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In vivo immunomodulatory profile of histamine receptors (H1, H2, H3 and H4): a comparative antagonists study

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ABSTRACT

Objective: To delineate the comparative immunomodulatory roles of H1R-H4R in antibody generation profile in rabbit model. **Methods:** The cohort comprised of eight groups containing 18 (9 male and 9 female) rabbits in each group. Group I remained non-immunized and received only vehicle (sterile distilled water, 1 mL/kg × b.i.d.) intramuscularly. Group II received vehicle (1 mL/kg × b.i.d.) while Groups III-VII (drugs-treated) received subcutaneous histamine (100 μg/kg × b.i.d.), and intramuscular H1R-antagonist (pheniramine, 10 mg/kg × b.i.d.), H2R-antagonist (ranitidine, 10 mg/kg × b.i.d.), H3R-antagonist (iodophenpropit, 1 μg/kg× b.i.d.) and H4R-antagonist (JNJ 7777120, 10 μg/kg×b.i.d.), and Group VIII DMSO (1 mL/kg × b.i.d.), respectively for 10 days (starting from day 1). They were subsequently immunized with intravenous injection of sheep red blood cells (SRBC) at day 3. The estimation of serum Igs, IgM and IgG were done by ELISA, and observed at day 0 (pre-immunization), and 7, 14, 21, 28 and 58 (post-immunization). **Results:** It was shown that histamine and HRs-antagonists could influence a detectable antibody response to SRBC as early as day 7-post-immunization (post-I), which lasted until day 58 post-I. The results were found statistically significant ($P<0.05$). **Conclusions:** This study suggests that histamine receptors play important roles in modulation of antibody generation in which H1R, H2R and H4R have immunosuppressive roles and conversely, H3R playes an immune enhancing role. The findings of this study may have clinical significance and provide the baseline information for future study.

1. Introduction

Histamine has incredible influence over an assortment of pathophysiological processes through the activation of four receptors: H1, H2, H3 and H4, and is known to participate in allergy, inflammation, secretion of gastric acid, immunomodulation and neurotransmission[1]. Moreover, histamine immunobiology pertaining to histamine receptors is elementary in contrast to increasing frequency of allergic diseases. Accumulating evidences have also been postulated its important role in other pathological conditions such as increased level in bronchoalveolar lavage fluid from patients with allergic asthma, and this increase is negatively correlated with airway function. An increase in histamine level has been observed in skin and plasma of patients of atopic dermatitis (AD), chronic urticaria (CU), multiple sclerosis (MS) and in psoriatic skin[2]. Histamine receptors (H1, H2, H3 and H4) transducing extracellular signals through different G-proteins: Gq/11 for H1, Gαs for H2, Gi/o for H3 and H4-receptors[1]. Specific activation or inhibition of histamine receptors has led to a tremendous increase in the knowledge of the roles of histamine in physiology and pathology of disease conditions[1, 3]. Histamine receptors have been revealed to increase delayed hypersensitivity and antibody mediated immune responses in several pathological processes regulating several essential events in allergies and autoimmune diseases in experimental animals, especially in knock out mice (either H1- or H2-deficient)[4-6].

Thus, after a century of histamine discovery, the existing literature has provided intensive knowledge about its synthesis, metabolism, receptors, signal transduction, and physiological and pathological effects. However, the roles of the complex interrelationship and cross talk

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by histamine and its receptors on antibody generation in immunobiology remained to be elucidated. Our recent studies have confirmed that histamine has a short-term effect on antibody generation (until its presence in the body), and the antibody generation titer in vivo was affected by the concentration of histamine[7, 8]. Notably, H1-specific antagonist tripelennamine inhibited histamine binding in T-helper1 (Th1) but not in T-helper2 (Th2) cells and demonstrated predominant H1 expression on Th1 cells. Neither H2-antagonist ranitidine nor H3-antagonist/H4-partial agonist clobenpropit had any impact on histamine binding to Th1 cells. H1-receptors and H2-receptors are regulated by specific cytokines present in the immune system, and the expression of H1 on Th1 cells and H2 on Th2 cells were confirmed by antibodies generated against the H1- and H2-receptor[9].

