



Review Article

IN-VIVO AND IN-VITRO SCREENING MODELS OF ASTHMA: AN OVERVIEW

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ABSTRACT

There are significant unmet medical needs in the Asthma. No single animal model of asthma gives a complete idea about all features of the human asthma. In-vivo and in-vitro screening methods are essential to transform the promising drug candidate from preclinical studies to human being. In this article, we discuss various in-vivo & in-vitro models of asthma which may describe the various mechanisms & pathophysiology involved in asthma & also provides the way for investigation of new therapeutic agents.

Keywords: Asthma, Screening models, Histamine, Acetylcholine.

INTRODUCTION

Asthma may be defined as an inflammatory condition with recurrent reversible obstruction of the airflow in the airways in response to stimuli which are not in themselves noxious and which do not affect non-asthmatic subjects ^[1].

Asthma is widely known as a chronic inflammatory lung disease characterized by reversible bronchoconstriction, elevated basal airway tone, eosinophils and lymphocyte accumulation and activation, epithelial cell dysfunction and damage, smooth muscle and submucosal gland hypertrophy, submucosal fibrosis, airway wall edema, mucus overproduction and episodes of non-specific airway hyper responsiveness to spasmogens ^[2]. Asthma is also determined as a complex chronic inflammatory disease of the respiratory tract that involves the activation of many inflammatory and structural cells, all of which release inflammatory mediators ^[3]. This condition affects over 5-10 % of the population in industrialized countries, and it is increasing in prevalence and severity ^[1].

Symptoms

- Breathlessness
- Wheezing
- Sputum Production
- Difficulty in speaking
- Dyspnoea
- Tightness of Neck Muscle
- Coughing after physical activity
- Whistling Sound while breathing
- Frequent coughing
- Feeling Frightened, exhaustion
- Chest Tightness
- Grayish or bluish colouring of lips ^[4].

Causes of Asthma

Researchers are not exactly sure why some people get asthma and others don't, but there are a number of factors that may increase a person's chances of developing the disease. These include:

- Being in an urban region, especially the inner city, which may increase vulnerability to environmental pollutants
- Smoking or exposure to secondhand smoke
- Exposure to occupational pollutants, such as chemicals used in farming and hairdressing, and in paint, steel, plastics and electronics manufacturing
- Having one or both parents with asthma
- Respiratory infections in childhood
- Low birth weight
- Obesity
- Gastro esophageal reflux disease (GERD) [5].

Asthma Triggers

People with asthma have very sensitive airways that react negatively to a variety of irritants in the environment. These irritants are called “triggers.” Contact with triggers cause asthma symptoms to initiate or worsen [5].

Need of Models in Asthma

The huge efforts that have been put into asthma research in the past have clearly increased insight into the pathogenesis of the disease. At the same time, current gene technology identifies an ever increasing range of particles that could be taken in the sensitization process to an allergen and/or the evolution of a chronic inflammatory process in the mucous membrane of the lower air passages. For obvious ethical reasons, studies in humans are restricted to a combination of morphological assessment and in vitro experimentation. Hence the continuing need for in vivo animal models, since they set aside for a more functional assessment of a given molecule, relating it, amongst other features, to the dynamics of the inflammatory process or to alterations in airway behavior.

The mouse is increasingly being used for the development of in vivo asthma models. An obvious advantage is that it is a non endangered species that offers the wide availability of genetically characterized inbred strains at relatively low cost [5].

IN-VIVO MODELS

There is lack of standardized animal model of asthma which resembles human asthma. At present there is no standardized experimental protocol. Various laboratories have developed their own model of asthma with major or minor modifications [6].

Histamine and Acetylcholine induced Bronchoconstriction in Guinea pigs

This is the traditional immunological model of antigen induced airway obstruction. Inhalation of histamine or other spasmogens can induce symptoms like asphyctic convulsions resembling bronchial asthma in guinea pigs [7]. Histamine and acetylcholine when inhaled causes hypoxia and leads to convulsion in guinea pigs. Histamine causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in cardiovascular system. In each species there occurs a characteristic predominant response to histamine, which is the cause of death [8].

