INTRODUCTION

Broncho-spasm is characterized by hyper responsiveness of tracheo-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. Calcium handling by the airway smooth muscles may be an important determinant of airway hyper responsiveness. The amplitude, frequency or localization of Ca^{2+} oscillation in the smooth muscle may determine the degree of airway sensitivity and reactivity, which are characteristic features of asthma. There is evidence of reduced bronchial hyper reactivity as measured by responses to challenge with histamine or methacholine.

Interest in the treatment of airway obstruction with compounds of chromone class is long standing. Altounyan 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.

Azelastine, a phthalizinone derivative as a new effective and long acting anti-allergic agent inhibits
the passive cutaneous anaphylaxis and allergic bronchoconstriction. 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. 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 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. 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°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, EC50 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 EC50 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 ± 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 EC50 i.e. 0.3x10⁻⁶ ±0.12x10⁻⁶ g/ml (Figure I) and EC50 induced contraction of isolated strips of lung parenchymal tissue (n=6) 9mm±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 EC50 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 EC50. 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 EC50 ovalbumin, when compared before treatment with Azelastine and the concentration of 10⁻⁷ g/ml antagonized the effect of EC50 (Figure II and Table I).

FIGURE I: Ovalbumin induced contractile responses expressed in percentage of sensitized...
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 including the leukotrienes. Mediator release due to immediate type of hypersensitivity is one of the proposed reasons in the pathogenesis of allergic broncho-constriction. 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. Clinical trials with Sodium cromoglycate have shown a strong carryover effect after long-term treatment.

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 \(\beta\) adrenergic bronchodilators. 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.

REFERENCES