Therefore, our present comparative study account for exploration of the regulatory mechanisms in the control of immune process through effector cells derived histamine, exogenous histamine and histamine H1-, H2-, H3- & H4-receptors-antagonist in immunomodulation in rabbit model.

2. Materials and methods

2.1. Experimental design

To evaluate the systemic antibody response, 144 (72 male and 72 female) New Zealand adult healthy rabbits of either sex weighing 1.63±0.36 kg were randomized equally into eight treatment groups, i.e. 18 rabbits (9 male and 9 female) in each group.

Control group: Group I (negative control) remained non-immunized and received only vehicle (sterile distilled water, 1 mL/kg × b.i.d.). Group II was vehicle (sterile distilled water, 1 mL/kg × b.i.d.) treated and immunized as a positive control.

Experimental group: Group III was histamine-treated and immunized, group IV was H1R-antagonist-treated and immunized, group V was H2R-antagonist-treated and immunized, group VI was H3R-antagonist-treated and immunized, group VII was H4R-antagonist-treated and immunized, and group VIII was dimethyl sulphoxide treated (DMSO-treated) (control group for H4R-antagonist) and immunized.

The animals were housed in well-maintained animal facility at central animal house, J. N. Medical College & Hospital, Aligarh Muslim University, Aligarh, in the Bioresources unit under a 12 hr light/dark cycle, temperature of (22±2) ℃ and were allowed free access to standard laboratory diet including green vegetables and tap water until experimentation. Each animal was used only once. All studies were carried out during the light cycle and were approved by the Institutional Animal Ethical Committee.

2.2. Materials

All materials were obtained from the following manufacturers: Monoclonal-anti-rabbit-immunoglobulins-horseradish peroxidase (HRP) conjugate and monoclonal-anti-rabbit-IgG-HRP conjugate from Sigma (USA), anti-rabbit-IgM-HRP conjugate from G Biosciences from Maryland heights (USA), tetramethyl benzidine (TMB) and TMB diluent from J. Mitra and Co. (India), Polystyrene MaxiSorp microtitre flat and round bottom ELISA plates from NUNC (Denmark), Glutaraldehyde solution from Central Drug House (India), Skim milk from Nestle India Ltd. (New Delhi), and DMSO was obtained from Qualigen, Glaxo, India. All chemicals were of analytical grade.

2.3. Drugs

Following drugs were used: histamine dihydrochloride from Himedia laboratories Pvt Limited, India; H1R-antagonist: Avil® (pheniramine maleate) in injection I.P. by Unimark Remedies, India and H2R-antagonist: Rantac® (ranitidine hydrochloride) in injection I.P. by J. B. Chemicals and Pharmaceuticals, India; H3R-antagonist (iodophenpropit dihydrobromide) was kindly donated by Tocris Bioscience, Tocris Cookson Ltd., United Kingdom; and H4R-antagonist (JNJ 7777120) from Sigma (USA).

2.4. Doses

Histamine (100 μg/kg× b.i.d.) was administered through subcutaneous (s.c.), and pheniramine maleate (10 mg/kg× b.i.d.), ranitidine hydrochloride (10 mg/kg ×b.i.d.), iodophenpropit dihydrobromide (1 μg/kg× b.i.d.), JNJ 7777120 (10 μg/kg×b.i.d.) and DMSO (1 mL/kg × b.i.d.) were administered through intramuscular (i.m.); starting from three days prior to immunization until 7 days after immunization. All doses were referred to the weight of the salts used.