In this model histamine (0.25%) and acetylcholine (10%) is to be given as aerosol under a constant pressure 40 mm/Hg from the inbuilt nebulizer of the histamine chamber. A prominent effect caused by histamine and acetylcholine leads to severe bronchoconstriction in the guinea pig that causes asphyxia and convulsive dyspnoea. Bronchodilators can delay the occurrence of these symptoms. The time required for appearance of preconvulsive dyspnoea (PCD)

Table 1: List of agents responsible as triggers in asthma

List of agents	Events triggering asthma
Respiratory infection	Respiratory syncytial virus (RSV), Rhinovirus, Influenza, Para-influenza, Mycoplasma pneumonia
Allergens	Airborne pollens (grass, trees, weeds), House-dust, Mites, Animal dander, Cockroaches, Fungal spores
Environment	Cold air, Fog, Ozone, Sulfur dioxide, Nitrogen, Tobacco smoke, Wood smoke
Emotions	Anxiety, Stress, Laughter
Exercise	Particularly in cold, Dry climate
Drugs/preservatives	Aspirin, NSAIDs, Sulfites, Benzalkonium chloride, β blockers
Occupational stimuli	Bakers (flour dust), Farmers (Hay mold), Spice and enzyme workers, Printers (Arabic gum), Chemical workers (Azodyes, Anthraquinone, Ethylenediamine, Toluene, Diisocyanates, PVC), Plastics, Rubber and wood workers (Formaldehyde, Western cedar, Anhydrides, Dimethylethanolamine)

Table 2: Experimental design for Histamine and Acetylcholine induced Bronchoconstriction in Guinea pigs [8].

Group (n=6)	Treatment	Dose & route of administration	Recording parameter
Standard group	Chlorpheniramine maleate (Atropine sulphate in case of Acetylcholine)	2 mg/kg p.o.	Preconvulsion period
Test group	Test drug	Test dose p.o.	

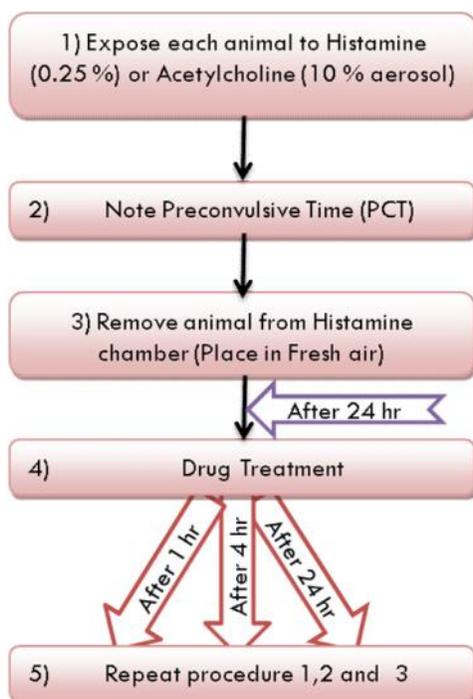


Fig 1: Flowchart showing study procedure of Histamine and Acetylcholine induced Bronchoconstriction in Guinea pigs [8]

The protection offered by treatment calculated by using the following formula:

$$\text{Percentage Protection} = (1 - T1/T2) \times 100$$

Where,

T1 = The mean of PCT before administration of test drugs.

T2 = The mean of PCT after administration of test drugs at 1 hr, 4 hr and 24 hrs.

Table 3: Experimental design for Clonidine-induced Catalepsy in Mice [14,15].

Group (n=6)	Treatment + Clonidine	Dose and route of administration	Recording parameter
Control group	Distilled water	10 ml/kg p.o.	Duration of catalepsy
Standard group	Chlorpheniramine maleate	10 mg/kg i.p.	
Test group	Test drug	Test dose p.o.	

