EFFECT OF CORTICOSTEROIDS ON ELEVATED INTRACELLULAR SODIUM, PLASMA LIPID PEROXIDE LEVELS AND REDUCED Na^+ , K^+ -ATPASE ACTIVITY IN PATIENTS OF BRONCHIAL ASTHMA

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ABSTRACT

In view of several reports that there is a lack of balance in free radicals in case of bronchial asthma (1) the effect of free radicals on cell membrane was studied by estimating the membrane bound protein Na^+ , K^+ -ATPase activity and the intracellular sodium level in patients of bronchial asthma before and after a short course (one week) of oral corticosteroid (prednisolone 0.75-1mg/kg body weight) therapy. Results showed that there is a definite statistically significant rise in free radical level and intracellular sodium level and a significant lowering of Na^+ , K^+ -ATPase activity in case of untreated bronchial asthma. After short course of therapy with oral corticosteroids, the free radical level and intracellular sodium level decreased significantly, together with a significant rise of the Na^+ , K^+ -ATPase activity. Also, a significant negative correlation ($r=-0.74$) between the lipid peroxide level and the Na^+ , K^+ -ATPase activity was found in these cases.

KEY WORDS: Corticosteroid, Na^+ , K^+ -ATPase, Lipid Peroxide

INTRODUCTION

Asthma has become one of the most common chronic disease in both developed and developing countries (1) and epidemiological studies suggest that its prevalence, severity and mortality are rising at a time when mortality from other treatable conditions is falling (2). The mechanisms of this disease are poorly understood and much less is known about the biochemical aspects of asthma. Bronchial asthma is an inflammatory airways disease characterized by the recruitment and migration of inflammatory cells into airways. Biological membranes contain considerable amounts of highly unsaturated lipids and cholesterol. The regulation of the degree of phospholipid unsaturation serves to maintain the membrane homeoviscosity required for the proper function of membrane – associated enzymes and

channels. A prevalence of poly-unsaturated fatty acids, makes the membrane phospholipids potentially susceptible to lipid peroxidation and cause oxidative membrane injury and increase of membrane permeability. An increased oxygen free radical generation has been reported in asthmatics. Activity of membrane associated enzyme Na^+ , K^+ -ATPase provides a functional assessment of injury that is related to lipid peroxidation (3). A significant correlation between lipid peroxidation and Na^+ , K^+ -ATPase activity has been reported in preeclamptic toxemia and essential hypertension (4,5). The aim of this study was to investigate the possibility whether plasma lipid peroxide and intracellular sodium concentrations are raised in patients of bronchial asthma. Also, the activity of the membrane bound enzyme Na^+ , K^+ -ATPase and its correlation with the intracellular sodium levels and plasma lipid peroxide level was investigated.

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Corticosteroids are now generally considered as

front-line drugs for controlling asthma. The effect of treatment with these group of drugs on leukocyte Na⁺,K⁺-ATPase activity, intracellular sodium levels and plasma lipid peroxide levels was estimated.

MATERIALS AND METHODS

The studies were carried out in leukocytes, as the leukocyte in an inflammatory cell and asthma is an inflammatory airway disease. Subjects included in the study were patients of Bronchial asthma and normal healthy controls and selection of subjects was made from the patients attending OPD at the clinical research center, V.P. Chest Institute, University of Delhi, Delhi and Maulana Azad Medical College and associated hospitals, University of Delhi, Delhi.

Asthma is diagnosed on the basis of: (1) Clinical history: Wheezing, Dyspnea, Cough. (2) (a) Airway function, (b) Reversibility of airway obstruction, if present and (c) Airway responsiveness to histamine.

Leukocytes were separated from human peripheral venous blood by dextran sedimentation of erythrocytes, centrifugation and rapid lysis of the remaining erythrocytes (6). The cell pellet was suspended in Tris buffer (100 mM, pH=7.4) for

estimation Na⁺, K⁺-ATPase activity (30 x 10⁶ cells/ml) and HEPES buffer (10 mM, pH=7.4) for [Na⁺]_i estimation. Na⁺, K⁺-ATPase activity was estimated spectrophotometrically (7). Intracellular sodium was determined using the novel fluorescent indicator "sodium binding fluorescent indicator" (SBFI) (8). Plasma lipid peroxide levels were estimated spectrophotometrically by the method of Jain et. al. (9).

RESULTS

The results are summarized in Table 1. Compared to age and sex matched controls, a highly significant rise of leukocyte sodium (intracellular) level (P<0.0001), a significantly low (P < 0.0001) Na⁺, K⁺-ATPase activity was found in acute asthmatics. Also, a highly significant rise (P<0.0001) of plasma lipid peroxide levels was found.

After treatment with a short course (1 week) of oral corticosteroids leukocyte (Na⁺), decreased significantly (P < 0.01) and Na⁺, K⁺-ATPase activity increased very significantly (P < 0.0001) from pre treatment values. The plasma lipid peroxide levels also showed significant decrease (P < 0.0001) after treatment. A significant negative correlation was found

Table-1 : Leukocyte Na⁺,K⁺-ATPase activity, intracellular sodium levels & plasma lipid peroxides

	Na ⁺ ,K ⁺ -ATPase activity (ug Pi liberated/mg pro/30 min)	[Na ⁺] _i , (mM)	Plasma lipid peroxide (nmol/ml)
1. Normal Subjects	3.10 ± 0.40 (n=31)	27 ± 8.1 (n=22)	2.9 ± 0.2 (n=10)
2. Acute asthma patients (Pretreatment)	1.10 ± 0.35*** (n=22)	56 ± 14*** (n=16)	7.7 ± 0.9*** (n=10)
3. Asthma patients (Post-treatment)	2.6 ± 0.8*** (n=16)	38 ± 8.1** (n=8)	4.4 ± 0.6*** (n=10)

*** P < 0.0001, ** P < .01

n. No. of samples in each group

All values are expressed as Mean ± S.D.

between leukocyte Na⁺,K⁺-ATPase activity and intracellular Na⁺, ($r=-0.68$) and plasma lipid peroxide levels ($r=-0.68$) and also between lipid peroxide level and Na⁺,K⁺-ATPase activity ($r = -0.71$).

DISCUSSION

The action of corticosteroids are now well established to be due to induction of the synthesis of a group of proteins, the lipocortins, which cause inhibition of phospholipase A₂ (10, 11). Phospholipase A₂ releases an unsaturated fatty acid and a lysophospholipid. Increased level of lysophosphatidylcholine has been reported in patients of asthma (12, 13). Lysophosphatidylcholine is known to inhibit the Na⁺,K⁺-ATPase activity (7).

More of the unsaturated fatty acids would lead to formation of more free radicals. Free radicals cause peroxidation of membrane lipids which initiate the loss of membrane integrity and membrane bound enzyme Na⁺,K⁺-ATPase and consequently an increase of intracellular sodium.

The increase in the activity of Na⁺,K⁺-ATPase after treatment with steroids observed in the present study may be due to lowering of lysophospholipid levels following inhibition of Phospholipase A₂. With a lesser release of unsaturated fatty acids, there is less formation of lipid peroxides, and the intactness of the biomembrane resulting in pumping out of sodium ions and consequent lowering of intracellular sodium levels.

Conclusions

The present study indicates that corticosteroids can play an important role in bronchial asthma probably by reducing the lipid peroxidation level and thereby restoring the membrane damage.

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