2.5. Antigen

Sheep blood diluted 1:1 in sterile Alsevier's solution was obtained from Department of Microbiology, J. N. Medical College & Hospital, A.M.U., Aligarh, and washed with PBS (10 mM sodium phosphate buffer containing 150 mM NaCl, pH-7.4) thrice by centrifugation. The cell suspensions were adjusted to the desired concentration in terms of hemoglobin, lysis of a 1% sheep red blood cells (SRBC) suspension (2×10^8 cells/mL) with 14 volumes of 0.1% Na_2CO_3 develops an optical density of 0.135 at 541 nm in a spectrophotometer (Systronics, UV visible double beam spectrophotometer-2101, India), as described Franzl[10]. Finally the concentration was adjusted to 5% (1×10^9 cells/mL) in PBS for immunization before use.

2.6. Immunization of rabbits

The rabbits in groups II-VIII were immunized intravenously via marginal ear vein with 1 mL of 5% (1×10^9 cells/mL) SRBC in PBS.

2.7. Sample collection

To determine the systemic antibody response, blood samples were collected from rabbits through the marginal ear veins into labelled sterile bottles prior to immunization (day 0), as well as on days 7, 14, 21, 28 and 58 post-immunization. Blood samples were kept at room temperature for 2 hr and then at 4 ℃ overnight. Blood samples were centrifuged for 10 minutes at 580 × g, and serum was isolated and heated at 56 ℃ for 30 minutes to inactivate complement proteins and stored in aliquots containing sodium azide as preservative at -20 ℃ till tested further[11].

2.8. Serological analysis

2.8.1. ELISA using whole SRBC

The whole SRBC-ELISA[7,8,12,13] was used to determine anti-SRBC-Igs, anti-SRBC-IgM and anti-SRBC-IgG response. In brief, polystryrene MaxiSorp immunoplates were coated with SRBC suspension (5×10^6/100 μL PBS). The plates were held overnight at 4 ℃. Each sample was coated in duplicate

and half of the plates served as controls devoid of antigen coating. Without disturbing the cell layer, 20 μL of 1.8% glutaraldehyde solution was then gently added to plates inoculated with SRBC and the plates were held at 25 ℃ for 30 minutes. Unbound SRBC was washed four times with 200 μL of PBS and non-specific binding sites were blocked with 1% fat-free milk in PBS for 120 minutes at 37 ℃. After incubation, the plates were washed four times with 200 μL of PBS. Each rabbit serum diluted 1:100 in PBS (100 μL/well) was adsorbed for 90 minutes at 37 ℃, and then overnight at 4 ℃ followed by washing as earlier. The secondary antibody, HRP conjugated monoclonal-anti-rabbit-immunoglobulins, monoclonal-anti-rabbit-IgM and monoclonal-anti-rabbit-IgG was then added (100 μL/well) in respective plates and incubated at 37 ℃ for 60 minutes. The washing stage was repeated as before and 100 μL/well TMB substrate was added and the plates were incubated at 25 ℃ for 60 minutes. The enzymatic reaction was stopped by adding 50 μL/well of 5% H_2SO_4. The absorbance (A) was determined at 405 nm on an automatic ELISA plate reader (Micro scan MS5608A, ECIL, India). Each rabbit serum sample was run in duplicate. The control wells were treated similarly but were devoid of antigen. Results were expressed as a mean of $A_{test-control}$.

2.9. Statistical analysis

Data were summarized as Mean±SD. Groups were compared by using repeated measures (subjects within groups) two way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test. A two-tailed ($\alpha = 2$) probability $P<0.05$ was considered to be statistically significant. Analyses were performed on SPSS for windows (version 15.0, Inc., Chicago, IL).

3. Results

To evaluate the effects of histamine receptors-antagonist on the immunomodulation, antibody-mediated responses to SRBC were assessed. Total serum immunoglobulins (Igs), total immunoglobulin-M (IgM) and total immunoglobulin-G (IgG) generation profiles were studied *in vivo* in eight experimental groups at days 0 (pre-immunization, pre-I), 7, 14, 21, 28 and 58 (post-immunization, post-I).

