Original Article

THE EFFECTS OF SODIUM CROMOGLYCATE ON OVALBUMIN SENSITIZED GUINEA PIGS LUNG PARENCHYMAL TISSUES

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ABSTRACT

This study was conducted at Basic Medical Sciences Institute (BMSI) Karachi in the year 1998. Antigen induced histamine released from sensitized lung parenchymal tissues was observed within the initial 15% of the duration of contraction, responsible for the initial phase contraction and protracted phase were mediated by the release of other autacoids from sensitized mast cells. Ovalbumin sensitized guinea pigs lung parenchymal tissue was exposed to specific antigen in vitro, resulted in a rapidly developing, prolonged contraction. Treatment of the tissue with sodium cromoglycate before antigen exposure inhibited or delayed the onset and decreased the amplitude of contraction the response was dose dependent.

The data suggested that sodium glycate treatment of the tissues prior to antigen exposure stabilizes the mast cell membrane with the resultant inhibition of mast cell disruption and release of chemical mediators that are responsible for the early and late phase contraction. Sodium cromoglycate in concentration of 10^6 g/m (1μg/ml) completely antagonizes the antigen induce contraction.

KEY WORDS: *Ovalbumin. Sodium-Cromoglycate. Guinea Pig.*

INTRODUCTION

Interest in the treatment of airways obstruction with compounds of the chromone class is of long standing. Altounyan[1] using him as the subject found that some of the chromones protected against experimentally induced asthma. Further investigations showed that cromoglycate inhibits the passive cutaneous anaphylaxis in several species of animals as well as the response of passively sensitized human lung to allergic challenge[2].

The mechanism of action of cromolyn remains relatively poorly defined. Most attention has been focused on the ability of cromolyn to reduce the accumulation of intracellular Ca^{++} induced by antigen in sensitized mast cells[3].

One important action of cromolyn is believed to be the inhibition of pulmonary mast cell degranulation in response to a variety of stimuli, including the interaction between cells bound IgE and specific antigen[4].

We here present findings of our study which was designed to see the effect of sodium cromoglycate on ovalbumin sensitized Guinea Pigs Lungs Parenchymal tissues in vitro.

METHODS

This study was conducted at Basic Medical Sciences Institute (BMSI) Karachi from January to July 1998. Guinea pigs of either sex weighting 300-380gm were sensitized according to the protocol of Andersson[5]. As per protocol injection of 5mg of ovalbumin were given on day O, followed on day 2 by 10mg intra-peritoneal and kept in the animal house for 21 days and then killed by decapitation and exsanguinations. The trachea, heart and lungs were removed en block. Initially lung parenchymal strips (3x3x20mm) were cut, with care being taken to avoid large airways and blood vessels[6].

Lung parenchymal strips were suspended in an organ bath containing Krebs solution, maintained at 37^0C and continuously bubbled with oxygen and placed under a baseline tension of 1gm with equilibration times of 90 minutes, and response were recorded under resting tension of 0.5gm.

After the confirmation of immunological sensitization (contraction of tissue in response to the addition of ovalbumin 20mg) on one strip, the remaining strips of lung parenchyma were used to determine the

concentration effect relationship for ovalbumin. All contractions were expressed as percentage and placed against the ovalbumin concentration response of 50% of maximal contraction on the initial concentration effect curve was taken and mean of contractile effect curve was taken and mean of contractile response of EC_{50} were evaluated (mm) as EC_{50}.

EC_{50} ovalbumin induced contraction was again generated after the strips had been incubated with sodium cromoglycate in concentration range of (10^{8} g/ml -10^{6} g/ml) for 10 minutes in organ bath and contractile tracing were recorded on polygraph.

RESULTS

In sensitized animals, the effectiveness of immunization was confirmed by demonstrating ovalbumin-induced contraction of lung parenchymal strips. In the initial series of experiment of sensitized guinea pigs, lung parenchymal tissues were exposed to ovalbumin (10^{5} g/ml-10^{3} g/ml) and contraction of lung parenchymal strip were recorded to determine the EC_{50} of ovalbumin ie. $0.3x10^{-6} \pm 0.16x10^{-6}$ than EC_{50} ovalbumin induced contraction in 10 parenchymal strips were recorded and the mean response of amplitude was 9 mm $\pm$ 0.44.

Six strips from sensitized lung parenchymal tissues were prepared as per protocol. In each strip after stress relaxation serial concentration of sodium cromoglycate 10^{-8}-10^{-6} g/ml were added to the incubation medium for 10 minutes and antigen induced contractile response recorded for 3 minutes. Sodium cromoglycate in concentration 10^{-8} g/ml in parenchymal tissue, did not exhibit any inhibition of contraction to ovalbumin EC_{50} i.e. EC_{50} contractile response before drug administration was 9 mm $\pm$ 0.44 and after drug administration 11.3 mm $\pm$ 0.33. While in concentration 10^{-7} g/ml of sodium cromoglycate showed inhibition in contractile response of ovalbumin EC_{50} i.e. contractile effect was less than 85% in the presence of sodium cromoglycate 10^{-7} g/ml in comparison to EC_{50} ovalbumin response before treatment. Sodium cromoglycate in concentration 10^{-6} g/ml or 1µg/ml completely antagonized the ovalbumin EC_{50} induced contraction **(Table I and Figure II)**.

