

***In vivo* Study of Histamine H3-Receptor in Immunomodulation**

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Abstract: Histamine via its receptors (H1, H2 and H4) modulates several immunopathological reactions. However, H3-receptor's role is still unclear. Present study was designed to delineate the immunomodulatory role of histamine by H3-receptor on antibody generation profile in rabbit model. The cohort comprised of three groups (III, IV and V), containing 18 rabbits each and received subcutaneous histamine ($100\mu\text{gkg}^{-1}\times\text{b.i.d.}$), H3-agonist (R-[-]- α -methylhistamine dihydrobromide, $10\mu\text{gkg}^{-1}\times\text{b.i.d.}$) and intramuscular H3-antagonist (iodophenpropit dihydrobromide, $1\mu\text{gkg}^{-1}\times\text{b.i.d.}$), respectively for 10 days (starting from day 1). They were subsequently immunized with intravenous injection of SRBC at day 3. Group-II positive-control (n=18) received vehicle (sterile distilled water, $1\text{mlkg}^{-1}\times\text{b.i.d.}$) intramuscularly and immunized in a similar manner, while group-I negative-control (n=18) remained non-immunized and received only vehicle ($1\text{mlkg}^{-1}\times\text{b.i.d.}$). The estimation of serum immunoglobulins (Ig), IgM and IgG were done by ELISA and Hemagglutination Assay (HA) and observed at day 0 (pre-immunization) and day 7, 14, 21, 28 and 58 (post-immunization). The ELISA and HA both showed similar production of Ig, IgM and IgG but the results were found comparatively more significant by ELISA as opposed to HA. Histamine could influence a detectable antibody response to SRBC as early as day 7-post-I, which lasted until day 58-post-I. Moreover, H3R-antagonist showed modulatory activity by enhancing the antibody generation levels. In contrast, H3R-agonist showed modulatory activity by suppressing the antibody generation levels. The results were statistically significant ($P < 0.05$). Histamine through H3-receptors modulates antibody generation. The findings of this study may have clinical significance and provide the baseline information in future.

Key words: Histamine receptors • Immunomodulation • T-cell dependent antibody response • Humoral immune response • H3-antagonists • H3-agonists

INTRODUCTION

Histamine is considered a mediator of chronic inflammation and hypersensitivity and also a regulator of several essential events in the immune response. Histamine regulates dendritic cells, T-lymphocytes, B-

lymphocytes, as well as related antibody isotype responses. Recently, accumulating evidence has highlighted the importance of histamine receptors in immunomodulation [1-6]. Immunosuppressive and immunomodulatory effects on both humoral-and cell-mediated immune responses have been observed [7, 8].

Table 1: Comparative activities of H3R and H4R ligands at the human histamine receptors (hH3R and hH4R), for detailed study kindly see Lim *et al.* [14]

Ligands	hH3R			hH4R		
	pKi at H3R	pEC ₅₀	α	pKi at H4R	pEC ₅₀	α
Histamine	8.0±0.1	8.3±0.1	1	7.8±0.1	7.7±0.1	1
(R)- α -Methylhistamine**	8.2±0.1	9.5±0.1	1	6.6±0.1	6.2±0.1	1
Immepip	9.3±0.1	10.4±0.1	1	7.7±0.1	7.8±0.1	0.9
Imetit	8.8±0.1	9.9±0.1	1	8.2±0.1	7.9±0.1	0.9
Burimamide	7.9±0.1	7.0±0.1	0.5	7.4±0.1	7.7±0.1	0.7
Clobenpropit	8.6±0.1	9.4±0.1	-1	8.1±0.1	7.7±0.1	0.8
Idophenpropit	8.2±0.1	8.5±0.1	-1	7.9±0.1	-	0
Impromidine	6.8±0.1	6.5±0.3	-0.5	7.6±0.1	7.6±0.1	0.5
Thioperamide	7.3±0.1	7.5±0.1	-1	6.9±0.1	7.0±0.1	-1
Proxyfan	7.9±0.1	8.5±0.1	1	7.3±0.1	7.2±0.1	0.5
Iodoproxyfan	9.2±0.1	10.3±0.1	1	7.9±0.1	7.9±0.1	0.7
JNJ 777120	5.3±0.1	6.0±0.1	-0.7	7.8±0.1	-	0
4-Methylhistamine	-	-	-	7.3±0.1	7.4±0.1	1

α = intrinsic activity (1 designated for full agonistic, 0 for neutral antagonist and -1 for full inverse agonistic activity).

pKi values for hH3R were determined with [³H]histamine displacement assay [14].

pKi values for hH4R were determined with [³H]*N* α -methylhistamine displacement assay [14].