3.1. Profile of total anti-SRBC-immunoglobulins (Igs) production

The profile of total anti-SRBC-Igs titer was studied by whole SRBC-ELISA method[7,8,12,13] (Figure 1). No anti-SRBC-Igs response was detected in all experimental groups (negative control, positive control and drug treated) at day 0 (pre-I). There was an initial increase and subsequent decrease in total serum Igs titer over a time span of 58 days in all the groups. The detailed summary of statistically analyzed Igs production by two way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test is shown in Figure 1. By day 7 post-I, anti-SRBC-Igs titer was significantly high, however it obtained a peak at day 14, and by days 21, 28 and 58 post-I. There was a gradual decrease or a plateau in all experimental groups as compared to histamine-treated and H3R-antagonist-treated groups (Figure 1). More extensive evaluation revealed that anti-SRBC-Igs raised steeply up to 7 days post-I, and there was a decrease in histamine-treated and H3R-antagonist-treated groups as compared to all other experimental groups.

Histamine-treated group showed initial suppression ($P< 0.01$) (as opposed to H3R-antagonist-treated group) and enhancement ($P< 0.01$) (as compared to H1R-,H2R- and H4R-antagonists-treated, positive control and DMSO-treated groups) of anti-SRBC-Igs generation titer at day 7 post-I, and later suppression was observed in the anti-SRBC-Igs production titer during the whole study as compared to all other experimental groups at days 14, 21, 28 & 58 post-I except H1R-antagonist-treated group (histamine showed enhanced Igs generation titer at days 28 and 58 post-I).

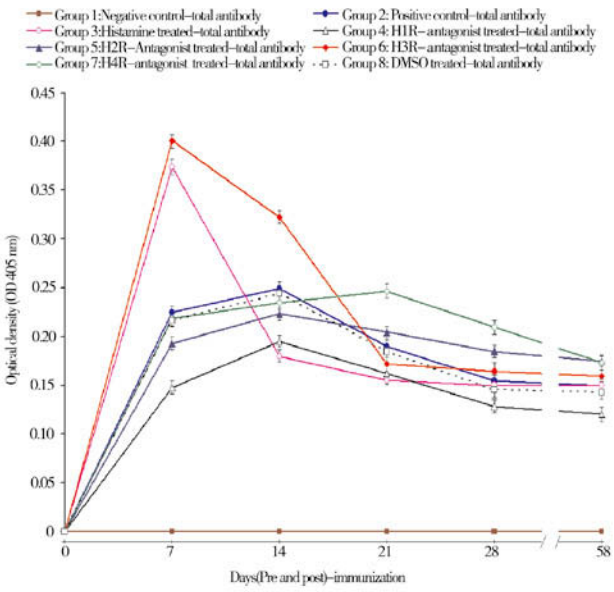

Figure 1. SRBC-specific immunoglobulins (Igs) production titers in H1R-, H2R-, H3R- & H4R-antagonist-treated rabbits by whole SRBC-ELISA method in duplicate 1:100 diluted sera.

The results demonstrate Mean±SD of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments (F=3 608.816, DF=7 136; $P<0.01$) and days (F=72 124.468, DF=5 680; $P<0.01$) on SRBC were statistically significant. The interaction (treatment×days) effect of (F=3 896.230, DF=35 680; $P<0.01$) these on SRBC were also found to be significant.

