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EFFECTS OF VERAPAMIL ON CENTRAL & PERIPHERAL AIRWAY FUNCTION OF GUINEA PIGS



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ABSTRACT... saleemhz@hotmail.com **Introduction:** Calcium ions play an important patho-physiological role in allergic reactions. It is therefore likely, Ca^{++} antagonist, Verapamil may modify the allergic broncho-pulmonary responses. Isolated preparations were used to examine the direct action of the Verapamil on bronchial and pulmonary smooth muscles. **Material and Methods:** The bronchial spirals and the lung parenchymal strips were prepared that enabled the investigation of the action of agents on peripheral and central airways in the same species and compare the responses. To observe the effects on ovalbumin sensitized guinea pigs bronchial and lung parenchymal tissue after 10 minutes exposure of verapamil in different concentration and to evaluate the antagonistic / inhibitory effect on antigen induced contractile responses. **Results:** The data showed that inhibition of antigen induced response with verapamil was better in central airways than to peripheral airways smooth muscle. **Conclusion:** Concluded from this study that verapamil can influence both central and peripheral airway function by modulating antigen induced broncho-constriction; however, it leaves query regarding the importance of this activity in clinical set up.

Key words: Guinea Pig, Verapamil, Bronchial Strip, Parenchymal Strip.

INTRODUCTION

Calcium dependent excitation – contraction and stimulus

secretion coupling mechanisms play a central role in the patho-physiology of airway obstruction. Calcium channel

blocking agents that inhibit calcium flux across membrane ionic channels are a focus of current interest for their therapeutic potentials in the treatment of broncho-constriction. Air way smooth muscles are known to switch from a contractile to a proliferate and synthetic phenotype despite the possible relationship between airway smooth muscle phenotype and airway remodeling in asthma¹. An inter-action between smooth muscles and inflammatory cells, especially mast cells may play a role in bronchial hyper-responsiveness *in vitro*².

The mast cells play a pivotal role in early asthmatic response via release of mediators, which directly influence airway smooth muscle tone. The increased response in sensitized tissues was inhibited by calcium voltage dependent channel antagonist, Verapamil³. The completeness of protection with the Ca⁺⁺ channel blockers might be related especially to inhibition of Ca⁺⁺ influx or release⁴. These agents could conceivably exert salutary effects via several mechanisms including inhibiting of airway smooth muscle contractile response to chemical mediator and neuro-humoral substances; inhibition of secretory processes of mast cells, other inflammatory cells or mucous glands and active relaxation of smooth muscle.

There have been a number of investigations of the broncho-dilator effects of calcium channel blockers as well as studies of their ability to inhibit airway responses to chemical agonists and allergens. The bio-active sphingolipid that is increased in airway of asthmatic subjects markedly induced contraction of human airway smooth muscles. L-type calcium channel blocker Verapamil markedly decreased sphingosine-1-phosphate (SIP) induced smooth muscle cell contraction, supporting the role of calcium influx from extracellular sources⁵. Verapamil and Nifedipine have been the most extensively studied agents for because of their availability for clinical use. Ca⁺⁺ handling of 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 the characteristic features of asthma⁶.

Therefore, it has been postulated that calcium antagonist (verapamil) might prevent broncho-constriction and stabilize that airways⁷. The goal of these studies was to determine whether verapamil could inhibit the contraction of airway smooth muscle by antigen..

MATERIAL AND METHODS

Male or female guinea pigs weighing 315-410gm (n=10) were sensitized according to the protocol of Andersson⁸ by intra peritoneal injection of ovalbumin 5mg on day 0 and 10mg on day 2. After 21 days, the animals were killed by decapitation and exsanguinations. The entire trachea, lung and heart were removed en block from the thoracic cavity.

The tracheobronchial tissue were separated and cleaned of excessive connective tissues and placed in Krebs solution continuously bubbled with oxygen. Bronchial strips were prepared by cutting the bronchus spirally either right or left in such a way that two or three segments of cartilage separated. Each turn of spiral as described by Constantine⁹ for tracheal spiral preparation each strip was suspended in a 20ml glass organ bath with initial tension of 3gm for 90 minutes and temperature maintained at 37°C and continuously bubbled with oxygen.