"*" = warranting further investigation.

pEC₅₀ = values show the inhibition of 1 μ M forskolin-induced CRE- β -galactosidase activity in SK-N-MC/hH4 cells [14].

** (R)- α -Methylhistamine exhibits agonist properties for H3R and also for H4R.

Histamine receptors (H1R and H2R) have been shown to enhance delayed hypersensitivity and antibody mediated immune responses in many pathological processes regulating several essential events in allergies and autoimmune diseases in experimental animals, especially in knock out mice (either H1 or H2-deficient) [3,7,9]. Our previous study on H1R and H2R demonstrated B-cell proliferation in response to anti-IgM is increased in H2R-antagonist treated rabbits and is diminished in H1R-antagonist treated rabbits. H1R-antagonist treated rabbits display diminished antibody production against a T cell-dependent antigen-SRBC as compared with H2R-antagonist treated and control rabbits [10].

However, studies evaluating the role with intact receptors, but blocked by respective agonists/antagonists and especially studying the immunomodulatory profile over a span of time are lacking in the existing literature. Moreover, the studies in rabbit model are elementary and the existing studies have demonstrated immunomodulatory role studying only single blood samples taken after immunizing the animals (except our earlier report on immunomodulatory profile [5,6,10-12]. Histamine shows agonist property and has different pKi values of its receptors as 4.2±0.1 for H1R, 4.3±0.1 for H2R, 7.8±0.1 for H3R and 8.1±0.1 for H4R (12, Table 1). HRs

belong to the G-protein-coupled receptor (GPCR) family: Gq/11 for H1R, G α s for H2R, Gi/o for H3R and H4R [4] however; they trigger different biochemical intracellular events upon activation [4, 13]. Jutel *et al.* [2] demonstrated that the H1R-specific antagonist tripeleminamine inhibits histamine binding in Th1 but not in Th2 cells which correlates to predominant H1R expression on Th1 cells. Neither H2R-antagonist (ranitidine) nor H3R-antagonist/H4R-partial agonist (clobenpropit) (Table 1) had any impact on histamine binding to Th1 cells. Rather, H1Rs and H2Rs are regulated by specific cytokines present in the immune system. Their study demonstrated the expression of H1R on Th1 cells and H2R on Th2 cells by antibodies generated against the H1R and H2R [2]. The distribution and role of H1R, H2R and H4R in several immunological cells are well described [4]. However, the role of histamine H3 receptor in immune modulation and regulation is still elementary.

Keeping in view the above facts, especially the paucity of literature (i.e., immunomodulatory role of histamine H3 receptors, defining the co-relation of histamine H3-receptor-agonist/-antagonist in immune regulation and fragmentary histamine literature describing existing immunomodulatory role of histamine *in vivo* system, the present study was designed.

MATERIALS AND METHODS

Experimental Design: To evaluate the systemic antibody response, 90 (45 Male and 45 Female) New Zealand adult healthy rabbits of either sex weighing 1-1.5 kg were randomized equally into five treatment groups, i.e. 18 rabbits (9 male and 9 female) in each group. Each group was immunized with sheep red blood cells (SRBC).

Control Group: Group I (negative control) remained non-immunized and received only vehicle (sterile distilled water, $1 \text{ ml kg}^{-1} \times \text{b.i.d.}$). Group II was vehicle (sterile distilled water, $1 \text{ ml kg}^{-1} \times \text{b.i.d.}$) treated and immunized as a positive control.

Experimental Group: Group III histamine-treated and immunized, group IV H3R-agonist-treated and immunized and group V H3R-antagonist-treated and immunized.

The animals were housed in well-maintained animal facility at Central Animal House, J. N. Medical College and Hospital, Aligarh Muslim University, Aligarh, in the Bioresources unit under a 12 hr light/dark cycle, temperature ($22 \pm 2^\circ\text{C}$) 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.