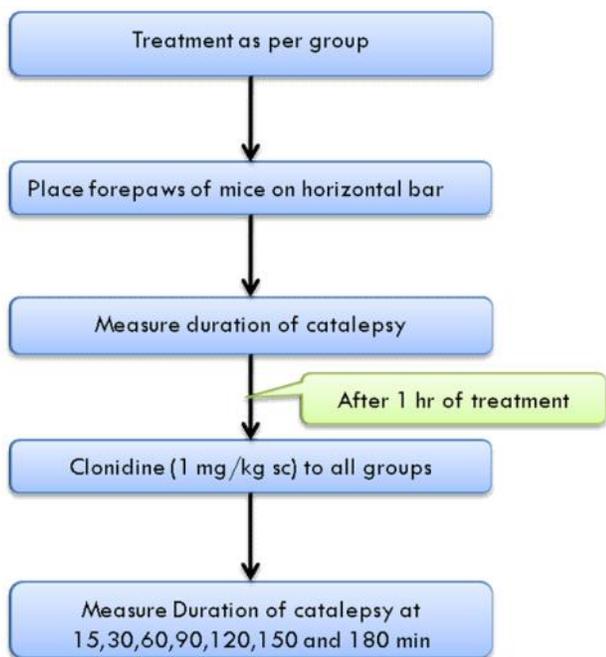


Fig 2: Flowchart showing study procedure of Clonidine-induced Catalepsy in Mice [14,15]

caused by the histamine and acetylcholine is recorded for each animal. This model is used to evaluate the bronchodilator activity of test drug against histamine and acetylcholine induced bronchoconstriction in guinea pigs [9,10,11].

Evaluation

Percent of increase of preconvulsive time is calculated versus control. ED₅₀ values can be found i.e. 50% of increase of preconvulsive time [7].

Clonidine-induced Catalepsy in Mice

Catalepsy is a condition in which the animal maintains imposed posture for long time before regaining the normal posture. Catalepsy is a sign of the extra pyramidal effect of drugs that inhibits dopaminergic transmission or increase/release histamine (inhibitory neurotransmitter) in the brain. Clonidine, a α_2 -adrenoreceptor agonist induces dose dependent catalepsy in mice, which is inhibited by histamine H₁ receptor antagonist but not by H₂ receptor antagonist [12,13]. Histamine acts as a modulator of pre-synaptic catecholamine processes in the CNS by causing depletion of the transmitter stores in the nerve terminals. Intracerebroventricular (i.c.v.) injection of histamine in conscious mice induces catalepsy, which was suppressed by

the H₁ receptor antagonist, Chlorcyclizine and not by metiamide, an H₂ receptor blocking agent [12].

There is histamine containing mast cells in brain. Brain histamine does play a definite role in the production of the extra pyramidal motor symptoms of catalepsy. Therefore it has been suggested that the cataleptic effect of Clonidine in the mouse be mediated by histamine (via H₁ receptors), which is released from the brain mast cells in response to stimulation of α_2 adrenoreceptors by Clonidine [12].

Bar test is the best way to study the effect of test drug on Clonidine-induced catalepsy. The forepaws of mice need to place on a horizontal bar (About 1 cm in diameter, 3 cm above the table) and the time required to remove the paws from bar has to be noted for each animal before and 1 hr after the test drug administration and measure the duration of catalepsy at 15, 30, 60, 90, 120, 150 and 180 min interval [14,15].

Evaluation: Decrease in the duration of catalepsy is calculated versus control. Standard and test groups are compared with control group.

Clonidine-induced Mast Cell Degranulation in Rats

The histamine concentration has been estimated to be about 0.3 m. in rat mast cell granules [16]. The clonidine and compound 48/80 act through the dynamic expulsion of granules without causing any damage to the cell wall. Clonidine releases histamine from mast cells in a similar style to a selective liberator like compound 48/80 [17]. Sodium cromoglycate a standard mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate [18]. This model is used to check mast cell stabilization potential of test drug.

Evaluation

Percent of decrease in clonidine induced mast cell degranulation is calculated versus control. Standard and test group are compared with control group and control group is compared with % of intact mast cells.

Milk-Induced Leucocytosis and Eosinophilia in Mice

Several medicinal properties have been attributed to the plants in the traditional system of medicine. The presence of adaptogenic properties in some plant materials is being one of them, as described to be tonics in the Ayurvedic system of medicine. Most important feature of an adaptogen is that it increases resistance to adverse influences of extensive range

Table 4: Experimental design for Clonidine-induced Mast Cell Degranulation in Rats [17].

