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Study of Cells Blebbing in Patients with Allergy Disorders

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ABSTRACT

Allergy is serious healthcare problem that affects large populations in all countries. Allergic disorders are a multifactorial disease with a wide spectrum of clinical manifestation and symptoms.

The aim of the present study was to investigate the immune status and analysis if the allergens can cause *in vitro* cell activation and membrane blebbing.

The study was used blood of healthy persons and persons with allergies, allergens: 5% solutions of glucose, sucrose, fructose, methylene blue dye. Blood cells were placed in a saline solution and subjected to alternating microelectrophoresis.

When comparing the blood of healthy patients and patients with allergies, it was found that the patient's healthy cell activity (CA) above, blebbing and aggregation hardly observed (CA=0.92). When introduced into a patient's blood glucose allergy (CA=0.6) and sucrose (CA=0.7) was observed enhanced blebbing and sludge erythrocyte hemolysis them - is an allergic reaction. Fructose such phenomena have been identified (CA=0.83).The study was conducted analysis of two leukocytic formulas: before and after application of riboxin. As a result, changes were slight (1-2%), shifts in the formula is almost not observed. Then compared the effects of antihistamine on blood cells. It was found that Suprastin increases the resistance of cells to the action of the allergen and improves their activity, alters the shape of the erythrocyte. Some red blood cells bind to monocytes and neutrophils and blebbing and aggregation not observed.

As a result, the bioelectrical properties of red blood cells are reduced in case of allergy. In the presence of the allergen, blood cells become less active, changing the shape and size observed blebbing lymphocytes and neutrophils, erythrocytes aggregation. Antihistamines increase cytophysiological characteristics of blood cells of patients with allergies.

Keywords: Immune status, Allergy, Cell activity (CA), Blebbing

INTRODUCTION

Allergy is serious healthcare problem that affects large populations in all countries [1]. The prevalence of them has dramatically increased last decades, with accompanying social costs due to increase morbidity and lost productivity from missed work or school. Allergic disorders are a multifactorial disease with a wide spectrum of clinical presentations and symptoms [2].

The prevalence of asthma in the United States 8.4% has asthma as compared with 4.3% of the population worldwide. The average annual asthma prevalence is higher in children in comparison to adults (9.5% vs7.7%) [3,4]. Atopic dermatitis (AD) is a chronic inflammatory skin disease than affects up to 20% of children and up to 3% of adults worldwide [3]. Allergic rhinitis affects 20 to 30% of adults in both the United States and Europe [5]. Allergic eye diseases are usually manifested as conjunctivitis with or without keratitis, in response to an allergen, and affect 10%-20% of people globally [6].

The etiology of allergic disorders is complex and multi-faceted [7,8]. Previous studies have indicated that several environmental factors such as air pollution and smoking, nutritional habits, chronic stress, genetics are key factors in asthma pathogenesis [2,9]. Moreover, it was demonstrated by previous studies than human microbiota, both intestinal and upper airway, play an important role in mediating the pathogenesis of childhood asthma [10].

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Blebs are blister like, typically spherical protrusions that occur in the surface of the cells [11,12]. Blebbing is a common phenomenon in human and animal cells and identified during various cellular processes such as the cytokinesis phase of cell division [13], apoptosis [14], cell migration [15,16] and angiogenesis [17]. Blebs kick-out by intracellular pressure and are initially cytoskeleton-free, but they subsequently assemble the cytoskeleton, which can drive bleb retraction [15]. Preciouses studies have shown that the blebbing in human cells may be divided into two types: the dynamic and apoptotic blebbing [18]. Apoptotic blebs are presented on the cells' surfaces during death [19,20], while the dynamic blebs are membrane protrusions that appear and disappear from the surface of healthy cells [11].

Based on previous results, the aim of the present study was to investigate if the allergens can cause *in vitro* cell activation and membrane blebbing.

