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## EVALUATION OF BRONCHIAL SMOOTH MUSCLE RELAXANT ACTIVITY OF FLUPIRTINE IN EXPERIMENTALLY INDUCED ASTHMA IN GUINEA PIGS

Sabreen Bashir\* and Md. Sibgatullah

Department of Pharmacology, JSS Medical College, Mysore, Karnataka, India.

### Keywords:

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### Correspondence to Author:

**Dr. Sabreen Bashir**

Post graduate and Tutor  
Department of Pharmacology, JSS  
Medical College, S.S Nagar, Mysore,  
Karnataka, India.


**E-mail:** Sabreenbhat.sb@gmail.com

**ABSTRACT: Objectives:** The study aimed at evaluating the broncho-relaxant effect of the test drug Flupirtine in experimentally induced Asthma in Guinea pigs & compares its efficacy with the Standard. **Materials and Methods:** Guinea pigs of both sexes weighing around 350-400 gm were randomly divided into 3 groups of 6 animals each. Groups were categorised into Control, Standard and test groups. Animals serving as controls received 10 ml/kg body weight of Normal saline; Standard group received 4.5 mg/kg body weight of salbutamol & the test group received the test drug flupirtine in doses of 46.5 mg/kg of body weight. All the compounds were administered once a day, per orally, through a gavage tube for 5 days. On day 5 experimental bronchial asthma was induced by exposing animals to 0.25% histamine acid phosphate under constant pressure (40mm/Hg) in an aerosol chamber. Animals exposed to histamine aerosol developed progressive dyspnoea. Pre-convulsive time (PCT) was determined from the time of exposure to onset of dyspnoea, immediately before the appearance of convulsions. Greater the PCT more is the bronchial smooth muscle relaxant effect. **Results:** There was a significant increase in Pre-convulsive time in the test group when compared to the control group suggesting bronchial smooth muscle relaxant activity of the test drug Flupirtine. **Conclusion:** Flupirtine capable of mediating a reduction in contractile & inflammatory responses would be a promising drug combining broncho-dilatation with anti-inflammatory action. However further preclinical & clinical studies are warranted.

**INTRODUCTION:** Chronic Respiratory Diseases (CRD) is one of the most common cause of disease burden, both globally and in India. CRD includes Bronchial Asthma & Chronic Obstructive Pulmonary Disease (COPD) which together may account for an estimated burden of about 100 million individuals in India currently<sup>1</sup>. Asthma is a chronic inflammatory disorder characterised by airway hyper-responsiveness to a variety of stimuli. It results from a complex interaction between inflammatory mediators & airways.

Short-term relief, mainly aiming at relieving broncho-constriction is most effectively achieved by agents that relax airway smooth muscle, of which  $\beta$ -adrenergic agonists are most potent & widely used. Long-term control is achieved with an anti-inflammatory agent such as an inhaled corticosteroid and in part with a leukotriene pathway antagonist or an inhibitor of mast cell degranulation<sup>2</sup>. Most asthmatics respond well to the available treatments of corticosteroids and  $\beta$ -adrenergic agonists; however, 5–10% has severe disease that responds poorly.

However, adverse effect profile and gradual non-responsiveness to the current pharmacotherapy in asthma necessitates evaluation of newer targets. Recent insights into the molecular mechanisms of airway inflammation & broncho-constriction has led to exploration of novel targets in drug therapy

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of Asthma and other disorders of airway requiring broncho-dilatation. Evidences of the presence of ions channels like  $K^+$  channels, receptors like GABA, NMDA & their essential role in the regulation of airway diameter, suggests that they may be targeted pharmacologically to relieve airway hyper-constriction.

**MATERIALS AND METHODS:** Healthy adult guinea pigs of either sex weighing around 350-400 grams were obtained from Central Animal Facility of JSS Medical College, Mysore, after having received the approval for the study proposal by Institutional Animal Ethical Committee. Animals were randomly divided into 3 groups of 6 guinea pigs each. Groups were categorised as Control, Standard and test groups. Animals serving as controls received 10 ml/kg body weight of Normal saline; Standard group received 4.5 mg/kg body weight of salbutamol and the test group received the test drug flupirtine in doses of 46.5 mg/kg of body weight. All the compounds were administered once a day, per orally, through a gavage tube for a period of 5 days. On 4<sup>th</sup> day, animals were housed in the aerosol chamber for acclimatization. On 5<sup>th</sup> day, one hour after drug administration experimental bronchial asthma was induced in guinea pigs by exposing them to 0.25% histamine acid phosphate aerosol under constant pressure (40mm/Hg) from an inbuilt nebuliser of the aerosol chamber<sup>3</sup> (M/s Inco, Ambala). Animals

