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COMPARISON OF THE EFFECTS OF AZALASTINE AND VERAPAMIL IN THE MODIFICA TION OF BRONCHO-CONSTRICTION OF OVALBUMIN SENSITIZED LUNG PARENCHYMAL TISSUES OF GUINEA PIGS *IN VITRO*

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ABSTRACT: Pharmacological prevention of antigen-antibody mediated reaction can be accomplished, at least in principle either to Prophylaxis achieved by inhibiting the release of chemical mediators andlor blocking the tissue receptors that served as target of the mediator action. The ability of azalastine and verapamil to influences antigen induced contractile responses in isolated sensitized parenchymal tissues of Guinea pig *in vitro.*

The Guinea pigs (n= I 0) were sensitized with ovalbumin and their parenchymal strips exposed to different concentration of ovalbumin to calculate the ECso. Each sensitized parenchymal strips treated with either Azalastine or verapamil in an organ bath for 10 minutes and treated with ECso ovalbumin and contraction recorded by Grass Polygraph model 78.

ECso (n=6) of parenchymal strips O.3x 10.6 + 0.16x1O.6g/ml and give a mean response of contraction (n=6) 9+0.44mm Azalastine in concentration 10.9 glml does not show any inhibitory effects but as the concentration increases to 10.8 glml marked inhibition recorded and at concentration 10.7 *glml* completely antagonizes the ECso induced contraction. While verapamil does not show any inhibition at concentration 10.10 *glml* and concentration 10.8 glml showed complete antagonism.

It is concluded that inhibition of ovalbumin-induced contraction of sensitized parenchymal tissues of Guinea pig in vitro is dose dependent and controlled better with vrepamil than Azalastine.

KEY WORDS: Ovalbumin, Azalastine, Verapamil, Guinea pig.

INTRODUCTION

Calcium dependent excitation - contraction and stimulus secretion coupling mechanisms playa 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 Fish (1984). An inter-action between smooth muscles and inflammatory cells, especially mast cells may playa role in bronchial hyper-responsiveness in vitro Ammit *et at.,* (1997). The mast cells playa 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, Verapamil Johnson *et at.,* (1997). The completeness of protection with the Ca^{++} channel blockers might be related especially to inhibition of Ca^{++} influx or release Ben Harari *et at.* (1992).

Azalastine, a phthalzinone derivative, a new effective and long acting anti-allergic agent, inhibits the passive cutaneous anaphylaxis and allergic bronchoconstriction Storm *et aI.,* (1985). Azalastine has also been shown to inhibits the allergic release of slow reacting substance of anaphylaxis and afford protection against anti-histamine resistant leukotriene mediated allergic broncho-spasm in Guinea pigs Chand *et at.* (1983a).

The goal of these studies was to determine whether verapamil and azalastine could inhibit the contraction of airway smooth muscle by antigen.

METHODS

Male or female Guinea pigs weighing 300-450 gm were sensitized according to protocol of Anderson (1980) by intra-peritoneal injection of 5mg ovalbumin on day 0 followed on day 2 by lOmg. 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.

Parenchymal strips approximately (3x3x20mm) were cut from the lower lobe Drazen *et at.,* (1979). Each strip was suspended in a 20ml organ bath. One end of the tissue held at the bottom of the glass hook in the organ bath and a silk thread to a force transducer fixed the other end. The tissue was bath with Krebs solution and gas continuously with oxygen at temperature of 37°C. Parenchymal strips were held with an initial tension of Igm and tissues were allowed for equilibration for 90 minutes. The bath solution

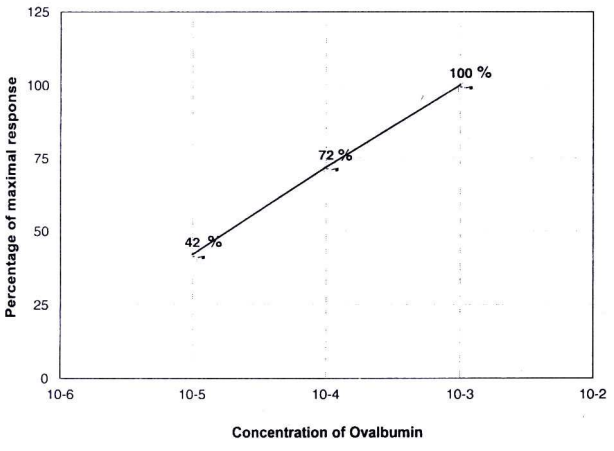
Table
Each reading represents mean of six observations

DRUGS	ECso Ovalbumin	Mean ECso (ovalbumin) response after sensitized tissues incubation for 10 minutes in different concentration *of* drugs			Antagonize
		lO-lOg/ml	10-9 o/ml *b*	10-8 *alml* *b*	
V erapami I	9mm SEM:!: 0.44	9.16mm SEM:!:0.31	3.3mm SEM:!:0.42	Omm	] 0-8 *glml*
		] 0:9 glml	10-8 glml	10-7 *glml*	
Azalastine		9mm SEM+0.31	l.8mm SEM+0.42	Omm	10-7 *glml*

Table showed inhibitory effects of Verapamil and Azalastine are concentration dependent against ECso ovalbumin induced contractions.

changed after every fifteen minutes interval. Under resting tension *of* 0.5gm, confirmation *of* sensitization *of* tissues by adding 20mg ovalbumin in the tissue bath and contraction of parenchymal smooth muscle recorded by Grass polygraph model 7B.