MATERIALS AND METHODS

The study was performed *in vitro*. For the experiments, 2 ml of heparinized whole blood were collected from patients with allergy disorders and age matched control individuals. Blood cells were placed in a 0.9% NaCl solution and exposed to alternating microelectrophoresis. At the same time, the electrokinetic properties of cells placed in an alternating constant electric field created by the "Cyto-Expert" apparatus are carried out as descripted previously [21]. To assess the electrokinetic properties of the cells, the amplitude of their vibrations was evaluated. Moreover, percentage of active cells was estimated. Next was carried out analytical processing of the data and leaving the formulas using the software [21].

Mediators and conditions used for stimulation/inhibition studies

To study blebing induction, as well as total cell activity, blood cells were treated with allergens glucose, sucrose, fructose (1:20 dilution) for 30 min at 37°C. To investigate the mechanism of bleb retraction and inhibition of cell activating, human cells were pre-treated with riboxin and chloropyramine hydrochloridein different concentrations (primary and diluted in 10%).

RESULTS

Blood cell activation and blebbing formation

Considering that glucose and sucrose can activate blood cells in patients with allergy, red blood cells blotting and sludge was observed. On the other hand, similar data were not observed after cells' exposure to fructose **(Table 1)**.

Table 1. Quantitative characteristics of the blood cells activity.

Patient		Saline 0.9%			Glucose 5%			Sucrose 5%			Fructose 5%	
CA%	A (μm)	B%	CA%	A (μm)	B%	CA%	A (μm)	B%	CA%	A (μm)		
Controls	0.94	3 ± 0.03	0.43	0.91	2 ± 0.02	0.44	0.86	2 ± 0.01	-	0.83	3 ± 0.01	
Patients	0.93	4 ± 0.02	0.5	0.6	0.4 ± 0.01	0.75	0.7	0.5 ± 0.01	0.83	0.87	3 ± 0.02	

CA%: Total Cell Activity; A (μm): Total Cell Oscillation Amplitude; B%: Number of Cells with Blebbing

Moreover, blebbing was observed in patients' blood cells after exposure to glucose and sucrose, but not detected tin fructose exposure **(Figure 1)**. Blebbing is one of the initial manifestations of cell damage caused by hypoxia, intoxication, the action of viruses, but not leading to cell death [4]. Sludge is a phenomenon characterized by adhesion, aggregation and agglutination of the fomrenic elements of the blood.

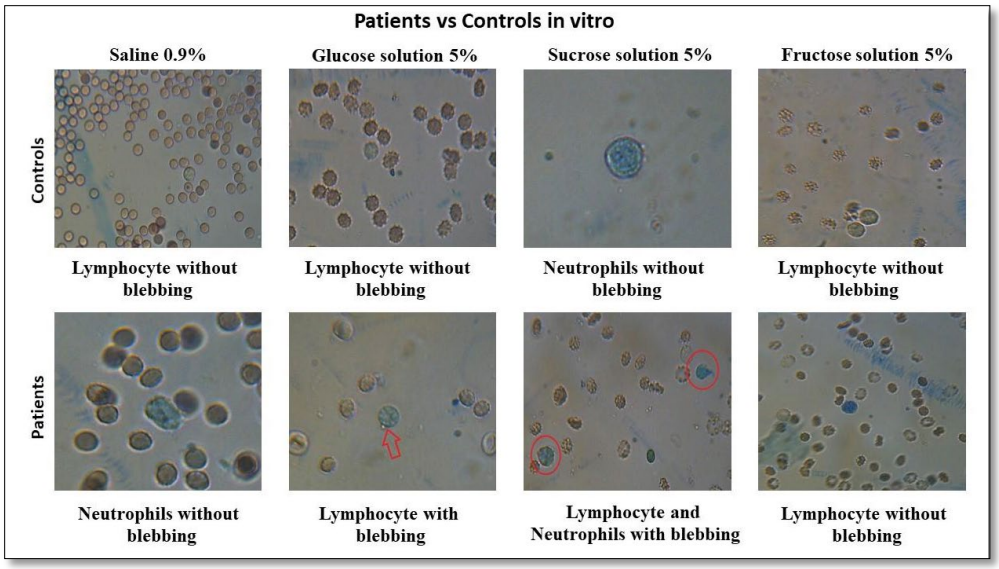

Figure 1. Results of exposure of glucose, sucrose and fructose in blood cells of patients and control.