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), 2-mercaptoethanol (2-ME) from Merck KGaA, Darmstadt (Germany). All chemicals were of analytical grade.

Drugs: In the present study following drugs were used: histamine dihydrochloride by Himedia laboratories Pvt Limited, India; H3R-agonist (R-(-)- α -methylhistamine dihydrobromide) and H3R-antagonist (iodophenpropit dihydrobromide) kindly donated by Tocris Bioscience, Tocris Cookson Ltd., United Kingdom.

Doses: Histamine ($100 \text{ } \mu\text{gkg}$) and R-(-)- α -methylhistamine dihydrobromide ($10 \text{ } \mu\text{gkg}$) were administered twice in a day through subcutaneous (s.c.) route and iodophenpropit dihydrobromide ($1 \text{ } \mu\text{gkg}$) was administered twice in a day through intramuscular (i.m.) route; starting from three days prior to immunization until 7 days after immunization. All doses referred to the weight of the salts used.

Lim *et al.* [14] have demonstrated comparative activities of HRs agonists / antagonists at the human histamine receptors (hHRs) (Table 1). Moreover, for *in vivo* histaminergic study, rabbit is an ideal animal model and expressed all histamine receptor subtypes [15-22], albeit do not address the selectivity of HRs compounds at the rabbit receptors. Thus, on the basis of pK_i and pEC_{50} values of drugs (Table 1), we have used the above doses of H3R-agonist / antagonist in present *in vivo* study.

Antigen: Sheep blood diluted 1:1 in sterile Alsevier's solution was obtained from Department of Microbiology, J. N. Medical College and 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% 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 [23]. Finally the concentration was adjusted to 5% (1×10^9 cells/ml) in PBS for immunization before use.

Immunization of Rabbits: The rabbits in all experimental groups (II-V) were immunized intravenously via marginal ear vein with 1ml of 5% (1×10^9 cells/ml) sheep red blood cells (SRBC) in PBS.

Sample Collection: To determine the systemic antibody response, blood samples were collected from rabbits through the marginal ear veins into labeled sterile bottles prior to immunization (day 0), as well as on days 7-, 14-, 21-, 28-and 58-post-immunization (post-I). Blood samples were kept at room temperature for 2 hr and then at 4°C overnight. Blood samples were centrifuged for 10 minutes at $580 \times g$ and serum was separated and heated at 56°C for 30 minutes to inactivate complement proteins [24] and stored in aliquots containing sodium azide as preservative at -20°C till tested further.

Serological Analysis

Hemagglutination Assay (HA): To determine the antibody response to SRBC, a direct hemagglutination technique [10,11,25] was used with some modifications in our laboratory. Briefly, 100 μ L of PBS was dispensed into each well of a round bottomed 96-well microplate. Serum samples (100 μ L) were then added using serial two-fold dilutions (1:2, 1:4, 1:8, 1:16, 1:32...) in the wells from columns 2 to 12. The first column (PBS only) of wells was considered blank. Then, 100 μ L of 2% sheep red blood cells (SRBC) in PBS was added to all wells to make a final volume of 200 μ L. Subsequently, the plates were shaken for 1 minutes and incubated at 37°C for 1 hr and then overnight at 4°C to determine agglutination titres. A positive result was recorded when at least 50% SRBC agglutination was observed. To measure anti-SRBC-immunoglobulin-M (IgM) and immunoglobulin-G (IgG), serum sample were treated with 0.2M 2-mercaptoethanol (2-ME) for 1 hr at 37°C. This treatment inactivates IgM antibody and as a result, hemagglutination observed after treatment with 2-ME is due mostly to the presence of IgG antibody. The difference between total antibodies and IgG antibody titres were taken as the titres of IgM antibody.