H3R-antagonist-treated group showed significant ($P< 0.01$) enhancement of total anti-SRBC-Igs at days 7- and 14 post-I as compared to all other experimental groups, while it showed significant ($P< 0.01$) suppression as compared to H2R-antagonist-and H4R-antagonist-treated groups at days 21, 28 & 58 post-I (whereas significant suppression was noticed as compared to positive control & DMSO-treated at day 21 post-I). This group further demonstrated significant ($P< 0.01$) enhancement as opposed to H1R-antagonist-treated and histamine-treated at days 21, 28 & 58 post-I, and also positive control & DMSO-treated groups at days 28 and 58 post-I. The anti-SRBC-Igs generation titer in H4R-antagonist-treated group showed significant suppression ($P< 0.01$) as compared to H3R-antagonist- & histamine-treated groups; however it showed similarity to DMSO-treated and positive control group at day 7 post-I. Furthermore, this group showed significant ($P< 0.01$) enhancement as opposed to H2R-antagonist- and H1R-antagonist-treated groups at days 7 post-I. At day 14 post-I, H4R-antagonist showed further suppression of anti-SRBC-Igs generation titer as opposed to H3R-antagonist-treated, positive control and DMSO-treated; while it demonstrated enhanced Igs titer as compared to H1R-antagonist-, H2R-antagonist- and histamine-treated groups. Furthermore, this group

showed enhancement of anti-SRBC-Igs generation titer as opposed to all other experimental groups at days 21, 28 and 58 post-I. H4R-antagonist- and H2R-antagonist-treated groups demonstrated similar profile of anti-SRBC-Igs generation titer; however H4R-antagonist-treated group showed enhanced profile as opposed to H2R-antagonist-treated group (Kindly refer to Figure 1 for statistical analysis). Moreover, H1R-antagonist-treated rabbits showed suppression of anti-SRBC-Igs level at days 7, 14, 21, 28 and 58 post-I in comparison to all other experimental groups ($P< 0.01$) except histamine-treated group (whereas this group demonstrated enhancement of total serum anti-SRBC-Igs levels at days 14 and 21 post-I which were found statistically significant). H1R-antagonist-treated rabbits showed significant ($P< 0.01$) suppression of total anti-SRBC-Igs as opposed to that of H2R-,H3R-&H4R-antagonist-treated groups and positive control rabbits (Figure 1).

3.2. Profile of anti-SRBC-immunoglobulin-M (IgM) production

Anti-SRBC-IgM was determined by whole SRBC-ELISA method [7,8,12,13] (Figure 2). No anti-SRBC-IgM response was observed in all experimental groups (negative control, positive control and HRs-antagonist-treated) at day 0-pre-I; however there was an initial increase and then gradual decrease in serum-IgM titer over time in all the groups. The detailed summary of statistically analyzed IgM production by two way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test is shown in Figure 2. By day 7 post-I, the IgM titer increased and obtained highest peak, but by days 14, 21, 28, and 58 post-I there was a gradual decrease in all experimental groups (except H1R-antagonist-treated, H2R-antagonist-treated and H3R-antagonist-treated groups, where the highest peak was obtained at day 14 post-I and then there was a gradual decrease).

Histamine-treated group showed initial suppression (as opposed to H3R-antagonist-treated group) and enhancement (as compared to H1R-,H2R-&H4R-antagonist-treated, positive control and DMSO-treated groups) of anti-SRBC-IgM generation titer at day 7 post-I, and later suppression was noticed in the anti-SRBC-IgM production titer during the whole study as compared to all other experimental groups at days 14, 21, 28 & 58 post-I except H1R-antagonist- and DMSO-treated group (histamine showed significant enhanced IgM generation titer at days 28 and 58 post-I as compared to H1R-antagonist-treated group, while histamine showed similar IgM production level to DMSO-treated group at days 28 and 58 post-I).

