Original Article

EFFECTS OF AZELASTINE AND SODIUM CROMOGLYCATE IN THE INHIBITION OF BRONCHO - CONSTRICTION OF OVALBUMIN SENSITIZED LUNG PARENCHYMAL TISSUES OF GUINEA PIGS *IN VITRO*

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ABSTRACT

OBJECTIVE: To see the ability of Azelastine and Sodium cromoglycate in influencing antigen induced contractile responses in isolated parenchymal tissues of Guinea pig *in vitro*.

DESIGN: An experimental study.

SETTING: Department of Pharmacology and Therapeutics of Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center (JPMC), Karachi during 1998.

METHODS: The Guinea pigs (n=10) were sensitized with ovalbumin and their parenchymal strips were exposed to different concentrations of ovalbumin to observe the EC_{50}. Each sensitized parenchymal strip was treated with either Azelastine or Sodium cromoglycte in an organ bath for 10 minutes and treated with EC_{50} ovalbumin and contraction was recorded by Grass Polygraph model 7B.

RESULTS: EC_{50} (n=6) of parenchymal strips ($0.3x10^{-6}$ + $0.16x10^{-6}$g/ml) produced a mean response of contraction 9+0.44mm. Azelastine in concentration of 10^{-9} g/ml did not show any inhibitory effect but as the concentration increased to 10^{-8} g/ml, marked inhibition was recorded and with further increase in concentration by 10^{-7} g/ml, it completely antagonized the EC_{50} induced contraction. Sodium cromoglycate did not show any inhibition at concentration 10^{-8} g/ml while at higher concentration of 10^{-6} g/ml, it showed complete antagonism.

CONCLUSION: Ovalbumin induced contraction of sensitized lung parenchymal tissues of Guinea pig *in vitro* is dose dependent and controlled better with Azelastine than Sodium cromoglycate.

INTRODUCTION

Broncho-spasm is characterized by hyper responsiveness of traceheo-bronchial smooth muscles to a variety of stimuli resulting in narrowing of airways. Asthma is primarily an inflammatory condition or inflammation underlying hyper reactivity; allergic basis has been demonstrated beside a variety of other factors.

Mast cell play a pivotal role in early asthmatic response via release of mediators which directly influence airway smooth muscle tone. The mast cell derived products act *in vitro* and human airway produce hyper responsive contraction in sensitized bronchi via a calcium related mechanism[1]. Calcium handling by the airway smooth muscles may be an important determinant of airway hyper responsiveness. The amplitude, frequency or localization of Ca^{++} oscillation in the smooth muscle may determine the degree of airway sensitivity and reactivity, which are characteristic features of asthma[2]. There is evidence of reduced bronchial hyper reactivity as measured by responses to challenge with histamine or methacholine[3].

Interest in the treatment of airway obstruction with compounds of chromone class is long standing. Altounyan[4] using himself as the subject, found some of the chromone-protected action against experimentally induced asthma. There is no direct evidence of anti-inflammatory effect of Sodium cromoglycate, but the number of eosinophils, mast cells, T lymphocytes and macrophages significantly reduce as a result of Sodium cromoglycate administration[5].

Azelastine, a phthalzinone derivative as a new effective and long acting anti-allergic agent inhibits

the passive cutaneous anaphylaxis and allergic bronchoconstriction[6]. Azelastine has also been shown to inhibit the allergic release of slow reacting substance of anaphylaxis and also afford protection against anti-histamine resistant leukotriene mediated allergic broncho-spasm in Guinea pig[7]. In this paper, comparative effects of Azelastine and Sodium cromoglycate in the inhibition of broncho-constriction of ovalbumin sensitized lung parenchymal tissues of Guinea pigs *in vitro* are presented.

MATERIAL AND METHODS

This study was conducted at Department of Pharmacology and Therapeutics of Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center Karachi during 1998. Male or female Guinea pigs weighing 300-450 gm were sensitized according to protocol of Andersson[8] by intra-peritoneal injection of 5 mg ovalbumin on day 0 followed by day 2 with 10 mg. On day 21 of sensitization, Guinea pigs were killed by decapitation and exsanguinations. The lungs were removed from the thoracic cavity and flushed with Krebs solution. Lung parenchymal strips approximately (3x3x20mm) were cut from the lower lobes[9]. Each strip was suspended in a 20 ml organ bath. One end of the tissue was held at the bottom of the glass hook in the organ bath and a silk thread to a force transducer was fixed with the other end. The tissue was bathed with Krebs solution and oxygen continuously at temperature of 37^{o}C. Parenchymal strips were held with an initial tension of 1gm and tissues were allowed for equilibration for 90 minutes. The bath solution was changed after every fifteen minutes interval. Under resting tension of 0.5gm, confirmation of sensitization of tissues was done by adding 20 mg ovalbumin in the tissue bath and contraction of parenchymal smooth muscle was recorded by Grass polygraph model 7B. Initial series of experiments of ovalbumin concentration effects was determined, EC_{50} calculated and ovalbumin induced contractions were recorded. In the second phase, the Sodium cromoglycate and Azelastine in different concentrations were made in contact to parenchymal tissues for 10 minutes and then EC_{50} induced contractions were developed and recorded. The results were analyzed statistically by applying Wilcoxon rank sum test.