Group (n=6)	Treatment	Dose and route of administration	Recording parameter
Control group	Distilled water	5 ml/kg p.o.	Cell disruption count
Standard group	Sodium cromoglycate	50 mg/kg i.p.	
Test group	Test drug	Test dose p.o.	

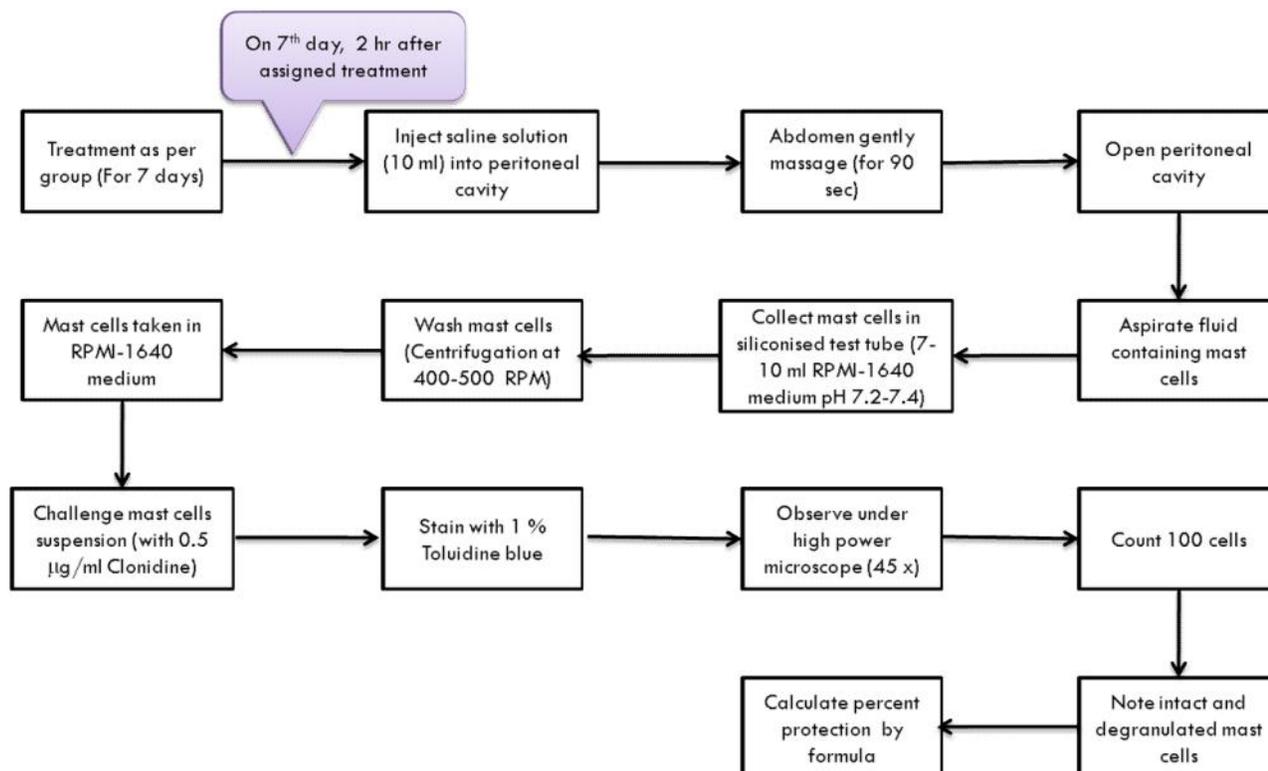


Fig 3: Flowchart showing study procedure of Clonidine-induced Mast Cell Degranulation in Rats [17]

Percent protection against clonidine induced mast cell degranulation can be calculated by following formula.

$$\% \text{ Protection} = \frac{T2-T1}{T2} \times 100.$$

T1 = Control group.

T2 = Test group.