H3R-antagonist-treated group showed significant ($P< 0.01$) enhancement of total anti-SRBC-IgM at days 7 post-I as compared to all the experimental groups, while it showed significant ($P<0.01$) suppression as compared to H4R-antagonist-treated groups at days 14, 21, 28 & 58 post-I and also showed significant ($P<0.01$) suppression as compared to H2R-antagonist-treated group at days 14, 21 & 28 post-I, whereas H3R-antagonist group showed suppression as compared to positive control group at day 21 post-I. This group further demonstrated significant ($P<0.01$) enhancement as opposed to H1R-antagonist-treated and histamine-treated at days 14, 21, 28 & 58 post-I, and also positive control & DMSO-treated groups at days 21, 28 & 58 post-I. The anti-SRBC-IgM generation titer in H4R-antagonist-treated group showed suppression as compared to positive control & DMSO-treated and also significant suppression ($P<0.01$)as compared to H3R-antagonist- & histamine-treated groups. Furthermore, this group showed significant ($P<0.01$)) enhancement as opposed to H1R-and H2R-antagonist-treated groups at days 7 post-I. Moreover at day 14, 21, 28 & 58 post-I, H4R-antagonist further showed significant ($P<0.01$)enhancement of anti-SRBC-IgM generation titer as opposed to all other experimental groups (Figure 2). H4R-antagonist- and H2R-antagonist-treated groups demonstrated similar profile of anti-SRBC-IgM generation titer; however H4R-antagonist-treated group showed enhanced profile as opposed to H2R-antagonist-treated group.

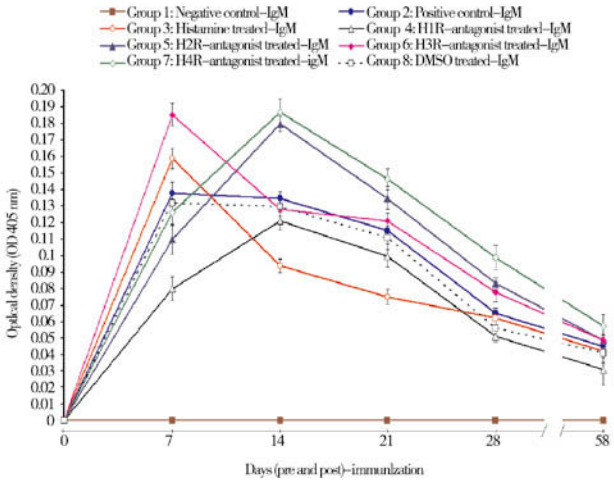

Figure 2. SRBC-specific immunoglobulin-M (IgM) production titers in H1R-, H2R-, H3R- & H4R-antagonist-treated rabbits by whole SRBC-ELISA method in duplicate 1:100 diluted sera.
The results demonstrate Mean±SD of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments (F=2 167.238, DF=7 136; $P<0.01$) and days (F=16405.067, DF=5680; $P<0.01$) on SRBC were statistically significant. The interaction (treatment×days) effect of (F=642.525, DF=35680; $P<0.01$) these on SRBC were also found to be significant.

Moreover, H1R-antagonist-treated rabbits showed suppression of anti-SRBC-IgM level at days 7, 14, 21, 28 and 58 post-I in comparison to all other experimental groups except histamine-treated group (this group demonstrated enhancement of total serum anti-SRBC-IgM level at days 14 and 21 post-I which were found statistically significant). H1R-antagonist-treated rabbits showed significant ($P<0.01$) suppression of total anti-SRBC-IgM as opposed to that of H2R-,H3R-&H4R-antagonist-treated and positive control rabbits (Figure 2).

3.3. Profile of anti-SRBC-immunoglobulin-G (IgG) production

The profile of total anti-SRBC-IgG titer was also studied by whole SRBC-ELISA method[7,8,12,13] (Figure 3). The detailed summary of statistically analyzed IgG production by two way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test is shown in Figure 3. More extensive evaluation revealed that anti-SRBC-IgG in histamine-treated groups raised steeply up to 7 days post-I as compared to positive control, DMSO-treated and H1R-,H2R-&H4R-antagonist groups, but showed suppression as opposed to H3R-antagonist-treated rabbits. By days 14, 21, 28 and 58

