

THE EFFECT OF KETOTIFEN ON EOSINOPHILIC CHEMOTACTIC FACTORS IN EXPERIENTIALLY ALLERGIC ASTHMA

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ABSTRACT

Ketotifen is a Histamin antagonist which inhibits the mast cells and has clinical use in treatment of allergic asthma. It's possible that one of the anti inflammatory mechanisms of this drug is preventing the migration of Eosinophils to guinea pigs' bronchus. The aim of this study was to examine the effect of Ketotifen in preventing Eosinophils from migrating into the bronchus in the experientially allergic asthma patients. In this experiment; 16 guinea pigs were divided into 4 groups. In group 1 after sensitization by Ovalbumin they were challenged by Ovalbumin and buffer, then they received Ketotifen; The second group was sensitized and challenged by Ovalbumin without receiving any Ketotifen. The third group was challenged only by buffer, and the last group was challenged without sensitization. The comparison of statistical analysis among groups showed that the number of Eosinophils in groups which received Ketotifen, significantly decreased in comparison with the Control group ($P < 0.05$). Comparison of the second and third groups showed that the second group had a significant increase in the number of Eosinophils. The data showed that Ketotifen had an important effect in preventing the migration of Eosinophils into the air pathway.

Keywords: Allergic Asthma, Eosinophilic chemotactic factors, Ketotifen

INTRODUCTION

Allergic diseases such as asthma, food borne allergies atopic dermatitis, allergic rhinitis are common diseases of which their incidence rates have increased during the last 30 years (wiqar et al, 2005). Asthma is a complicated multi-factor pulmonary disease which is diagnosed through reversible blockage, the increase in external bronchial responses and chronic inflammation of bronchial system.

So many Environmental and genetical factors are effective in this disease. Allergy is one of the immunological responses which is categorized under the hyper sensitivity reactions (Shaikh, 2001). There is a high genetic potential for evolution the allergy immediate hypersensitivity reaction. There are factors which increase the allergy, that in its most sever systematic form which is anaphylaxis, causes the cells to constrict the aerial ways until they cause asphyxia and heart strokes to death.

Asthma is defined with reversible and periodic contractions of brunches as a result of different stimulants. Asthma is divided into two groups which is based on the being or lack of a factor in immune disorders.

- A) External asthma: starts with hyper sensitivity reactions type which is caused by an external antigen. Three forms of external asthma have been diagnosed so far.
- B) Internal asthma: which deals with non-immune beginner mechanisms (Akids and blasé, 1999, Agertoft and Pedersen, 1995).

The presence of asthma allergy is one kind of the asthma, asthma with external origin that is called allergic asthma or atopic asthma which is the most common type of asthma. The diagnosis of allergic asthma is mainly based on the presence of IgE (type I hyper sensitivity). The allergic asthma like type I hyper sensitivity making the TCD⁺ Th₂ cells sensitive which release cytokines like interleukin 4 and 5 (IL-4 and IL-5) which support the making of IgE, the increase of growth of mast

cells (IL4) and the activation of Eosinophil (IL5) which have an important role in allergic asthma.

The gathered Eosinophils take effects through chemotactic factors of the cells PAF, IL-5 and Eotaxin and produce the basic protein and cationic protein which are poison for the epithelial cell. Therefore the Eosinophils can make the allergic response stable and sever (Carlsen, 2004). Eosinophils produce lipid mediators and cytokines which cause the increase in the allergic response. Eosinophils play a main role in the bronchial asthma.

Two mechanisms are involved in the Eosinophils activation:

- 1) The Th₂ cells and mast cells by producing cytokines like IL-5 and a chemokine called Eotaxin.
- 2) Eosinophils by the Eotaxins and mast cells productions like Histamine and product of braking it like acid immidiason acetic, leukoterian B₄ and Baf reabsorbed to the location of mast cells degranulation (Grant et al, 1990).