ELISA using whole SRBC Method: To determine the SRBC-specific-immunoglobulins (Ig), SRBC-specific-IgM and SRBC-specific-IgG response, the whole SRBC-enzyme linked immunosorbent assay (ELISA) [5,6,10-12,26] was carried out on polystyrene plates with some modifications. Briefly, polystyrene MaxiSorp immunoplates were coated with SRBC suspension (5×10^6 cells/100 μ L PBS [10 mM sodium phosphate buffer containing 150 mM NaCl, pH-7.4]). The plates were held overnight at 4°C. Each sample was coated in duplicate and half of the plates served as control devoid of antigen coating. Without disturbing the cell layer, 20 μ L of 1.8% glutaraldehyde solution was then gently added to plates inoculated with SRBC and the plates were held at 25°C for 30 min. Unbound SRBC were washed four times with 200 μ L of PBS and non-specific binding sites were blocked with 1% fat-free milk in PBS for 2 hr at 37°C. After incubation, the plates were washed four times with 200 μ L of PBS. Each rabbit serum diluted 1:100 in PBS (100 μ L well⁻¹) was adsorbed for 1.5 hr at 37°C and then overnight at 4°C 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 μ L well⁻¹) in respective plates and incubated at 37°C for 1 hr.

The washing step was repeated as before and 100 μ L well⁻¹ TMB substrate was added and the plates were incubated at 25°C for 1 hr. The enzymatic reaction was stopped by adding 50 μ Lwell⁻¹ of 5% H₂SO₄. 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 $A_{\text{test-control}}$.

Statistical Analysis: Data were summarized as Mean \pm 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 12.0, Inc., Chicago, IL).

RESULTS

To evaluate the effects of histamine H3-receptors-agonist and-antagonist on the immunomodulation, antibody-mediated responses to SRBC were assessed. Total serum immunoglobulins, total immunoglobulin-M (IgM) and total immunoglobulin G (IgG) generation profiles were studied *in vivo* in five experimental groups at days 0 [pre-immunization (pre-I)], 7, 14, 21, 28 and 58 [post-immunization (post-I)].

Profile of total anti-SRBC-immunoglobulins (Ig) production: The profile of total anti-SRBC-Ig titer was studied by whole SRBC-ELISA method [5,6,10-12,26] (Fig. 1) and direct HA [10,11,25] (Table 2). The ELISA and HA assay both showed similar Ig production but the results were found comparatively more significant by ELISA as opposed to HA. No anti-SRBC-Ig 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 serum anti-SRBC-Ig titer over a time span of 58 days in all the groups except unimmunized control group (Table 2 and Fig. 1). The detailed summary of statistically analyzed Ig production by two way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test are shown in Fig. 1 and Table 2. By the day 7-post-I, antibody titers were detected, reached a peak at day 14-post-I and by days 21-, 28-and 58-post-I there was a gradual decrease or

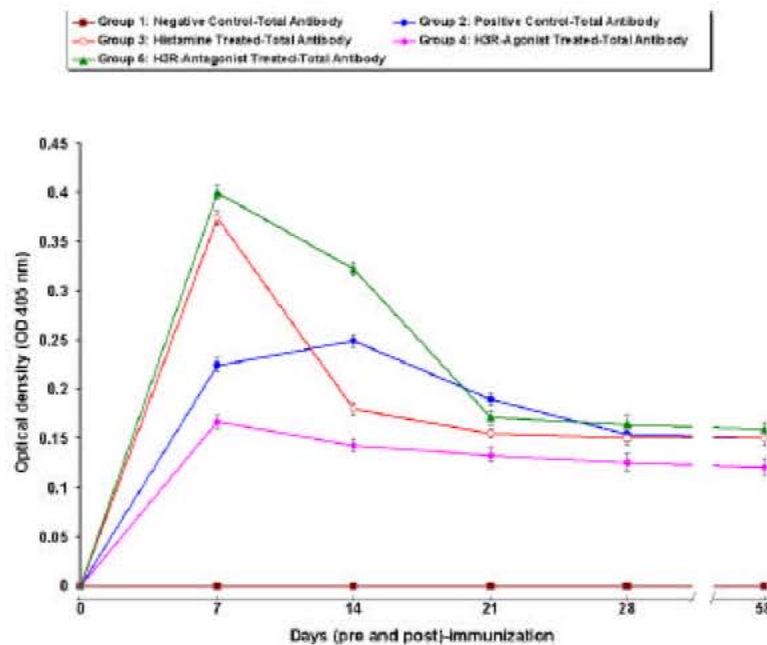


Fig. 1: SRBC-specific Immunoglobulins (Ig) production titers in H3R-agonist/-antagonist-treated rabbits by whole SRBC-ELISA method in duplicate 1:100 diluted sera. The results demonstrate mean \pm s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F=7188.097$, $DF=4,85$; $p<0.01$) and days ($F=33146.730$, $DF=5,425$; $p<0.01$) on SRBC were statistically significant. The interaction (treatments \times days) effect of ($F=4088.888$, $DF=20,425$; $p<0.01$) these on SRBC were also found to be significant