RESULTS

Confirmation of sensitization was done by recording the ovalbumin induced contraction i.e. 19mm $\pm$ 0.40 after exposure of 20mg of ovalbumin as per protocol. In the initial series of experiment, the concentration effect curve for ovalbumin (10^{-5} g/ml to 10^{-3} g/ml) was established. The ovalbumin induced contractile responses were expressed as percentage and placed on graph against ovalbumin concentration to calculate the EC_{50} i.e. $0.3x10^{-6}$ $\pm 0.12x10^{-6}$ g/ml (**Figure I**) and EC_{50} induced contraction of isolated strips of lung parenchymal tissue (n=6) 9mm$\pm$0.44.

Two sets of six strips from sensitized lung parenchyma were prepared as per protocol. Each strip after stress relaxation incubated for 10 minutes in serial concentration of Sodium cromoglycate and Azelastine and treated with ovalbumin EC_{50} produced contraction which was recorded for three minutes.

Sodium cromoglycate in concentration of 10^{-8} g/ml did not show any inhibition but as the concentration increased to 10^{-7} g/ml, it showed marked inhibition in contractile effect of ovalbumin $EC_{50.}$ Further increase in concentration of Sodium cromoglycate i.e. 10^{-6} g/ml (1ug/ml) completely antagonized the ovalbumin-induced contraction.

Azelastine in concentration of 10^{-9} g/ml (1ng/ml) did not exhibit any inhibition. As the concentration increased to 10^{-8} g/ml, it showed marked inhibition i.e. 20% contraction to EC_{50} ovalbumin, when compared before treatment with Azelastine and the concentration of 10^{-7} g/ml antagonized the effect of EC_{50} (**Figure II and Table I**).

FIGURE I: Ovalbumin induced contractile responses expressed in percentage of sensitized

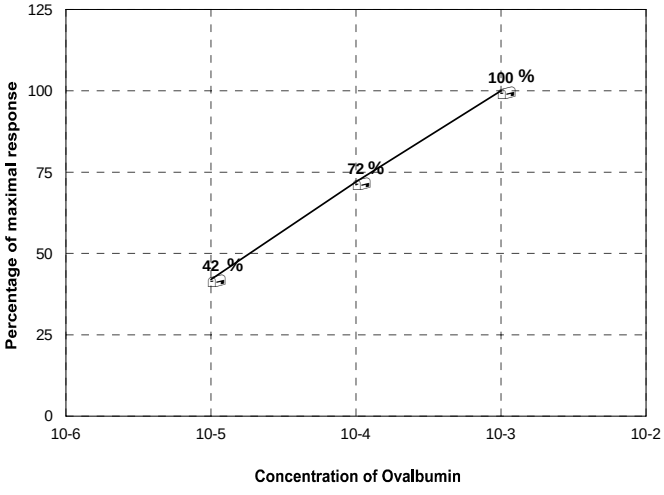

FIGURE II: Dose dependent inhibitory effects of Azelastine and Sodium cromoglycate of ovalbumin EC_{50} in sensitized parenchymal tissue. (Each point represents six experimental observations)