Eosinophils and their poisonous products can cause the increase in the aerial ways response. The levels of Eotaxin in epithelium and submucosal are related to the numbers of the Eosinophils and in the plasma of asthmatic individuals the increasing of Eotaxin are related directly to the severity of the disease. The main cell origins of the Eotaxin are the epithelial and the endothelial cells but smooth muscle, mast cells, fibroblast and Eosinophils can produce Eotaxin (McDonald, 1982). Allergic diseases of aerial ways have been known as a inflammatory disease (petheram eta al, 1981).

Ketotifen is the first anti asthma medication which has been successfully used as a preventive. this medication is used not only for curing asthma but also for allergic rhinitis and allergic conjunctivitis.

Ketotifen is vastly used as a prophylactic drug for currying the external asthma (mediei et al., 1989).

Ketotifen beneficial effects in asthma may be as a result of final inhibitory effects of inactivated Eosinophils and T cells which are in the aerial ways.

MATERIALS AND METHOD

In this study we dilute 10ml of pure sterile Ovalbumin in 50ml sterile saline buffer (in 0.5ml of this solution, there is 100 µg of Ovalbumin that is used in injection as 100 µg dose) and we sterile the solution with Heater-shaker and magnet and then we kept it in the refrigerator and in each injection into peritoneal 0.5ml of it was injected to guinea pigs. Ketotifen was prepared from the drugs in the form of 1mg tablets from the pharmacy and it was turned in to powder and solved in a 2.5ml of buffer.

Discharges and Bronchoalvoular fluid were taken from the guinea pig which was challenged and it was sensitive to Ovalbumin, it was then placed in a hanks buffer. The environment for the analysis of the results and comparison; and providing the slide and staining was worked, so that no changes accrued in them.

16 male guinea pigs were divided in to four groups and then one of these groups was chosen as the normal control and this process was done for the next 3 groups.

For the first group, after sensitization by 0.5ml Ovalbumin injection in peritoneal space with dose of (20 mg/ml) in the next step challenged through the nose and bronch, and then injection of Ketotifen through oral with 0.2ml per day in 0.5ml buffer for each animal for a week and then anesthetized animals, Bronchoalvoular fluid and mucosal secretion were taken through bronches and were put into Hank's buffer and then provided slide and stained, then Eosinophils were counted and finally results were analyzed and differences with the control groups were surveyed.

About the second group, like the first group at first, sensitization was done in 14 days and then guinea pigs were challenged through the nose and bronchi with Ovalbumin like the first group, then bronchoalvoular fluid was received and counting was done.

About the third group as the next control group, this group was sensitized with Ovalbumin like the first and the second group, then was challenged with buffer and was sampled like the rest.

About the fourth group as normal control group, was challenged only by buffer, slide was prepared and stained then Eosinophils were counted.

RESULTS

About the samples of bronch, the effect of the Ketotifen in decreasing of Eosinophils in groups that was received the drug had significantly meaningful difference with control ($p < 0.05$). The number of Eosinophils in the first group which received the drug was 14.8% and was decreased compared with the control group that was 32.5% (shown in chart 1). About the comparison of other groups, the statistical analysis interpretation with Donkan test, shoed that averages had significant differences ($p < 0.05$).

The data gathered from the blood samples was compared with Donkan test (chart2) that in this case also, like bronchoalvoular fluid samples, significant difference was observed between group 1 and group 3 (control) ($p < 0.05$).

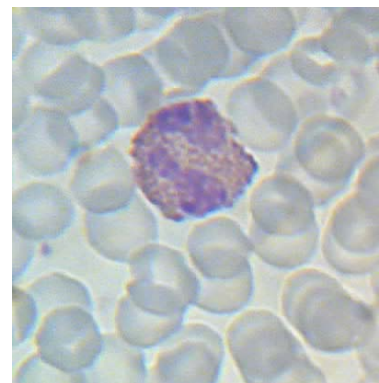
Chart 1: Samples of bronchoalvoular fluid

Group of the treatment	Eos %
One	14.8
Two	47.5
Three	32.5

Four	5
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Chart 2: samples of Blood

