect of these substances on surfactant system of lung in adult human beings, the findings in the present study clearly indicate a need to assay lecithin content in BAL fluid in patients receiving corticosteroids, as for example, in adult respiratory distress syndrome and bronchial asthma, and, also in patients with allergic disorders such as pulmonary aspergillosis.

INTRODUCTION

During breathing, energy is spent to overcome tissue resistance, airway resistance and elastic resistance. Tissue resistance is due to inertia of throcic cage and lungs which has to be overcome during expansion of thoracic cage and lungs. Airflow through the narrow airways encounters resistance i.e., airway resistance.The recoiling tendency of stretched elastic tissue of thorax and lungs constitutes elastic resistance. Grouped under elastic resistance also are the surface tension forces of alveolar lining that tend to collapse the alveoli. Two-thirds of total energy is spent to overcome elastic resistance even during normal breathing.

The surface tension forces of alveolar lining were discovered as early as in 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]. Soon, it's biochemical composition in various species including humanbeings was explored[4,5,6]. The methodology for extraction and measurement of it's surface tension lowering activity was refined.

The importance of pulmonary surfactant secreted by Type II alveolar cells in maintaining alveolar patency was first observed in respiratory distress syndrome in newborn. Hence, till recently, the research was focussed on the development of pulmonary surfactant system and the factors regulating it's secretion during fetal life. Currently, synthetic and natural surfactant are used in the treatment of hyaline membrane disese. However, in recent years, the research is extended to identify the other functions of surfactant, as for example-it's role in prevention of pulmonary edema, immune suppression etc[7,8]. Drugs which were at one time thought to relieve only airway resistance were found to have additional action of relieving elastic resistance as well by increasing pulmonary surfactant levels in alveoli[9]. The rationale of efficacy of frusemide in refractory cases of pulmonary edema was attributed to it's ability to increase surfactant concentration in the alveoli[10]. Decreased pulmonary surfactant activity was also observed in adult respiratory distress syndrome[11].

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 on pulmonary

surfactant system and the mechanism of action of these agents have been published. However, again, most of these studies were conducted on pulmonary surfactant system in fetal life. Thyroxine and cortisol were shown to be essential in the development of fetal pulmonary surfactant system[12]. The effect of glucocorticoids, if any, on surfactant system of lung in adults has not been explored despite corticosteroids being frequently used in various respiratory and other systemic disorders. There were no reports indicating the invivo effect of histamine on surfactant system of lung, though histamine is a comon mediator in many allergic disorders. The objective of this study is to observe the effect, if any, of gluco-corticoid (hydrocortisone) and histamine on surfactant system of lung in adult male rats.

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[13]. Lecithin is the major surface active phospholipid of pulmonary surfactant system. Thus, assay of lechitin in BAL fluid was employed in the present study to observe the effect of hydrocortisone and histamine on surfactant system of lung in adult rats.

Broncho alveolar lavage:

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 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 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. A total of eight samples which had none of the above defects were taken as control samples for assay of lecithin.

Assay of Lecithin:

Assay of lecithin was performed by enzymatic method using Boehringer - Mannheim kits. In brief, the steps are:

1. Hydrolysis of lecithin

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

   phospholopase c

   Lecithin + H_2O ----------> 1, 2 diglyceride + phosphorylcholine

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

   alkaline phosphatase

   Phosphorylcholine + H_2O----------> 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

   choline Kinase

   Choline + A.T.P ----------> phosphorylcholine + ADP .

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

   pyruvate kinase

   ADP + Phosphoenolpyruvate ----------->ATP + 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.

   LDH

   Pyruvate + NADH --------> Lactate + 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.