The lung parenchymal strip prepared according to the standard¹⁰ of approximate dimension (3x3x20) mm and suspended in tissue bath under initial tension of 1gm for 90 minutes. The tissue strips were bathed in a Krebs solution maintained at 37°C and continuous oxygenation and both solution changed after every 15 minutes interval. Ovalbumin concentration dependent effect relationships for both bronchial and parenchymal strips were determined. The contraction observed at highest ovalbumin concentration was taken as the maximal contraction for that tissue and all other contractions were expressed as percentage of it. The concentration of ovalbumin causing 50% of maximal contraction was

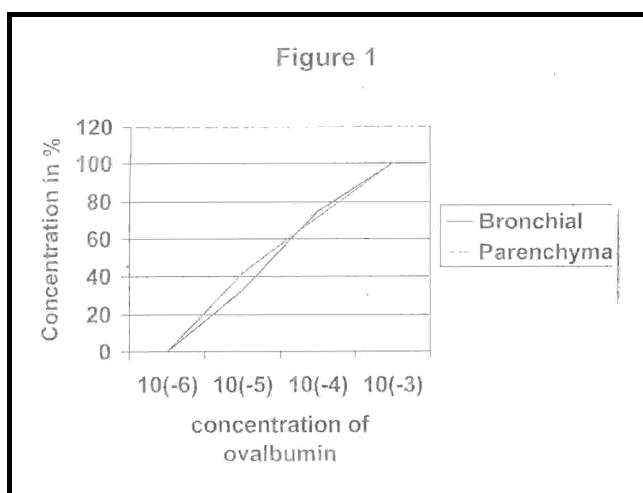
calculated as EC_{50} .

Concentration effects response were again generated after the strips had been incubated with verapamil in concentration range from (10^{-11} g/ml – 10^{-8} g/ml) for 10 minutes and EC_{50} ovalbumin induced responses were recorded to determined the dose dependent inhibitory/antagonistic effects of verapamil in an antigen induced sensitized bronchial and parenchymal strips.

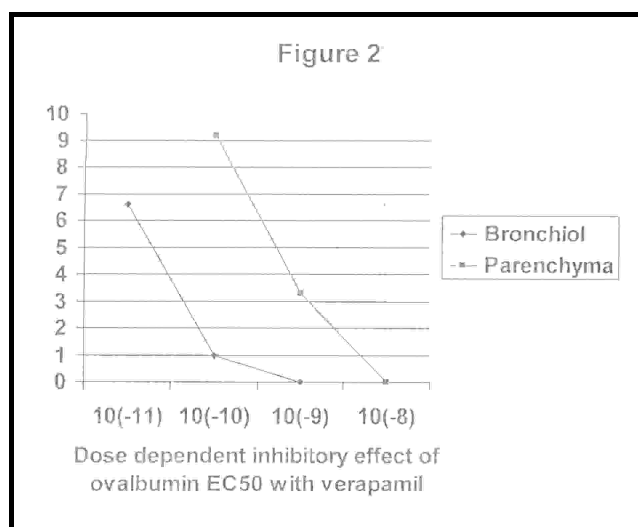
RESULTS

After the confirmation of sensitization by treating the isolated, bronchial and parenchymal strips (n=6) with 20mg of ovalbumin that produced a mean of contractile response $19 \text{ SEM} \pm 0.40\text{mm}$. The bronchial and parenchymal strips after an initial load with resting tension of 0.5gm, isometric contraction were recorded when both strips were exposed to ovalbumin in a concentrate range from (10^{-5} - 10^{-3} g/ml) and contractile effects were expressed in percentage. EC_{50} were calculated by percentage contraction against the ovalbumin concentration.