Group of the treatment	Eos %
One	16.2
Two	43
Three	33.1
Four	4



Eosinophils of the Quineapig in the Blood

CONCLUSION & DISCUSSION

In the current study, the results showed that Ketotifen as Anti-Histamine had its Anti-allergy effect through decreasing the number of Eosinophils in Blood and mucosa in spite of this fact that it's presentation accrued in a short time. the number of Eosinophils in bronchoalvoular fluid in the group that took the drug was significantly decreased in Comparison with control group, and comparison of the samples were obtained from bronchoalvoular fluid and blood samples confirms this effect of Ketotifen. Hoshino et al., (1998) studied the effects of Ketotifen on the symptoms, breathing acts and inflammation of the oral ways in 25 patients with allergic asthma. Results suggested that the fruitful effects on Ketotifen in asthma might be the results of the final effect of inhibitory on activated Eosinophils and T cells of the oral ways. Also Ketotifen might pacify the allergic inflammation in bronchial asthma. The study by Goble, Martin & Romer (1978) on guinea pig showed that the Ketotifen has the Anti- Anaphylaxis effect, also they concluded that Ketotifen is also effective in long period Prophylaxis of Allergic asthma. In a study by Hasale et al., (2005), they studied the effect of Ketotifen on human Eosinophils by light field microscope and they suggested that the effect of Ketotifen was mainly result of induction of the primary necrosis. The histological evaluation of the Eosinophils structures by electron microscope confirmed the symptoms of the necrosis. This research suggested that in the clinical concentration of drug, Ketotifen causes Eosinophils to be primary necrosis in Eosinophils. With regard to these studies in the human and animal samples and outcoming results in current study. It seems that Ketotifen is effective with decreasing chemotactic Eosinophils and induction of the necrosis in this cells. (Woerly et al., 2003; Hassle, 2005). The current study is conducted in a short time by supply of Ketotifen, but the long term studies of drug by Craps et al., 1979 showed that the main effect (for a complete recovery) of Ketotifen did not take place for the first four weeks. They suggested another experiment that 6 to 12 weeks were needed for prophylactic effect of Ketotifen. Therefore some reports about effects of Ketotifen, which were conducted in shorter times present some contradictory results, for example Mattson et al., 1981 in a controlled experiment with the asthma patients, examined the effects of Ketotifen on them and with

using of Ketotifen as a pretreatment for four weeks, no improvement (recovery) were observed in breathing signs. Van Ganse et al, 1997 confirmed the Anti-Histamine effect of Ketotifen on basophiles and mast cells with controlled experiments.

In the study conducted by Kurosawa, 1990 with 36 kids suffering from allergic asthma resulted suitable recovery by prescribing Ketotifen, in a similar study conducted by Klein et al, 1980 it reported that after stimulating by breathing allergen, Ketotifen is much more effective than other medicines in preventing asthma. Dyson & Machay 1980 observed normal recovery in breathing of the patients, who had used corticosteroid drugs through aspiration, with supplying Ketotifen.

About the effect of Ketotifen in preventing Allergic asthma, an experiment was conducted by Medici et al, 1989 on 131 men and 124 women among 6 to 51 years old, in the end of the experiment asthma attacks, significantly decreased in the group under treatment compared with the others. Clarke & May, 1980 conducted a comparative experiment between Ketotifen and other drugs, and Ketotifen was more effective than the others.

Long term studies in this field was conducted by Green wood, 1982 They concluded that there is a decrease in the quantity and the period of asthma attacks in the treatment with Ketotifen. Generally it showed that Ketotifen had an active role in prevention of asthma.

Ketotifen is a suitable drug, that is an except for the side effects of sedative, and that is obvious for short time, but the long time experiments did not confirm its role as sedative also in this study and the previous similar observation, specialized that part of the preventing effects of Ketotifen is in reducing of Eosinophils chemotactic factors. Therefore, using of Ketotifen for gaining and obtaining it's main effects, it seems that we can expect some good results.

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