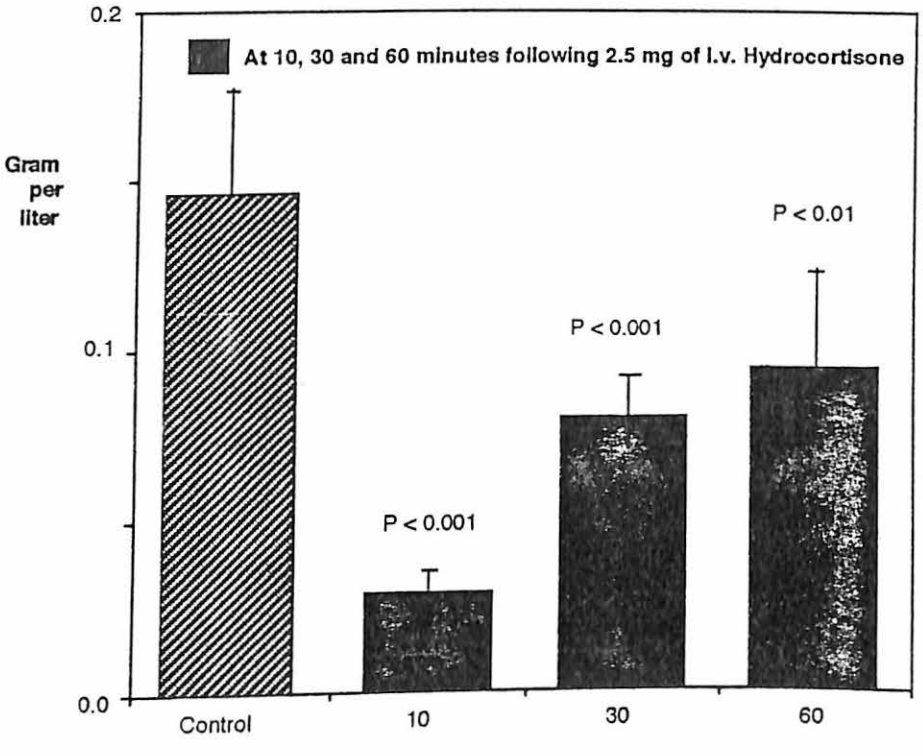
To observe the effect of hydrocortisone on surfactant system of lung in adult rats, BAL was performed in three groups of animals (8 in each group) following intravenous administration of 2.5 mg of hydrocortisone sodium succinate (sigma chemical company) at 10 minute, 30 minute and 60 minute intervals respectively. The internal jugular vein was cannulated under anaesthesia and hydrocortisone was injected into jugular vein of each animal. Later, lecithin content in BAL fluid was performed as in control group.

To observe the effect of histamine on surfactant system of lung in adult rats, BAL was performed in three groups of animals (8 in each group) following subcutanesus administration of 0.06 ng of histamine diphosphaste (sigma chemical company) at 10 min, 30 min and 60 min intervals respectively. The dose of histamine administered did not produce any untoward effects in the pilot experiments. Later, lecithin content was estimated as in control group.

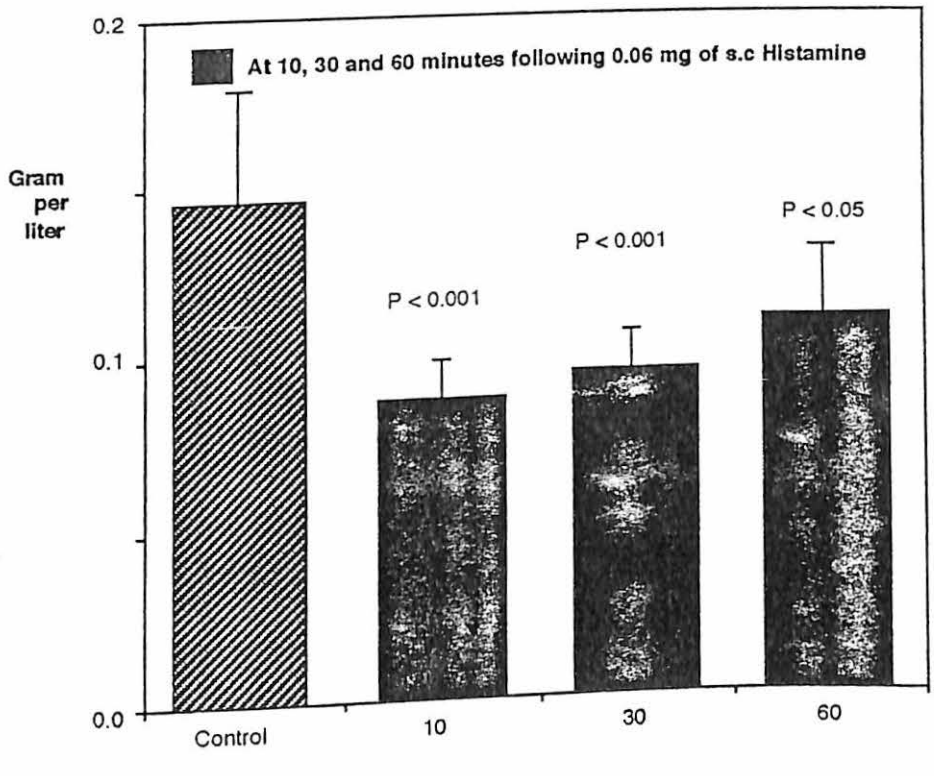
Statistical analysis of the results obtained was done using student's unpaired `t' test.