post-I there was a significant(P<0.01) decrease in anti-SRBC-IgG levels in histamine-treated as compared to all other experimental groups except H1R-antagonist-treated (whereas it showed enhancement of IgG as opposed to H1R-antagonist-treated at day 14 post-I) and this was found statistically significant (Figure 3). H3R-antagonist-treated group showed significant(P<0.01) enhancement of total anti-SRBC-IgG at days 7 post-I as compared to all other experimental groups, while it showed significant(P<0.01) suppression as compared to H4R-and H2R-antagonist-treated groups, positive control and DMSO-treated groups and also showed significant (P<0.01) enhancement as compared to H1R-antagonist- & histamine-treated groups at days 14 post-I. This group further demonstrated enhancement as opposed to all other experimental groups except H4R-antagonist-treated group (whereas it showed suppressed IgG level) at days 21, 28 & 58 post-I). The anti-SRBC-IgG generation titer in H4R-antagonist-treated group showed significant suppression as compared to histamine- & H3R-antagonist-treated groups. However, it showed similar IgG production level near to positive control & DMSO-treated and also H1R-and H2R-antagonist-treated groups at day 7 post-I. H4R-antagonist-treated group further demonstrated similar IgG generation titer near to positive control & DMSO-treated, while it showed significant (P<0.01) enhancement as compared to histamine- & H1R/H3R-antagonist-treated groups at day 14 post-I. Moreover at day 21, 28 & 58 post-I, H4R-antagonist further showed significant (P<0.01) enhancement of anti-SRBC-IgG generation titer as opposed to all other experimental groups (Figure 3).

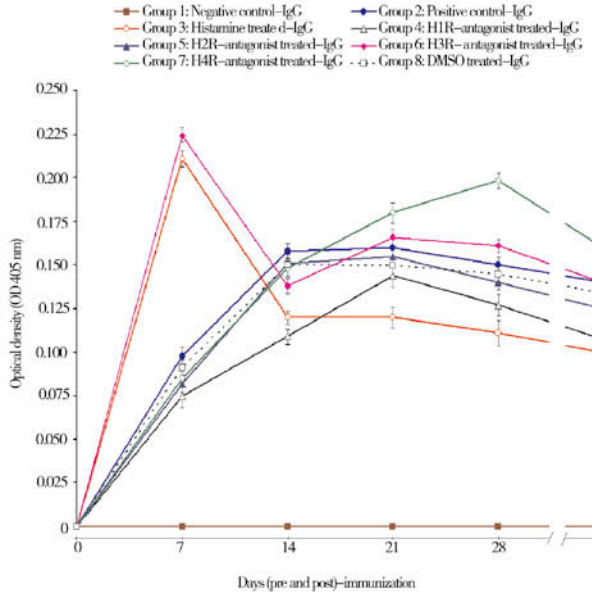

Figure 3. SRBC-specific immunoglobulin-G (IgG) production titers in H1R-, H2R-, H3R- & H4R-antagonist-treated rabbits by whole SRBC-ELISA method in duplicate 1:100 diluted sera.

The results demonstrate Mean±SD of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments (F=2 425.731, DF=7 136; P<0.01) and days (F=8 166.595, DF=5 680; P<0.01) on SRBC were statistically significant. The interaction (treatment×days) effect of (F=507.794, DF=35 680; P<0.01) these on SRBC were also found to be significant.

H2R-antagonist-treated and H4R-antagonist-treated rabbits showed similar anti-SRBC-IgG generation titer at days 7 & 14 post-I, while on further analysis, H2R-antagonist-treated group showed significant enhancement as compared to histamine-treated & H1R-antagonist-treated group and also demonstrated significant suppression as compared to H4R-antagonist-, H3R-antagonist-treated & positive control groups at days 21, 28 & 58 post-I (Figure 3).

H1R-antagonist-treated rabbits showed significant (P<0.01) suppression of anti-SRBC-IgG level at days 7, 14, 21, 28 and 58 post-I in comparison to all other experimental groups except histamine-treated group and demonstrated enhancement of total serum anti-SRBC-IgG level at days 21, 28 & 58 post-I (Figure 3).