The mean EC_{50} ovalbumin for bronchial strip (n=6) $0.3 \times 10^{-6} \text{ SEM} \pm 0.12 \times 10^{-6} \text{ g/ml}$ and for parenchymal strip (n=6) $0.3 \times 10^{-6} \text{ SEM} \pm 0.16 \times 10^{-6} \text{ g/ml}$ (Fig-1).



The mean concentration of EC_{50} ovalbumin was added on the isolated strips of bronchial and parenchymal tissue and contraction amplitude in millimeter were recorded i.e. for bronchial strip (n=6) $5 \text{ SEM} \pm 0.26 \text{ mm}$ and for parenchymal strip (n=6) $9 \text{ SEM} \pm 0.44 \text{ mm}$ (Fig 2).



In bronchial strip verapamil in concentration 10^{-11} g/ml had no inhibitory effect while at concentration 10^{-10} g/ml it showed marked inhibitory effect i.e. bronchial smooth muscle contraction reduced to 80% of control (EC_{50} induced contraction) and in the same concentration parenchymal strips did not showed any inhibitory contractile effect.

At concentration 10^{-9} g/ml showed complete inhibition of contractile response of bronchial smooth muscles while parenchymal smooth muscles showed partial inhibition of muscle contractility i.e. less than 36% to control ovalbumin EC_{50} . In parenchymal strips concentration of verapamil 10^{-8} g/ml showed complete inhibition or antagonist effect of EC_{50} ovalbumin induced contraction (Table). Table shows inhibitory/antagonize responses of ovalbumin EC_{50} on both bronchial and parenchymal tissues with different concentration of verapamil.

DISCUSSION

The role of calcium in biological system is well established and it is accepted that elevation of the free intracellular calcium serves to link many membrane initiated events with cellular responses¹¹.

The cell membrane is a phospholipid barrier that is relatively impermeable to cations, such as Ca^{++} , and

allows this trans-membrane concentration difference to persist. During physiological or patho-physiological events, the membrane structure change to allow passage of Ca^{++} , which function as messenger for cellular contraction (muscle) or secretion (glands and mast cell).

Table						
Drugs	Tissue Strip	Contraction by EC ₅₀ ovalbumin (0.3x10 ⁻⁶)	Mean EC ₅₀ (ovalbumin) response after drug incubation for 10 minutes in concentration			Antagonize
			10 ⁻¹¹ g/ml	10 ⁻¹⁰ g/ml	10 ⁻⁹ g/ml	
Verapamil	Bronchial	n = 8 (5±0.26mm)	6.6±0.42mm	1±0.51mm	00mm	10 ⁻⁹ g/ml
			10 ⁻¹⁰ g/ml	10 ⁻⁹ g/ml	10 ⁻⁸ g/ml	
	Parenchymal	n = 10 (9±0.44mm)	9.16±0.31mm	3.3±0.42mm	00mm	10 ⁻⁸ g/ml

Russi et al¹², have demonstrated the inhibition of release of chemical mediators from mast cells by Ca^{++} channel blocker in animals in vivo, the inhibition of antigen-induced broncho-constriction by verapamil in sheep, allergic to ascaris summ antigen, but verapamil failed to block in the same, non-sensitized animal. Study conducted in the department of Chinese medicine by Ko.Ch et al concluded that relaxing effects of magnolol on porcine tracheal smooth muscle suggest an association with the blockade of Ca^{++} influx through voltage operated Ca^{++} channel instead of Ca^{++} release from intra-cellular Ca^{++} stores¹³.

It is speculated that calcium channel blocker protected against the allergic broncho-constriction pre-dominantly by preventing the release of chemical mediators from the mast cells. On the other hand, Henderson and associate¹⁴ found significant inhibition of allergic response with nifedipine and Lee et al¹⁵ also supported the finding, which observed inhibition of mediator release from human lung in vitro by verapamil. The data reported here indicate that pre-incubation with verapamil, inhibits antigen-induced contraction of both bronchial and lung parenchymal strips and that this inhibition is in general

concentration dependent.

CONCLUSION

Based on our observations and the results of previous studies, it seems likely that calcium channel blocker verapamil play a role in the treatment of allergic induced broncho-constriction. 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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