Review Article

Various Screening Methods for Anti-allergic Activity: An Overview

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ABSTRACT: Development of compounds having diverse antiallergic activities to be used in the treatment of various conditions like rhinitis, dermatitis, asthma, food allergies and pollenosis, requires screening methods with efficient reproducibility and perfection. In such process some series of steps were followed; Antiasthmatic and relative activities were performed on albino guinea pig and isolated trachea through sensitization of animals with well known antigens such as egg albumin and Bordetella pertussis vaccine. Bronchodilator activities for both compound and standard (aminophyline) against contraction were produced by standard agonists like acetylcholine, histamine and egg albumin. Bronchoprotective activities were performed against various mediators. Experiments on sensitized isolated ileum, mast cell stabilizing activities on rat mesenteries, homologous passive cutaneous anaphylaxis (PCA) using above mentioned antigens were followed. Histamine release measurement using human basophilic cell line, KU812 suspension, biological assays using goat antimouse Immunoglobulin E (GAME) and biotinylated GAME, murine and human recombinant Interleukin 4(IL4) antiamouse and antihuman CD40 antibodies for ex vivo Immunoglobulin E (IgE) assay, IgE response in human cells and finally in vivo IgE response, Ig ELISA and cytokine assay were performed. Thus after successful completion of such studies it might be possible to establish a versatile antiallergic compound. Some of these studies are discussed here.

KEY WORDS: Antiallergic; IgE; Interleukin; Histamine; Assay; Screening

Introduction

Allergic disorders such as rhinitis, sinusitis, atopic dermatitis, asthma, pollenosis and food allergy are most common causes of human diseases (Rodrigo et al., 2004; Kelly., 1997). Hypersensitivity of the immune system to a specific antigen (allergens) plays a central role in the initiation of asthma and allergic rhinitis. Key components of this process include Th2 lymphocytes, which are a major cellular infiltrate in asthmatic lung (Walker et al., 1992; Robinson, 1992) and the antibody, immunoglobulin E (IgE), which is over produced in majority of people who suffer from allergic condition (Matsushita et al., 1993). However, numerous other components implicated for controlling IgE response are not always translated to prevent asthma (Milgrim et al., 1999; Haak-Frendscho et al., 1994; Lin et al., 2004). Low affinity receptor for IgE (CD23) has been reported to have direct effects on IgE regulation, antigen presentation and airway hyper responsiveness (Yu et al., 1994; Flores-Romo et al., 1993; Saxon et al., 1991; Stief et al., 1994; Texido et al., 1994; Haczkó et al., 1997; Ten et al., 1999; Kehry et al., 1989; Pirron et al., 1990). Interleukin 4 (IL-4) and IL-13 also are required for IgE responses in-vitro and in-vivo, and have other putative roles in the development of allergy beside from their direct activation of IgE (Clark et al., 1994; Bacharier et al., 2000; Grunig et al., 1998; Miyahara et al., 2004; Brusselle et al., 1995; Corry et al., 1996; Webb et al., 2000). Degranulation of mast cells caused by antigen-antibody reactions triggers such type-I allergic diseases and hypersensitivity of the immune system to a specific antigens like Th2 lymphocytes, IgE, IL-4 and 13, required for IgE responses plays a central role in the initiation. There are a number of pharmacological agents available for the treatment of allergic conditions such as asthma and allergic rhinitis. For a drug to be effective against allergic conditions, an action on a target that influences multiple mediators within the allergy cascade is required. Activity of IgE interfering compounds against a diverse group of allergy mediators will provide us to establish compounds as powerful tools for the treatment of allergy based diseases. Consequently novel approaches are needed as efficient search for useful candidates to be screened as anti-allergic drugs, so that few novel therapeutic candidates can be discovered which can serve the purpose best. Here in the present study we overviewed on the few well established screening methods for anti allergic activity as an essential step to the development of effective anti allergic agents.

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Antiasthmatic and Related Activities

Albino guinea pigs (350 to 400 g) and Albino rats (160 to 200 g) of either sex were used for the experiments. They were kept in standardized environmental conditions and maintained on a standard diet and water ad libitum and starved over night before the experiments. For anti-asthmatic activity, guinea pigs were sensitized with three doses of egg albumin (200 mg/kg) intraperitoneally as well as subcutaneously. The egg albumin doses were given on 1st, 3rd and 5th days. Three weeks after the last dose, the animals were utilized for the experiments and effects of test drugs were investigated on egg albumin challenged guinea pig and their isolated tracheal strip or ileum (Dale., 1913; Nair et al., 1994). For anti-passive cutaneous anaphylaxis (PCA) activity, rats were injected intramuscularly with 0.5 ml of a physiological saline solution containing 10 mg egg albumin. In addition, Bordetella pertussis vaccine (0.2 ml containing 1.8 x 10^9 cells) was injected intraperitoneally to each rat on 1st, 3rd, and 5th days. Four weeks after the first immunization, the blood was collected from heart and serum containing IgE type antibodies was separated out by cooling centrifugation and stored at -8 ± 10°C and utilized for the experiments (Overy et al., 1958; Dai et al., 1997).

