EFFECTS OF VERAPAMIL ON OVALBUMIN INDUCED CONTRACTION OF SENSITIZED GUINEA PIGS LUNG PARENCHYMAL TISSUES, IN VITRO

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ABSTRACT

Calcium ions play an important patho-physiological role in allergic reactions. The release of mediators from mast cells, synthesis of some newly formed chemical mediators, airway smooth muscle contraction and nerve impulse conduction are all dependent on the availability and influx of Ca++ ions. It is therefore likely, that Ca++ antagonist, verapamil may modify the allergic broncho-pulmunary responses.

Investigate the effects of verapamil on ovalbumin induced contractile responses on lung parenchymal tissue strip in vitro. Guinea pigs treated with two high doses of ovalbumin i.e. 5 mg on day 0 and 10mg on day 2, intra-peritoneal. Twenty-one days after sensitization the effect of verapamil on guinea pigs, parenchymal tissue was evaluated by incubation of strip with verapamil for 10 minutes and treated with EC50 ovalbumin.

Verapamil exhibits dose dependent inhibition of ovalbumin-induced contraction with significant effect at concentration 10-9 g/ml.

On the basis of these observations two possible mechanisms for this protective effects were suggested, firstly verapamil may have suppressed mediator release and second verapamil may have inhibited the contractile effect of mediators on parenchymal smooth muscle. It is therefore suggested that verapamil may prove useful in the management of airway hyper-reactivity.

Keywords: Ovalbumnin, verapamil, parenchymal strips, guinea pig

INTRODUCTION

To investigate the mechanism underlying airway hyper responsiveness, murine model was developed, sensitized intraperetonially with ovalbumin and challenged with ovalbumin, presence of mast cell degranulation was determined. Sensitization induced high antigen – specific IgE levels in serum. Ovalbumin in sensitized animal induce an immediate but not late bronchoconstructive response, it is hypothesized that mast cells are involved in the induction of airway hyper responsiveness [1]. An inter-action between smooth muscles and inflammatory cells, especially mast cells may play a role in bronchial hyper-responsiveness in vitro [2]. Mast cell plays a pivotal role in early asthmatic response via a release of mediators, which directly influence airway smooth muscle tone. The mast cell derived products act in vitro human airway, hyper responsiveness contraction in sensitized bronchi via calcium related mechanisms [3]. There have been a number of studies of, bronchodilator effects of calcium channel airway responses to chemical agonists and allergens. Verapamil and Nifedipine have been the most extensively studied agents because of their availability for clinical use. Although both of these drugs are capable of inhibiting exercise-induced bronchospasm [4].

Therefore, it has been postulated that calcium antagonist (verapamil) might prevent bronco-constriction and stabilize the airways.

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METHODS

Male and female guinea pigs weighing 350-475 grams were sensitized to ovalbumin by intra-peritoneal injection, 5 mg on day 0 and 10 mg on day 2. After 21 days, the animals were killed by decapitation and lungs were dissected out, form the thoracic cavity and lung parenchymal strip of approximate dimension (3x3x20) mm were immediately removed and prepared according to the standard technique [5]. The parenchymal strips were placed in 20 ml organ bath and connected via a silk suture to Grass Model FT 03C force displacement transducer (Grass instrument Co) for recording isometric contraction. The tissue strips were bathed in a Krebs solution and tissue bath temperature was maintained at 370C with continuous oxygenation. The parenchymal strips were placed under initial load of 1gm and equilibrated for 90 minutes. After an initial period of 90 minutes, the ovalbumin-induced responses for each strip were obtained in dose dependent order and all results were expressed in percentages (the contraction observed at the highest ovalbumin dose was taken as maximum contraction for that tissue and all other contraction were expressed as percentage of it). The dose of ovalbumin causing 50% of maximum contraction on the initial concentration effect curve was calculated and designated as EC50 (fig. 1).

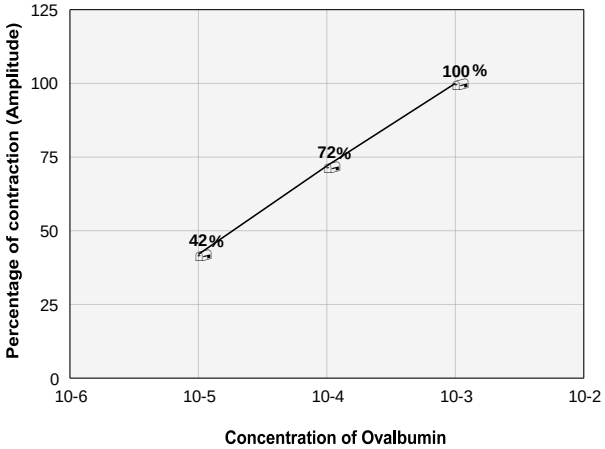

Fig. 1: (EC_{50} ovalbumin) Graph shows mean of percentage of 6 parenchymal tissue strips.