Table 5: Experimental design for Milk-Induced Leucocytosis and Eosinophilia in Mice [19,24]

Group (n=6)	Treatment	Dose and route of administration	Recording parameter
Control group	Distilled water	10 ml/kg p.o.	Total leucocyte count and Eosinophil count
Positive control group	Distilled water + Boiled & cooled milk	10 ml/kg p.o. + 4 ml/kg s.c.	
Test group	Test drug + Boiled & cooled milk	Test dose p.o. + 4 ml/kg s.c.	

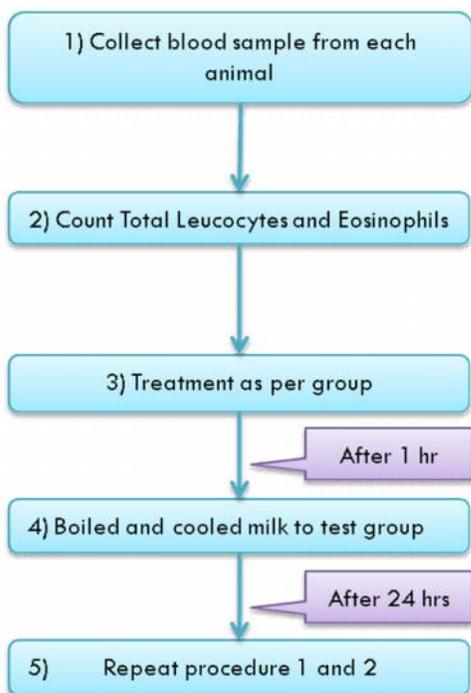


Fig 4: Flowchart showing study procedure of Milk-Induced Leucocytosis and Eosinophilia in Mice ^[19,24]

of factors of physical, chemical and biological nature which reveals itself irrespective of the direction of the previous pathologic shifts. Ayurveda provides a number of herbs for the treatment of asthma and herbal formulations used for the treatment of asthma include some adaptogenic (Nervine support) herbs to enable adoption to stress, since excessive stress or nervous debility may aggravate the symptoms of asthma. After parenteral administration of milk there is an increase in total leukocyte count (TLC) and this stressful condition can be normalized by administration of an antistress or adaptogenic drug ^[19]. Furthermore leukocytes recruited during asthmatic inflammation, release the inflammatory mediators like cytokines, histamine, and major basic protein and promote the ongoing inflammation. This model is used to evaluate the protective effect of test drug against milk-induced leukocytosis.

Eosinophilia is an abnormal increase in the peripheral eosinophil count of more than 4 % of total leukocytes. In the late phase, especially in the development of allergic asthma, eosinophils play role as an inflammatory cell. Eosinophil secretes mediators such as eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDNT), granulocyte

macrophage colony stimulating factor (GM-CSF), tumor necrosis factor (TNF) and Prostaglandin (PG), which results in epithelial shedding, bronchoconstriction and promotion of inflammation in respiratory tract ^[20]. Eosinophilia is associated with respiratory disorder, often allergic in nature together with pulmonary infiltrates that are detectable on chest films ^[21]. Parenteral administration of milk produces a marked and significant increase in the leukocytes/eosinophils count after 24 hr ^[22].

In this model of milk induced eosinophilia in mice, boiled and cooled milk (4 ml/kg, s.c.) is administered and the absolute eosinophil count is recorded before and after administration of milk ^[23].

Evaluation

A blood eosinophilia is hallmark of both allergic and non allergic asthma ^[24]. By noting the number of leukocytes and eosinophils before and after treatment, the difference is calculated. Standard group is compared with control group and test group is compared with standard group.

Passive Paw Anaphylaxis in Rats: Allergic asthma is a chronic inflammatory condition occurring due to exposure of allergen resulting in the activation of T-lymphocytes with subsequent release of inflammatory mediators. Immunomodulating agents are useful in asthma by inhibiting the antigen-antibody (AG: AB) reaction, thereby inhibiting the release of inflammatory mediators ^[25]. In passive paw anaphylaxis in rats, antibodies against egg albumin are raised in rats. Injecting these antibodies sensitize the animals. 24 hours after sensitization, test drug is administered. After 1 hour of test drug administration animals are challenged with egg albumin. This model is used to evaluate the protective effect of test drug against allergen-induced passive paw anaphylaxis and thus to study the effect of test drug on AG:AB reaction mediated inflammatory response.