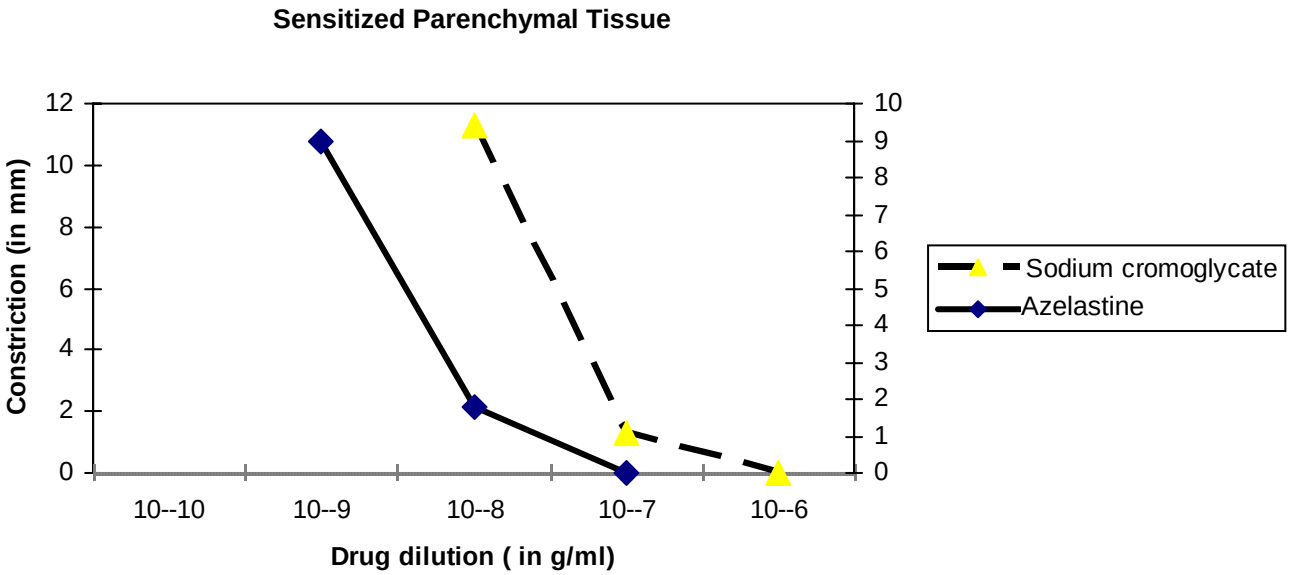

TABLE I: DOSE DEPENDENT INHIBITORY EFFECTS OF SODIUM CROMOGLYCATE AND AZELASTINE AGAINST EC_{50} OVALBUMIN. (EACH READING REPRESENTS MEAN OF SIX OBSERVATIONS)

DRUG	EC_{50} Ovalbumin $0.3x10^{-6}$ $\pm 0.12 \times 10^{-6}$ g/ml	Mean EC_{50} (ovalbumin) response after sensitized tissues incubation for 10 minutes in different concentrations of drugs			Antago-nize
		10^{-8}g/ml	10^{-7} g/ml	10^{-6} g/ml	
Sodium cromoglycate	9mm SEM $\pm$ 0.44	11.3mm SEM$\pm$0.33	1.3mm SEM$\pm$0.66	0mm	10^{-6} g/ml
		10^{-9} g/ml	10^{-8} g/ml	10^{-7} g/ml	
Azelastine		9mm SEM$\pm$0.31	1.8mm SEM$\pm$0.42	0mm	10^{-7} g/ml

guinea pig lung parenchymal tissues. (Each point represents mean of 6 observations)

DISCUSSION

The experimental system used in this study has incorporated several refinements not previously reported. Major significance was the utilization of several homogenous samples of Guinea pig lung parenchymal tissues, so that relatively subtle drug induced alteration of recording of parenchymal smooth muscles contraction could be detected. Secondly, we utilized a recently developed dual action anti-histamine (Azelastine) and compared with established mast cell stabilizer i.e. Sodium cromoglycate and observed the dose dependent inhibition of antigen induced broncho-constriction. The inhibition of mediator release by Azelastine may help to explain their protective action in anaphylaxis.

Our observations are in agreement that Azelastine exerts inhibitory effect on synthesis and release of chemical mediators from mast cell [10] including the leukotrienes[11]. Mediator release due to immediate type of hypersensitivity is one of the proposed reasons in the pathogenesis of allergic broncho-constriction[12]. Studies suggest that the acute effects of Sodium cromoglycate in extrinsic broncho-constriction are due to its ability to stabilize mast cells independently of stimulus[13]. Clinical trials with Sodium cromoglycate have shown a strong carryover effect after long-term treatment [14].

CONCLUSION

In vitro model of Sodium cromoglycate and Azelastine inhibits the antigen induced mediator release in dose dependent manner. Compounds

believed to raise intra-cellular level of cyclic AMP inhibit the mediator release by reducing Ca^{++} transport across the mast cell membrane resulting in the inhibition specifically the anaphylactic process initiated by reagenic antigen-antibody interaction. Hence, it can be inferred from the observation that responses produced by antigen can be controlled well with Azelastine than Sodium cromoglycate and emerging with similar activity regardless of exact mechanism involved. But, it remains to be determined that what affects these agents will posses clinically with antigen-induced broncho-constriction and whether any added benefit will be obtained by this class of agents over the β adrenergic broncho-dilators. However, further understanding of the mechanisms involved in producing the effects observed may allow pharmacological selectivity with more specific effects on bronchial pulmonary smooth muscle and mast cell.

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