Riboxin restores the *in vitro* cells activity and inhibit blebbing Riboxin (inosine) - purine nucleoside, the precursor of ATP. Riboxin is involved in metabolic processes, increases stamina, strengthens the immune system and stimulates biochemical processes in muscle tissue, increases protein synthesis. During the research, we analyzed two leukocyte formulas: before and after taking Riboxin. As a result, the changes were insignificant (1-2%); no changes were observed in the formula. After inosine pre-treatment, it was observed that cell activity was increased, blebbing was decreased and there was no sweeping observed **(Figure 2)**.

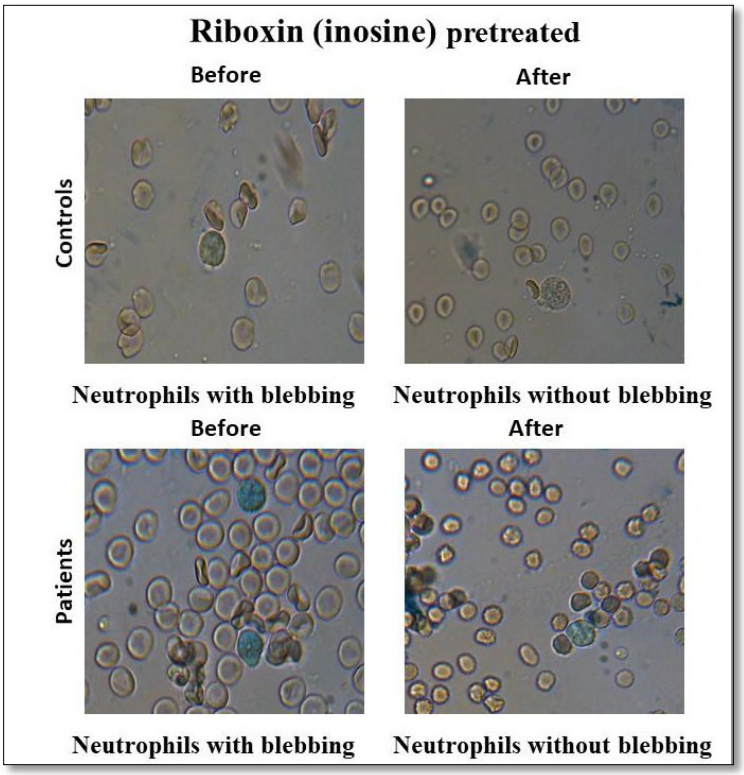

Figure 2. Results of inosine pre-treatment in blood cells of patients and control.

Chloropyramine hydrochloride reduces the *in vitro* cell activity. We next examined whether chloropyramine hydrochloride, a known antihistamine drug, could reduce the *in vitro* cell activation by glucose or sucrose. The

chloropyramine hydrochloride (Suprastin) is a classic antihistamine drug belonging to the group of ethylenediamine preparations. When conducting research, we compared the effect of different concentrations of Suprastin on blood cells. It was found that a high concentration of antihistamine drug leads to inactivation of cells, loss of membrane potential. There is a tendency to sludge, increases blebbing, lymphocytes die. The normal concentration of suprastin (dilution 10 times) improves the activity of the cells, changes the shape of red blood cells. Some red blood cells bind to monocytes and neutrophils. Thus, an overdose with an antihistamine can lead to a negative effect **(Figure 3 and Table 2)**.