4. Discussion

Histamine receptors are distributed in all parts of body and modulate several physiological and pathological reactions both *in vivo* and *in vitro* by the activation on releasing the histamine[1] and it has also been well documented that histamine shows the agonist properties of their receptors (H1R, H2R, H3R & H4R)[1,2,9]. Besides, histamine also modulates immunological reactions and directly affects B-cell antibody production as a co-stimulatory receptor on B-cells[3,6-8,14,15]. It is also documented in mice that histamine enhances anti-IgM induced proliferation of B-cells, which abolished in H1R deleted mice. In H1R-deleted mice antibody production against a T-cell independent antigen-TNP-Ficoll is decreased[16], suggesting an important role of H1R signaling in response triggered from B-cell receptors. A different pattern of antibody responses to T-cell dependent antigens like ovalbumin showed that H1R-deleted mice produced high ovalbumin-specific IgG1 and IgE in comparison to wild type mice[9].

Keeping in view the above facts, especially the paucity of literature (i.e., immunomodulatory role of histamine H1-, H2-, H3- and H4-receptors), defining the correlation of histamine receptors-antagonists in immune regulation & modulation and the fragmentary literature of histamine receptors (H1R, H2R, H3R and H4R) describing existing immunomodulatory role of histamine and histamine receptors in vivo system, the present study was proposed.

Therefore, the comparative study explored total serum anti-SRBC-Igs, IgM and IgG generation profile against SRBC (a T-cell-dependent test antigen)[13,17] in negative control (untreated) and treated groups (positive control, H1R-antagonist, H2R-antagonist, H3R-antagonist, H4R-antagonist, histamine, and DMSO-treated-experimental groups) in healthy albino rabbits. The study reveals that H1R-, H2R-, H3R- & H4R-antagonists-treated rabbits were characterized by a marked deviation of the immune response as compared to control rabbits.

Our recent studies demonstrated that the histamine released from immunological stimuli from effector cells *in vivo*[1], could influence a detectable antibody response to SRBC[7,8]. These studies showed that the histamine treated-rabbits presented immunopotentiating properties by enhancing the anti-SRBC-antibodies levels as compared to

positive control group.

The comparative study, with the presence of H1-, H2-, H3- & H4-antagonist in the body, *in vivo* immunoregulatory processes demonstrated enhanced generation profile of anti-SRBC-antibody (total-anti-SRBC-Igs, IgM and IgG) in H3R-antagonist-treated rabbits as compared to positive control rabbits, while H4R-antagonist, H2R-antagonist- and H1R-antagonist-treated rabbits revealed suppressed generation profile of total-anti-SRBC-Igs, IgM and IgG as compared to positive control rabbits. Whereas, study on further metabolism of antagonists in the body showed enhanced anti-SRBC-Igs, IgM and IgG generation profile in H4R-, H3R- & H2R-antagonist-treated rabbits (H4R- showed more marked immune modulation as compared to H3R- & H2R-) as compared to positive control rabbits. H1R-antagonist-treated rabbits showed suppressed antibody generation profile overall the study period as compared to other experimental groups. Furthermore, the effects of histamine & H1R-H4R antagonist disappeared on the antibody generation profile bringing them in the range of control antibody level at the final phase of study due to complete metabolism.

To conclude, our results provide evidence that histamine receptors (H1R, H2R, H3R & H4R) on antagonized have important roles in modulation of antibody generation, in which H1R has a dominant suppressive role as compared to H2R and H4R (H2R revealed more marked suppressive capability as compared to H4R). Conversely, H3R has a dominant enhancing role, this receptor thus has a regulatory role for histamine receptors (H1, H2 and H4) and an auto-regulatory role for itself in immuno-biological system. To the best of our knowledge, this is the first report describing the potential comparative immunomodulatory and immunoregulatory roles of H1-, H2-, H3- and H4-receptors.

Conflict of interest statement

We declare that we have no conflict of interest.

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