Bronchodilator Activities on Different Models

Isolated Tracheal Strip of Guinea pigs

The guinea pigs were killed by exsanguinations and trachea was dissected out and cut into strips (Emmerson et al., 1979). The tracheal preparation was mounted in an organ bath maintained at 37 ± 0.5°C, containing Krebs physiological solution and constantly gassed with air. The isolated tracheal strip was connected to force displacement transducer (Bio-Device, India). All tracheal preparations were allowed 1 hour for equilibration before testing of compounds or standard (Aminophyline). The contraction responses of standard agonist like acetylcholine (4.42 x 10^-7 M), histamine (4.08 x 10^-5 M) and egg albumin, 200 µg/ml of bath concentration (only in sensitized tissue) on tracheal strip were recorded on student physiograph (Farmer et al). The same responses were re-elicited in the presence of compounds or aminophyline at different concentrations.

In another set of experimental investigation, a sustained contraction of the tracheal strip was elicited using carbamylcholine chloride (4.10 x 10^-5 M). After 10 minutes, the tracheal strip was exposed to different concentrations of compounds or aminophyline (5.95 x 10^-4 to 1.78 x 10^-5 M) (Silver et al., 1984) and the relaxation in tracheal strip was recorded on student physiograph (Bio-devices, India).

Bronchoprotective Activity in Guinea pigs Against Various Mediators

Experimental bronchospasm was induced in guinea pigs by exposing them to either 4 % w/v acetylcholine bromide in 5 % NaH2PO4 or 2 % w/v histamine dihydrochloride or 5 % egg-albumin (in sensitized guinea pigs) aerosol at a constant pressure using histamine chamber. The guinea pigs under exposure to acetylcholine or histamine or egg-albumin shown progressive dyspnea. The end point of pre-convulsive dyspnea (PCD) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsion. As soon as PCD commenced, the animals were removed from the chamber and placed in fresh air for recovery (Bahekar et al., 2000). After two days interval the same group of guinea pigs was administered with different doses of compounds or aminophyline intraperitoneally, 45 min. after the drug administration, the time for the onset of PCD was recorded against the experimental bronchospasm induced by acetylcholine or histamine or egg-albumin aerosol (Mitra et al., 1999). The protection offered by different drug treatment was calculated by following formula (Singh et al., 2001).

Percentage protection = [1-(T1/T2)] X 100, Where T1 was a mean of control PCD time and T2 was a PCD time after drug treatment.

Experiments on Sensitized Guinea pig Using Various Mediators

Sensitized guinea pigs were killed by exsanguinations. The ileum was dissected out and cut in to small pieces (2 cm. long) and connected to force displacement transducer (Bio-devices, India) in a 20 ml. capacity of organ bath containing Tyrode solution, maintained at 37 ± 0.5°C and gassed with air. All isolated ileum preparations were allowed 1 hour for equilibration before the testing of the drugs (Dai et al., 1997). The contraction responses of standard agonist like acetylcholine (3.75 x 10^-5 M), histamine (2.71 x 10^-7 M), egg albumin (100 µg/ ml) and barium chloride (2.05 x 10^-5 M) on isolated ileum were recorded on student physiograph (Dale, 1913; Schultz et al., 1910). The same responses were re-elicited in the presence of compounds or aminophyline at different concentrations.

Mast Cell Stabilizing Activity on Rat Mesentery

Albino rats were killed by exsanguinations. The piece of small intestine along with mesentery of rat was excised and connecting lobes of fat and blood vessels were rapidly dissected out. The piece of mesenteries was spread on petri-dish containing Ringer-Locke physiological solution (Tripathi et al., 1979). Compounds at different concentration on standard disodium cromoglycate incubated mesenteries were challenged with 1 µg/ml of compound 48/80(stdandard granulator) for 10 minutes.
(Geetha et al.,1981). Pieces of mesentery were stained with 0.1 % toluidine blue solution containing 4% formaldehyde for 20 to 30 minutes and then treated through acetone and xylene and mounted on slides (Norton et al., 1954). The percentage of degranulated mast cells was counted under microscope and protection offered by various drugs treatment was calculated.