exposed to acetylcholine aerosol developed progressive dyspnoea. Pre-convulsive time (PCT) was determined from the time of exposure to onset of dyspnoea, immediately before the appearance of convulsions. Just after the PCT was noted, the animals were removed from the aerosol chamber, placed in fresh air & allowed time to recover. Greater the PCT more is the bronchial smooth muscle relaxant effect<sup>4</sup>. The Percentage protection offered by the treatment was calculated by using the formula:

$$\text{Percentage Protection} = (1 - T_1/T_2) \times 100$$

Where  $T_1$  = the mean of PCT of control group.

$T_2$  = the mean of PCT of test group.

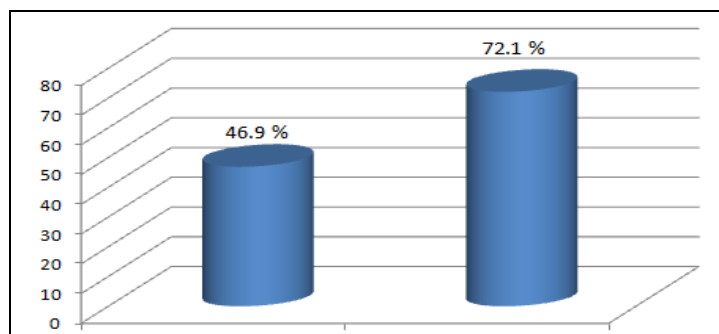
**RESULTS:** Data analysis was done by one-way analysis of variance (ANOVA). Probability values of 0.05 or less were considered statistically significant. The mean exposition time on acetylcholine exposure which corresponds to the time the animals withstood the acetylcholine exposure before the development of dyspnoea leading to the appearance of convulsions, was greater in the test group ( $95.03 \pm 3.70$ ) when compared to the control group ( $50.5 \pm 2.42$ ) as shown in the **Table 1**. Percentage protection offered by the treatment was found to be 46.9 % in the test group while it was calculated to be 72.9 % in the standard group, depicted in the **Fig. 1**.

**TABLE 1: EFFECT OF THE TEST DRUG, FLUPIRTINE ON THE EXPOSITION TIME OF GUINEA PIGS AFTER EXPOSURE TO 10% ACETYLCHOLINE AEROSOL**

Group	Drugs Administered and their Doses	Mean Exposition Time on Histamine Exposure/Latent Period (in secs)	Percentage Protection Offered
Control	Normal saline 10 mg/kg body weight, P.O	$50.5 \pm 2.42$	-----
Test	flupirtine 46.5 mg/kg body weight, P.O	$95.03 \pm 3.70^*$	46.9 %
Standard	salbutamol 4.5 mg/kg body weight, P.O	$180.66 \pm 3.26^*$	72.1 %

n = 6 in each group; Values are in Mean  $\pm$  SD. Data was analysed by one way ANOVA

\*P <0.05 was considered statistically significant.



**FIG. 1: REPRESENTS THE PERCENTAGE PROTECTION OFFERED BY THE TREATMENT IN TEST GROUP IN COMPARISON TO STANDARD**

Above findings suggest a significant broncho-relaxant activity of the test drug, flupirtine.

**DISCUSSION:** In asthma and other lung diseases, airways can become hyperconstricted because of increase in local bronchoconstrictor concentrations {e.g., histamine released from mast cells or acetylcholine released as a neurotransmitter.} These substances activate  $G_{q/11}$ -coupled receptors on airway smooth muscle cells (ASMCs) in the walls of the bronchioles, resulting in contraction of the ASMCs and hence constriction of the airways.

Flupirtine is a neuronal potassium channel opener that also possesses *N*-methyl-D-aspartate (NMDA) receptor antagonist and Gamma- Amino- butyric Acid( GABA) receptor modulatory properties<sup>5</sup>.