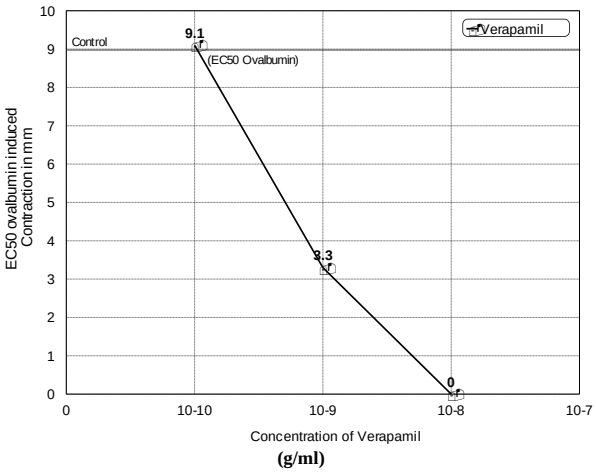

Fig. 2: Each point is mean of six experiments. Dose dependent inhibitory effect of ovalbumin EC_{50} with verapamil.

For experimental analysis, verapamil in concentration (10-10g/ml, 10-9g/ml, 10-8g/ml) was added to the tissue bath and each concentration was studied in six experiments. After 10 minutes of incubation, ovalbumin EC50 induced contraction was determined.

Table-1: Table shows inhibitory and antagonistic effect of verapamil on the isolated strip of parenchymal tissues of sensitized guinea pig when treated with EC_{50} (ovalbumin).

Drug	EC_{50} $0.3x10^{-6}$gm/ml ovalbumin	EC_{50} ovalbumin responses after incubation of parenchymal strip for 10 minutes in different concentrations of verapamil			Antagonizing concentration
		10^{-10} g/ml	10^{-9} g/ml	10^{-8} g/ml	
Verapamil	Mean ± SEM 9.0 ± 0.44	Mean ± SEM 9.16 ± 0.31	Mean ± SEM 3.3 ± 0.42	0 mm	10^{-8} g/ml

Each reading represents mean of six observations.

RESULTS

In the initial series of experiments, the dose dependent contraction on isolated sensitized parenchymal strips were obtained for ovalbumin and expressed in percentage. The percentage values were plotted on the graph against the ovalbumin concentration, in order to determine the EC50 i.e. 50% of the maximum response of that tissue. The mean concentration of ovalbumin EC50 i.e. 0.3x10-6 SEM + 0.16x10-6 gm/ml was calculated and was added on isolated strips of parenchymal tissue and mean contraction of amplitude in millimeters was determined (n=6) 9 SEM + 0.44.

As per protocol, each six strips of parenchymal tissue were incubated for 10 minutes in different concentration of verapamil and then

exposed to ovalbumin EC50. Inhibition was calculated from tracing of amplitude of contraction. Parenchymal strip in concentration of 10-10 g/ml did not show any inhibitory effect as compared to the effect of EC50 ovalbumin before verapamil incubation. As the concentration of verapamil was increased to 10-9 g/ml (1ng/ml) the parenchymal strip showed contractile effect less than 36% than that of EC50 ovalbumin and further increase in the concentration of verapamil i.e. 10-8 g/ml or 10ng/ml shows complete inhibition to antigen (ovalbumin EC50) induced contraction, depicted in (table-1) and (fig. 2). Percentage inhibition was calculated by comparing the amplitude of contraction of EC50 ovalbumin before and after treatment with verapamil

DISCUSSION

Available evidence indicates that the airway smooth muscle contraction depends mainly upon movement of Ca++ ions from extra cellular fluid to the cell interior and to a lesser degree from intracellular storage sites [6-8]. Because of importance of Ca++ movement in producing broncho-constriction, we studied the effect of verapamil on antigen-induced contraction of parenchymal lung tissues in vitro.

The data mentioned in the results, demonstrates that incubation with verapamil at certain concentration inhibits the allergen-induced contraction of parenchymal tissues. Previous studies have evaluated the ability of Ca++ blockers to relax the airway smooth muscles. Fanta et al [10] found that Nifedipine relaxed intrinsic tone of the airway muscles in vitro, while Himori and Taira [11] found that tracheal tone was decreased in vivo. Walter et al [12] have published an abstract which indicates that verapamil in a 0.2 to 0.4 micro-molar range could produced a 50% relaxation of canine trachealis, contracted with histamine, serotonin or methacholine and Weiss and Markowicz [13] found that 500 mg/ml could totally relax tracheal rings contracted with antigen. Together with the data reported here, these studies suggested that calcium antagonists similar to verapamil serve to stabilize the mast cell and prevent the smooth muscle contraction. However, relevance of these findings to the clinical use of calcium channel blockers is uncertain. It may be speculated that with the enhanced understanding of calcium channels in various tissues and their characterization, it may become possible in future to develop blockers having more selective action on the calcium channels in respiratory smooth muscle, which may open a new avenue in the treatment of bronchial asthma.

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