Evaluation

Percent of inhibition of paw edema is calculated versus control. Standard and test group are compared with control group.

Body plethysmography and respiratory parameters after histamine-induced bronchoconstriction in anesthetized guinea pigs

Guinea pigs can be placed in a plethysmograph for measurement of respiratory parameters.

Table 6: Experimental design for Passive Paw Anaphylaxis in Rats [25,26].

Group (n=6)	Treatment	Dose and route of administration	Recording parameter
Control group	Distilled water	10 ml/kg p.o.	Change in paw volume
Standard group	Dexamethasone	0.5 mg/kg i.p.	
Test group	Test drug	Test dose p.o.	

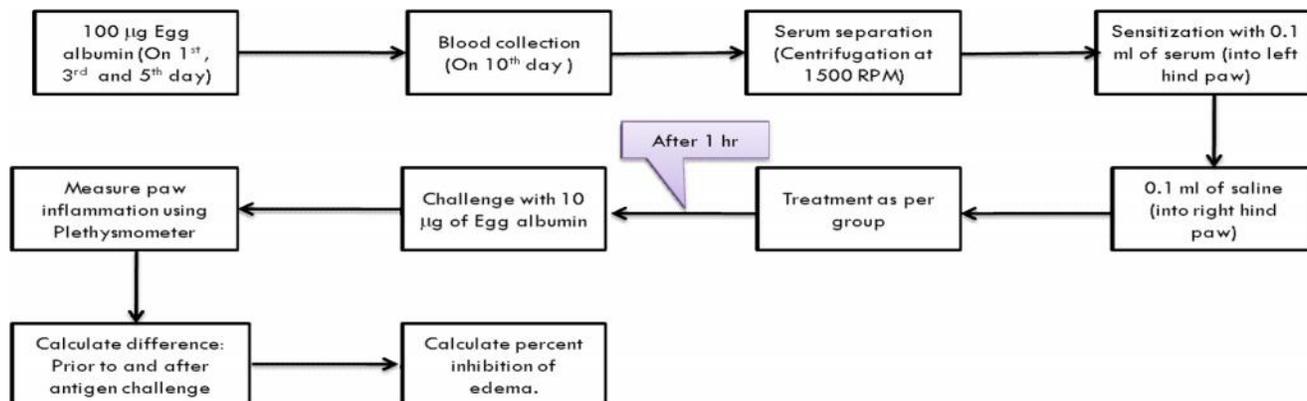


Fig 5: Flowchart showing study procedure of Passive Paw Anaphylaxis in Rats [26]

Table 7: Experimental design for Body plethysmography and respiratory parameters after histamine-induced bronchoconstriction in anesthetized guinea pigs

Group (n=6)	Sensitization (For 10 sec)	Treatment	Dose and route of administration	Recording parameter
Control	-----	Distilled water	10 ml/kg p.o.	Respiratory frequency and respiratory amplitude
Sensitized control	0.5% solution of Histamine operating pressure of approx. 1.5 bar	Distilled water	10 ml/kg p.o.	
Standard		Salbutamol	1 mg/kg p.o.	
Test		Test drug	Test dose p.o.	

Table 8: Experimental design for Effect on Broncho Alveolar Lavage Fluid in Egg Albumin Sensitized Guinea Pigs [37].

Group (n=6)	Sensitization (On 1 st day)	Treatment	Dose and route of administration	Recording parameter
Control	-----	Saline	10 ml/kg p.o.	Total WBC and differential WBC count
Sensitized control	Egg albumin (1 ml, 10% w/v, i.p.)	Saline	10 ml/kg p.o.	
Test		Test drug	Test dose p.o.	