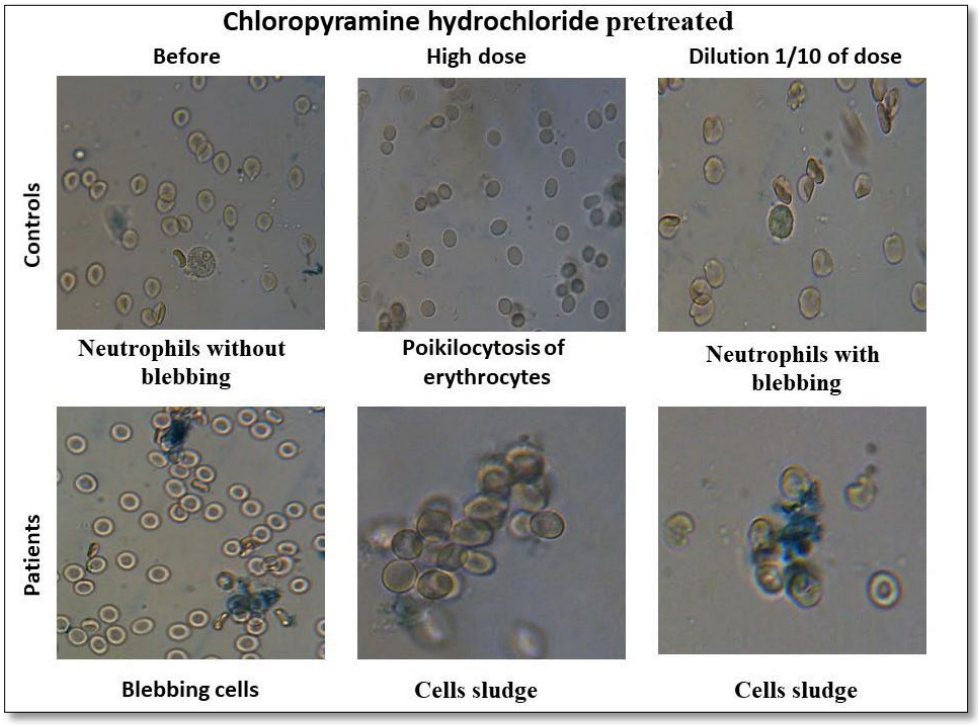

Figure 3. Results of chloropyramine hydrochloride pre-treatment in blood cells of patients and control.

Table 2. Quantitative characteristics of the activity of blood cells.

Before			Antihistamine pre-treatment (dilution 10 times)			Antihistamine pre-treatment (undiluted)	
CA%	A (μm)	B%	CA%	A (μm)	B%	CA%	A (μm)
0.05	0.5 ± 0.02	0.4	0.36	3 ± 0.5	0.3	0	0

CA%: Total Cell Activity; A (μm): Total Cell Oscillation Amplitude; B%: Number of Cells with Blebbing

As a result of the work, we determined that allergy reduces the protective reactions of the body. In the presence of an allergen, cells reduce activity, change shape and size. Antihistamines increase the body's resistance, improve blood counts. That is why in surveys improving immune status is important for every person.

DISCUSSION AND CONCLUSION

We have used the electrokinetic properties of cells placed in an alternating constant electric field created by the "Cyto-Expert" apparatus is carried out as descripted previously [21] to study the immune cells activation and formation of blebs. There is no doubt, that allergen-driven specific cell activation is critically required for allergic immune response. In the present experimental model, we shown that in the presence of an allergy, the protective abilities of the body are reduced, they are constantly wasted on fighting antigens. Interaction of allergen and immune response was studied by Borishe [22]. Moreover, Eckl-Dorna et al. [23] in their experiments have shown that cell activity is higher in a healthy patient, there was almost no blebbing and sludge.

Blebbing is one of the initial manifestations of cell damage caused by hypoxia, intoxication, the action of viruses, but not leading to cell death. Using blebs as detectors of cells activation in conjunction with riboxin treatment we were able to provide unequivocal proof of the experience that

riboxin can inhibit allergen induced cell activation. Indeed, riboxin pretreated cells, did not form bleb. Several *in vitro* and *in vivo* studies suggest that riboxin has an immunomodulatory and cell protective action [23,24]. Lazareva and Brovkina [25] in their animal model study in rats, have shown that riboxin, in combined injection with Essentiale and Elkar, restores activity of immune cells, hepatocytes and myocytes, that induced by phenylhydrazine. Additional studies are required to confirm our preliminary data that riboxin reduces of the allergen-inducted cell activation that may have clinical relevance. The role of the molecular mechanisms which induces the blebbing and their biological effects need further investigations.

Potential limitations of our study were that we had a small sample size of patients. Moreover, we do not use in our *in vitro* experiments glucocorticoids which play an important role in allergy therapy.

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