Initial series of experiments ovalbumin concentration effects were determined and calculate ECso and ECso ovalbumin induced contractions recorded. In the second phase, the Verapamil and Azalastine in different concentrations are in contact to parenchymal tissues for 10 minutes and than ECso induced contractions develop. Evaluate the concentration of the agents that influence the ECso ovalbumin induced contractions.



Figure]: Graph shows ovalbumin induced contractile responses expressed in percentage *of* sensitized guinea pigs parenchyma] tissues. Each point represent mean of six observations

RESULTS

Confirmation of sensitization 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 *glml* to 10-3 *g/ml)* established. The ovalbumin induced contractile responses were express as percentage and placed on graph against ovalbumin concentration and to calculate the ECso i.e. 0.3x 10-6 :!: 0.12x] 0-6 glml. Fig 1.

ECso induced contraction in millimeter of isolated strips of 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 Verapamil and Azalastine and treated with ovalbumin ECso to produced contraction, recorded for three minutes.

Verapamil in concentration 10-10 *glml* did not exhibit any inhibition but as the concentration increases to] 0-9 glml showed marked inhibition in contractile effect of ovalbumin ECso. Further increases in concentration of Verapamil i.e. 10-8 *glml* completely antagonize the ovalbumin-induced contraction.

Azalastine in concentration of 10-9 *glml* (1 ng/ml) does not exhibit any inhibition as the concentration increases to 10-8 glml showed mark inhibition i.e. 20% contraction to ECso ovalbumin, when compare before treatment with Azalastine and the concentration 10-7

g/ml antagonizes the effect of EC_{50} (Table and Figure 2).

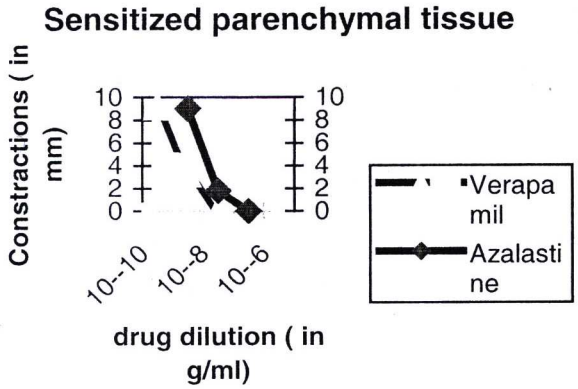


Figure 2: Graph shows dose dependent inhibitory effects of Azalastine and Verapamil of EC_{50} Each points represent six experimental observations.

DISCUSSION

The experimental system used in this study presented have incorporate several refinement not previously reported. Major significance was the utilization of several homogenous samples of Guinea pig parenchymal tissues, so that relatively subtle drug induced alteration of recording of parenchymal smooth muscles contraction could be detected and secondly we utilize a recently develop dual action anti-histamine (Azalastine) and compare with Calcium channel blocker Verapamil and observed the dose dependent inhibition of antigen induced broncho-constriction.

The inhibition of mediator release by Azalastine may help to explain their protective action in anaphylaxis. Our observations are in agreement that Azalastine exerts inhibitory effect on synthesis and release of chemical mediators from mast cell (Chand *et al.*), including the leukotrienes (Hamasaki *et al.)*.

The role of calcium in biological system is well established and it is accepted that elevation of the free intracellular calcium serve to link many membrane initiated events with cellular responses. The cell membrane is a phospholipid barrier that is relatively impermeable to cat-ions, such as Ca^{++}, and allows this tarns-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).

Russi *et al* (1980), have demonstrated the inhibition of release of chemical mediators from mast cells by Ca++ channel blocker in animals in vivo. Demonstrate 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. 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 *et al.* (1983) found significant inhibition of allergic response with Nifedipine and Lee *et al* (1983) also supported the finding, which observed inhibition of mediator release from human lung in vitro by verapamil.

CONCLUSION

In vitro model Verapamil and Azalastine inhibits the antigen induced mediator release in dose dependent. Compounds believed to raised intra-cellular level of cyclic AMP, inhibits 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.

It can be inferred from the observation that responses produced by antigen can be controlled well with Verapamil than Azalastine and emerging with similar activity regardless of exact mechanism involved.

It remains to be determined what affects these agents will posses clinically vs. antigen-induced broncho-constriction. Whether any added benefit will be, realize by this class of agents over the β adrenergic broncho-dilators.

Based on our observations and the results of previous studies, it seems likely that calcium channel blocker verapamil and Azalastine 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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