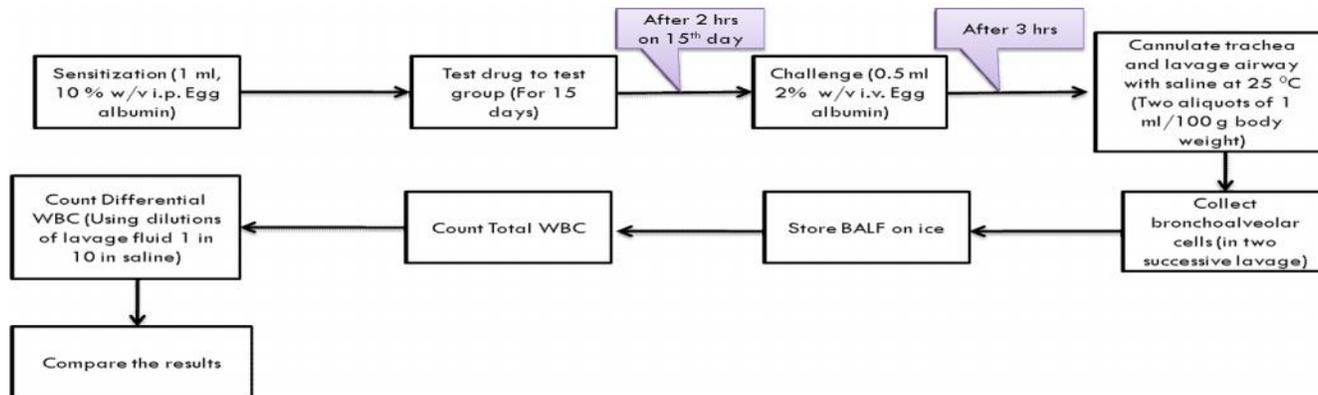


Fig 6: Flowchart showing study procedure of Effect on Broncho Alveolar Lavage Fluid in Egg Albumin Sensitized Guinea Pigs [37]

Respiratory frequency and respiratory amplitude are recorded. The decrease of respiratory amplitude (diminished respiratory volume due to bronchoconstriction) and the reflectory increase of respiratory frequency after histamine inhalation are attenuated by bronchodilatory drugs. Additional respiratory parameters can be recorded using a Fleisch tube and a catheter inserted into the pleural cavity [27].

In double chamber plethysmograph, or whole body restrained measurement of the airway resistance, animals are exposed to histamine or methacholine aerosol respectively to observe the airway responsiveness to these most frequently used mediators [28].

The method can be used for various purposes, e.g., to evaluate the antagonism against bradykinin-induced bronchoconstriction or the bronchodilator effects of potassium channel openers or to measure the effect of morphine on respiration in rats [7].

Evaluation

Inhibition of histamine induced bronchoconstriction by various doses of test compound and the standard is recorded. ED50 values for inhibition in pulmonary resistance (RL) are calculated. Furthermore, the time course of histamine antagonism can be measured. Compounds can be tested either after IV injection of histamine (prevention) or during intravenous infusion of histamine (intervention) [5].

Effect on Broncho Alveolar Lavage Fluid in Egg Albumin Sensitized Guinea Pigs

Bronchoalveolar lavage (BAL) has proved to be a useful tool to study the airways inflammation seen in conjunction with mild asthma [29]. BAL fluid from patients with asthma is a rich mixture of inflammatory cells and cytokines, eicosanoid mediators, neuropeptides, and soluble adhesion molecules [30]. Eosinophils, lymphocytes, and mast cells are present in increased numbers in persons with asthma [31,32,33]. The number of eosinophils, which appear to be increased in BAL fluid at baseline, appear to increase even more during periods of inflammation after exposure to allergen [34,35,36]. Lymphocytes and basophils have also been reported to increase in number in BAL fluid 19 hours after endobronchial instillation of allergen [36].

Evaluation: Total WBC and differential WBC are counted. The result obtained where compared with controlled with

sensitized group and sensitized with treated groups [37].

IN – VITRO MODELS

Isolated goat tracheal chain preparation:

This method is applied for the subject of the action of antispasmodic drugs on the tracheal musculature. The method is based upon the findings that the excised goat trachea respond to many drugs with the characteristic actions for which the drugs are well known, and that with proper magnification, the response can be recorded and measured for comparative purposes. Although, this method is known for its suitability in the study of antispasmodic drugs in general, emphasis is given on its use in the testing of bronchodilators. This is because of the close anatomical and physiological association, which exists between tracheal and bronchial musculature [38].