The 3 most important  $K^+$  channels in airway smooth muscle are the  $K$  ATP (activated by a fall in intracellular ATP), BK Ca (Big conductance Ca dependant K channels activated by a rise in intracellular  $Ca^{2+}$ ) and Kv (activated by depolarization). Opening of such channels leads to  $K^+$  efflux, membrane hyperpolarization, reduced excitability and smooth muscle relaxation<sup>6</sup>. Kv7 potassium channel are known to be expressed in airway smooth muscle cells and play a role in modification of airway diameter. Flupirtine, a voltage gated Kv7 type of potassium channel activator, is thus expected to serve as a potential therapeutic drug in relieving airway smooth muscle constriction<sup>7</sup>.

Flupirtine acts indirectly as *N*-methyl-D-aspartate (NMDA) receptor antagonist by activation of  $K^+$  channels. It causes a dose-dependent reduction of NMDA receptor mediated glutamate induced rise in intracellular  $Ca^{++}$  concentration, binds to and activates G- protein coupled inwardly rectifying  $K^+$  channels<sup>8</sup>. NMDA-receptors are found to be present in Human Airway Smooth Muscle and mediate contractile responses via glutamatergic mechanisms. These findings suggest that accumulation of glutamate-like ligands for NMDA-receptors are associated with airway inflammation & contributes directly to airway hyperreactivity.

Recent studies suggest presence of GABAergic signaling in airways. Airway epithelium is found to be a predominant cellular source of endogenous GABA in the airway contributing significant pro-relaxant GABA effects on airway smooth muscle

force. Flupirtine by virtue of possessing GABA modulatory properties can possibly be effective in mediating airway smooth muscle relaxation<sup>9</sup>.

**CONCLUSION:** Flupirtine, a Kv7 channel activator possessing NMDA & GABA receptor modulatory properties was found to reverse the airway smooth muscle contraction induced by bronchoconstrictor agonist like acetylcholine. Hence, Flupirtine capable of mediating a reduction in contractile & inflammatory responses would be a promising drug combining bronchodilatation with anti-inflammatory action. However further preclinical & clinical studies are warranted.

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**CONFLICTS OF INTEREST:** None

## REFERENCES:

1. Jindal SK, Aggarwal AN, Gupta D, Agarwal R, Kumar R, Kaur T, Chaudhry K et al Indian study on epidemiology of asthma, respiratory symptoms and chronic bronchitis in adults. *Int J Tuberc Lung Dis.* 2012 Sep; 16(9):1270-7.
2. Bertram G, Katzung, Susan B. Masters, Anthony J. Trevor” Drugs used in Asthma”. Basic & clinical Pharmacology. 12 ed, New Delhi, Tata McGraw –Hill 2012; 339-353.
3. Chapman RW, Danko G, Siegel MI. Effect of extra and intracellular calcium blockers on histamine and antigen induced bronchospasm in guinea pigs and rats. *Pharmacology.* 1984;29(5):282-91
4. Anil KD, P Ramu. Effect of methanolic extract of *benincasa hispida* against histamine and acetylcholine induced bronchospasm in guinea pigs. *Indian J of Pharmacol* 2002; 34: 365-366.
5. Kornhuber J, Bleich S, Wiltfang J, Maler M, Parsons CG. "Flupirtine shows functional NMDA receptor antagonism by enhancing  $Mg^{2+}$  block via activation of voltage independent potassium channels. *Journal of neural transmission.* 1996; 106 (9–10): 857–867.
6. Potassium channel modulation: a new drug principle for regulation of smooth muscle contractility. *Studies on isolated airways and arteries. Danish Medical Bulletin,* 1996; 43(5):429-431.
7. Liubov I. Brueggemann, Priyanka PK, Robert BL, Julian S et al. Kv7 potassium channels in airway smooth muscle cells: signal transduction intermediates and pharmacological targets for bronchodilator therapy, *ajplung* 2012; 302 (1): 120-132.
8. S Harish, K Bhuvana, Girish M Bengalorkar, TN Kumar Flupirtine: Clinical pharmacology *Anaesthesiol Clin Pharmacol.* 2012 Apr-Jun; 28(2): 172–177.

9. Gallos G, Townsend E, Yim P, Virag L, Zhang Y, Xu D, Bacchetta M et al Airway epithelium is a predominant source of endogenous airway GABA and

contributes to relaxation of airway smooth muscle tone, *ajplung*. Feb 2013; 304(3):191-197.

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