RESULTS

Lecithin levels in broncho-alveolar lavage fluid



Lecithin levels in broncho-alveolar lavage fluid



DISCUSSION

A highly significant reduction in lecithin content in broncho-alveolar lavage fluid (BAL) was observed within 10 minutes of administration of hydrocortisone or histamine. However, by one hour, there was a gradual increase in lecithin content in both cases. Thus, there is an acute effect of decrease in pulmonary surfactant activity with both these agents. The lecithin levels increased to near normal levels by one hour after administration of histamine. However, with hydrocortisone, though there is a gradual increase in levels of lecithin at hour, they were still significantly lower compared to control animals. The difference in lecithin levels at 1 hour may be a reflection of plasma half lives of these compounds. Half life of hydrocortisone is about 90 minutes, whereas that of histamine is much shorter.

Mechanism of decreased lecithin levels following administration of these compounds can not be ascertained from this study. Type II alveolar cells constantly secrete pulmonary surfactant in to alveoli. Reuptake of this substance from the alveoli by the same cells maintains homeostasis of this surface active material in the alveoli[14]. Thus, the administered hydrocortisone or histamine might have inhibited the secretion or stimulated the uptake or both. Some of the surfactant associated proteins inhibit secretion and some facilitate uptake [15, 16]. The effects observed in this study could also be due to secretion of these proteins. Further studies may help identification of the mechanisms 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. The dose of hydrocortisone employed in this study was equivalent to therapeutic doses employed in patients with status asthmaticus. Biological half life of hydrocostisone being 8 - 12 hours, 6 hrly injections can lead to a cumulative effect even when the dose is in minimum range. Clinical improvement is delayed in cases of status asthmaticus even with intravenous administration of glucocorticoids. Decreased lecithin levels in alveoli can lead to undesirable effect of increasing resistance to breathing. This may also be a factor responsible for the delayed therapeutic response seen in those patients.

High doses of corticosteroids are employed in the management of adult respiratory distres syndrome (ARDS). Some investigators observed no beneficial effect whereas some others reported an increase in mortility with the use of corticosteroids in some cases of ARDS [17, 18]. The decreased lecithin content in alveoli may precipitate or worsen any existing lung pathology in ARDS. The present study is in experimental animals and it may not be possible to extrapolate data from experimental animals to human beings. However, the findings indicate a need to study the effect of hydrocortisone on surfactant system of lung in adult human beings. Estimation of lecithin content in BAL fluid in asthmatics, ARDS and in other interstitial lung diseases in whom corticosteroids are frequently used may provide a clue in this aspect.

Histamine was reported to cause stimulation of type II cells invitro [19]. However, in the present study a decreased pulmonary surfactant activity was observed following histamine administration. This is not surprising since the invitro and invivo 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 adult human beings.

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