In isolated goat tracheal preparation, there is preponderance of H₁ excitatory and a scanty population of H₂ inhibitory receptors. Acetylcholine, histamine, 5-hydroxytryptamine and bradykinin show dose relative contractile responses on isolated goat trachea. With these agonists, the concentration necessary to produce contraction is less with goat tracheal chain than with guinea pig tracheal chain. Both goat tracheal chain and strip preparation were suitable for screening spasmogenic activity on respiratory smooth muscle and goat tracheal chain is easier to handle and prepare and is also much more sensitive than guinea-pig tracheal chain [39,40].

It is reported that isolated goat trachea contracts in response to acetylcholine (0.1- 12.8 µg), histamine (0.1- 102.4 µg), and barium chloride (0.1-51.2 µg) in a dose dependent manner and to 5-HT in a narrow dose range. Chlorpheniramine maleate (H₁- receptor antagonist) blocks contractions due to histamine while cimetidine (H₂- receptor antagonist) potentiates the contraction. These observations suggest the presence of both H₁-excitatory and H₂-inhibitory receptors for histamine on the isolated goat trachea [39].

The study of dose response curve indicates relative potency of the drug or agonist, when the curve is more towards the left; it indicates that the drug is more potent. The vice-versa is also true, and slope of the curve indicates error and reliability (precision) of the bioassay. Steeper the slope more precise is the assay and vice-versa is also true [41].

Evaluation

Height of the response is measured and dose response graph of acetylcholine and histamine is drawn in the absence and presence of test drug.

Histamine, Acetylcholine, Serotonin and Bradykinin Induced Contraction in Guinea Pig Ileum

Histamine is an autocoid having profound physiological effects in the body. It is an important mediator of immediate allergic (type-I) and inflammatory reactions. Besides the triple response caused by it, histamine has spasmogenic response on intestinal smooth muscle. By acting on H₁ receptor, it causes the contraction of guinea pig intestinal smooth muscle [41]. Acetylcholine, serotonin and bradykinin also cause contraction of guinea pig ileum similar to histamine. Acetylcholine is cholinergic agonist that causes contraction by acting on muscarinic receptors. This model used to screen the effect of test drug on Histamine, Acetylcholine, Serotonin and Bradykinin induced contraction of intestinal smooth muscle.

Evaluation

Height of the response is measured and dose response graph of acetylcholine and histamine is drawn in the absence and presence of test drug.

Vascular and airway responses in the isolated lung

The isolated perfused rat lung allows the simultaneous registration of pulmonary vascular and airway responses to several drugs. Pulmonary arterial perfusion pressure, airway pressure, and reservoir blood level are continuously monitored, electronically averaged and recorded with a polygraph [5].

Evaluation

Changes (increase or decrease) in pulmonary arterial pressure and in airway pressure after injection of test compounds are measured in mm Hg and compared with baseline values [5].

Bronchial perfusion of isolated lung

Bronchial perfusion of the isolated lung is a simple method for studying pharmacological reactions of bronchiolar muscle. The method consists in perfusing fluid down the trachea through the bronchi, and allowing it to escape from the alveoli through scratches on the surface of the lungs. Bronchoconstriction results in a reduced rate of flow. Bronchodilatation is indicated by an increased flow. The

method has been applied to evaluate sympathomimetic drugs [5].

Evaluation

Activity ratios of bronchodilating agents versus the standard can be calculated with a 3 + 3 point assay including confidence limits [5].

CONCLUSION

Various categories of agents are employed in the symptomatic relief of Asthma such as β_2 agonist, antimuscarinic, antihistaminics, anti-inflammatory corticosteroids, anti-allergic etc. In the present article, we have tried to cover most of categories of agents in the screening models for the evaluation of antiasthmatic activity. Probable mechanisms related to these screening models are also discussed here. A short effort has also been made to discuss symptoms, causes and triggers of asthma. This review article will be useful for students, teachers, researchers and young scientists in the field of respiratory research.

Exhaustive survey of several screening methods for antiasthmatic activity is essential to provide some precious input towards further research so that it can result into the development of some new therapeutic candidate as potent antiasthmatic agents.

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