**Experiments on Homologous PCA in Rats**

The homologous anti- PCA technique was used (Dai et al., 1997). The rats were injected intramuscularly with 0.5 ml of a physiological saline solution containing 10 mg egg-albumin. In addition, *Bordetella pertussis* vaccine (0.2 ml containing 1.8 x 10⁷ cells) is injected intraperitoneally to each rat on 1st, 3rd and 5th day. Four weeks after the first immunization, the blood was collected from heart and serum containing IgE type antibodies, was separated out by cooling centrifugation and stored at -8 ± 1°C, till being used for homologous PCA testing. The serum containing IgE antibodies was injected intradermally to rats at four shaved sites (0.1 ml/site) on the clipped dorsal skin. Three hours later, compounds or DSCG (50 mg/kg.) was administered intraperitoneally while control group was given an equal quantity of distilled water. One hour after the drug administration, 0.5 ml solution containing 1 % egg albumin along with 0.5 % Evan’s blue dye solution was injected into tail vein of rats of all groups. Forty five minutes after antigen (egg-albumin) challenge, the rats were sacrificed and skins were removed and blue colored area due dye leakage on the each testing site of inner aspect skin was measured with the help of scale and caliper. The anti PCA protection offered by different drugs was calculated by following formula:

\[
\text{Percentage protection} = 100 - \frac{\text{Blue coloured area in treated group}}{\text{Blue coloured area in control group}} \times 100
\]

**Histamine Release Measurement**

**Method of Junko et al., 2006**

The anti allergic effect of the test samples on the induced histamine release from the human basophilic cell line, KU812 was evaluated (Tachibana et al., 2000. Briefly, KU812 cells (1.5x10⁶ cells) were washed and resuspended in a Tyrode buffer. 5.0 mM calcium ionophore A23187 (Wako, Osaka, Japan) was incubated with the test compounds (1.5m g/ml) and then added to the cell suspension. The mixture was incubated at 37 °C for 20 min, and the reaction was terminated by cooling at 4 °C for 15 min. The cell suspension was then centrifuged and the amount of histamine in the supernatant was measured by the HPLC peak area using Cosmosil 5C18-PAQ. The percentage histamine release was calculated as follows:

\[
\text{Histamine release (％) = (test-negative control) / (positive control-negative control) \times 100.}
\]

The supernatant from the nonresponsive cells was used as the negative control, and the supernatant from the stimulated cells only with A23187 was the positive control. IC₅₀, the concentration that inhibited histamine release from the KU812 cells by 50% relative to control, was interpolated from graphed dose response results (1.5, 30, 75, 105mg/ml). IC₅₀ values were determined graphically.

**Modified Method of Sollman and Von Oettingh (1928)**

Twenty four albino rats (150-250 g) of either sex of Haffkine’s strain were sensitized with Freund’s adjuvant (Calbiochem U. S. A.) and bovine albumin (Sigma Chemical Co. U. S. A.) 0.05 ml each. Ten days later the animals were sacrificed and the lungs were removed and perfused. The changes in the rate of flow were recorded after challenging with bovine albumin 0.05 ml in the perfusion system. In one group of 6 rats, 0.5 ml/100 g of the test compounds were injected intraperitoneally daily for 3 days prior to sensitization of rats (Haranath et al., 1975). In the second group of sensitized Rats test compounds were administered directly into the perfusion system in a dose of 0.5 ml/100 g of lung tissues. In the third group of rats the test compounds were added into the perfusion system in normal rats. In the fourth group of rats, disodium chromoglycate was added into the perfusion system (5 mg/100 g of lung tissue). In all the groups of rats, changes in the rate of flow were measured and results were analysed.

**Biological Assay**

**Materials**

Goat anti-mouse IgE (GAME) and biotinylated GAME (b-GAME), murine and human recombinant IL-4Anti-mouse, anti-human CD40 antibodies and female 8–12-week-old BALB/cByj mice were collected. Spleen cells were obtained after sacrificing the mice by cervical dislocation, removal of the spleens, dispersing cells in a tissue grinder, filtering through nylon, washing twice with phosphate buffered saline (PBS), and finally culturing in DMEM/10% fetal bovine serum (FBS) at 37 °C, 10% CO₂(Richards Mark et al.,2006).