Figure I Responses of Guinea pig parenchymal strips to ovalbumin. Contractions are expressed in percentage against the ovalbumin concentration

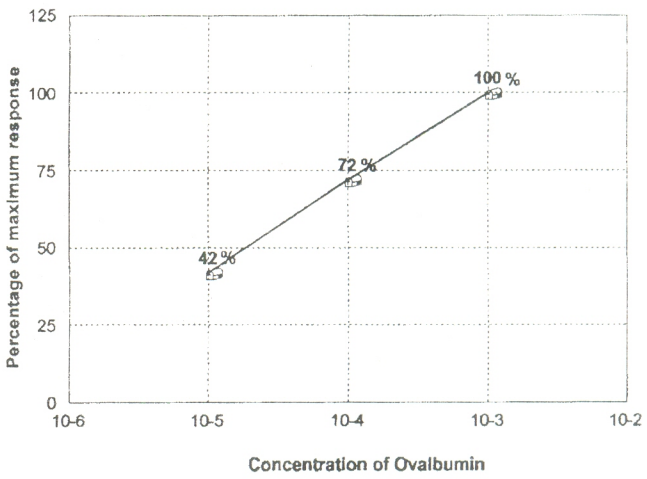

Figure II Graph showing dose dependent inhibition of contractile responses of parenchymal smooth muscle in the presence of sodiumcromoglycate when treated with ovalbumin EC_{50}

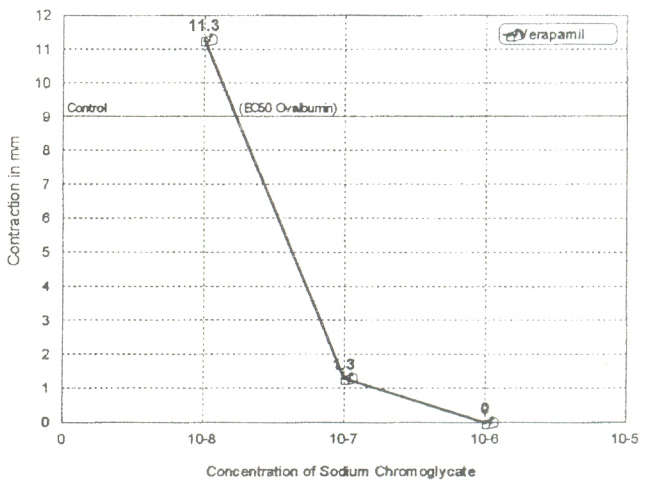

Table I Showing ovalbumin EC_{50} induced contractile response in millimeter before and after incubation with sodium cromoglycate in different concentration

	EC_{50} $0.3x10^{6}$	EC_{50} ovalbumin response after drug incubation for 10 minutes in concentration			Antagonise
Drug	Ovalbumin	10^{8}g/ml	10^{7}g/ml	10^{6}g/ml	
Verapamil	9mm SEM±0.44	11.3mm SEM±0.33	1.3mm SEM±0.66	0mm	10^{6}g/ml

DISCUSSION

Guinea pigs model in vitro were used to observe the inhibition of ovalbumin induced parencymal anaphylaxis using different concentration of sodium cromoglycate, which stabilizes the mast cell membrane. Our object was to review of that is known about the pharmacology of cromoglycate but also to postulate the mechanism of action in terms of findings, related to the release of mediator during immediate type hypersensitivity reactions.

Most manifestations of immediate type hypersensitivity reaction appears to result, from the release of mediators by antigen antibody interaction fixed to mast cell membrane. In this study Ovalbumin sensitized isolated parencymal strip, exposed to specific antigen, stimulated the supposed events leading to liberation of chemical mediators. Sodium cromoglycate in concentration 10^{7} gm/ml showed marked inhibitory effect and in concentration 10^{6} g/ml (1 μg/ml) revealed complete inhibitory response to EC_{50} ovalbumin induced contraction.

Earlier studies suggested that sodium cromoglycate was active only as a specific inhibitor of IgE mediated reaction[7]. Other studies also suggest that the acute effects of sodium cromoglycate in extrinsic broncho spasm are due to its ability to stabilize mast cells independently of the stimulus[8]. Clinical trials with cromoglycate have also shown a strong carry over effect after long term treatment[9].

Mediator released due to immediate type of hypersensitivity is one of the proposed reason in the pathogenesis of allergic broncho-constriction[10]. The effect of sodium cromoglycate could be assumed to reverse the changed reactivity to mediators participating in the anaphylactic reactions[11].

CONCLUSION

Sodium cromoglycate inhibits antigen induced mediator release by interfering with calcium transport across the mast cell membrane. The compound believed to raise intracellular level of cyclic AMP, inhibits mediator release by Ca^{++} transport across the mast cell membrane. Sodium cromoglycate inhibits specifically the anaphylactic process initiated by reagnic antigen antibody interaction probably due to interference in Ca^{++} transport.

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