**Ex vivo IgE Assay**

The ex vivo IgE response assay involved in vivo antigen priming and measurement of secondary antibody responses in vitro. The basic protocol was thoroughly documented and optimized for a range of parameters including: antigen dose for priming and time span following priming; number of cells cultured in vitro, antigen concentrations for eliciting secondary IgE (and other Igs) response in vitro, FBS batch that will permit optimal IgE response in vitro, showing the importance of primed CD4-positive T cells
and hapten-specific B cells and specificity of the ELISA assay for IgE (Texido et al., 1994). Female BALB/cByj mice were immunized intraperitoneally with 10 μg dinitrophenol-conjugated keyhole limpet haemocyanin (DNP6-KLH) adsorbed onto 4 mg alum and sacrificed after 14–20 days. Spleens were removed and homogenized in a tissue grinder, washed twice. Spleen cell cultures (3 × 10⁶ lymphocytes per ml) were established in 96-well round-bottom plates in the presence or absence of DNP6-KLH (10 ng/ml). Test compounds were added to spleen cell cultures immediately prior to the addition of antigen. Cultures were prepared in quadruplicate and incubated for 7–12 days.

IgE Response in Human Cells

Freshly drawn human peripheral blood was diluted 1:1 with sterile PBS and layered over 10 ml of Histopaque. The tubes were centrifuged at 1800 rpm (400 × g) for 30 min at room temperature. The plasma layer (yellow) was aspirated and the white cells were collected by removing the interphase. That layer was transferred to a new 50 ml conical tube containing 20 ml of sterile PBS. White blood cells were counted, centrifuged at 1000 rpm (250 × g) for 10 min, and re-suspended in 15 ml of sterile PBS. Human PBL cultures (5 × 10⁵ cells per ml) were established in quadruplicate in the presence of human IL-4 and anti-human CD40 Ab (Pharmingen), and incubated at 37 °C; 10% CO₂ for 10–14 days before harvesting supernatants for the ELISA.

In vivo IgE Response

Female BALB/c mice (5–10 per group) were administered 250 rads (except control group) followed by immunizing with 2 μg KLH in alum (4 mg) i.p. 2 h later. 1ml was injected intraperitoneally on 3 consecutive days starting 6 days later. On day 2 of the drug injections, 2 μg of DNP6-KLH with alum (4 mg) was injected intraperitoneally. Periorbital bleeds was obtained 21 days following DNP6-KLH challenge and the serum was processed for quantification of antigen-specific antibody by ELISA.

Ig ELISA

To detect DNP-specific antibodies in cultures of mouse spleen cells ELISA plates were prepared by coating with specific antigen (DNP-OVA) overnight. After washing and blocking the plates with 200 μl bovine serum albumin (BSA) in PBS, an aliquot (1:4 dilution in PBS with 1% BSA 0.1% azide 0.5% Tween 20) of each culture supernatant were transferred directly to the ELISA plates and incubated overnight in a humidified box at 4 °C. IgE was quantified following successive 90 min incubations with biotinylated GAME (prepared in-house), alkaline phosphatase-streptavidin (Zymed), and 100 μl of phenolphthalein monophosphate (PPMP, DCHA salt, 40 mg/ml). Absorption was measured at 540nm. The level of detection for IgE is about 200–400 pg/ml and cross-reactivity was less than 0.001% with any other Ig isotype in the ELISA for IgE (Marcelletti et al., 1991)

Cytokine Assays

Drug activity was tested in two distinct assay systems utilizing spleen cells from female BALB/c mice. For the in vitro assay, T cells were isolated using Pan-T (CD3ε) magnetic bead kit (Miltenyi Biotec) and cultured for 48 h in the presence of ConA (5 μg/ml) in the presence and absence of drug. Culture supernatants were tested for IL-4 and IL-5 content using fluorescent beads from a cytokine detection kit (Upstate) and analyzed by a Luminex 100 flow analyzer. IL-4 and IL-5 to be quantified by comparison to standard curves. For ex vivo cytokine response assay spleens were removed from mice that had been sensitized to DNP6-KLH. Two weeks following sensitization, mice were sacrificed and spleen cell cultures were established in the presence of DNP6-KLH. After 4 days, ConA (5 mg/ml) was added to cultures and the cells were incubated for an additional 4 h. Cytokine levels in the culture supernatants were quantified by ELISA using reagents and protocols supplied by Pharmingen. Control responses were determined from cultures that contained no drug (Richards Mark et al., 2006).

Conclusion

Thorough study of various screening methods for anti-allergic activity might render some valuable contribution towards further research resulting development of some novel therapeutic candidates to be effective as potent anti-allergic agents.

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