Table 2: Immunomodulatory study of histamine and H3R-agonist/-antagonist-treated rabbits determined by hemagglutination assay

Groups of Experimental Rabbits (n = 18)	Mean antibody titre \pm s.d.*											
	Total Anti-SRBC-Immunoglobulins [†]						Anti-SRBC-Immunoglobulin-M [‡]					
	Pre-I		Post-I				Pre-I		Post-I			
	0 Day	7 th Day	14 th Day	21 st Day	28 th Day	58 th Day	0 Day	7 th Day	14 th Day	21 st Day	28 th Day	58 th Day
Negative Control (Group I)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Positive Control (Group II)	0.00 \pm 0.00	4.67 \pm 0.52	5.00 \pm 0.63	4.00 \pm 0.89	3.50 \pm 0.55	2.33 \pm 0.52	0.00 \pm 0.00	4.13 \pm 0.88	4.00 \pm 0.63	2.33 \pm 1.21	1.50 \pm 0.52	0.00 \pm 0.00
Histamine (Group III)	0.00 \pm 0.00	5.87 \pm 0.63	4.17 \pm 0.41	3.83 \pm 0.41	3.47 \pm 0.52	1.67 \pm 0.51	0.00 \pm 0.00	5.29 \pm 0.14	3.17 \pm 0.41	2.33 \pm 1.19	1.67 \pm 0.63	0.00 \pm 0.00
H3R-agonist (Group IV)	0.00 \pm 0.00	4.33 \pm 0.52	4.17 \pm 0.41	3.5 \pm 0.55	2.83 \pm 0.41	2.33 \pm 0.55	0.00 \pm 0.00	3.86 \pm 0.69	2.56 \pm 0.32	2.26 \pm 1.27	0.33 \pm 0.81	0.00 \pm 0.00
H3R-antagonist (Group V)	0.00 \pm 0.00	6.00 \pm 0.41	5.34 \pm 0.41	4.00 \pm 0.81	3.85 \pm 0.54	2.67 \pm 0.63	0.00 \pm 0.00	5.47 \pm 0.92	4.00 \pm 0.63	3.00 \pm 0.81	1.87 \pm 0.87	0.00 \pm 0.00

Table 2 continued

Groups of Rabbits Experimental (n = 18)	Mean antibody titre \pm s.d.*					
	Anti-SRBC-Immunoglobulin-G ???					
	Pre-I		Post-I			
	0 Day	7 th Day	14 th Day	21 st Day	28 th Day	58 th Day
Negative Control (Group I)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Positive Control (Group II)	0.00 \pm 0.00	2.33 \pm 0.51	4.00 \pm 0.63	3.33 \pm 1.03	3.00 \pm 0.89	2.33 \pm 0.52
Histamine (Group III) H3R-agonist (Group IV)	0.00 \pm 0.00	4.27 \pm 0.17	3.17 \pm 0.41	3.00 \pm 0.63	2.17 \pm 0.87	1.67 \pm 0.51
(R-(-)- α -methyl-histamine) H3R-antagonist (Group V)	0.00 \pm 0.00	2.33 \pm 0.52	2.5 \pm 0.84	2.5 \pm 0.55	2.67 \pm 0.52	2.5 \pm 0.55
H3R-antagonist (Group V) (iodophenpropit)	0.00 \pm 0.00	4.32 \pm 0.52	3.50 \pm 0.52	3.37 \pm 0.82	3.17 \pm 0.41	2.57 \pm 0.63

*Results demonstrate means \pm s.d. of log₁₀ titers of three independent experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F=223.329$, $DF=4,85$; $p<0.01$), days ($F=3862.90$, $DF=5,425$; $p<0.01$) and the interaction (treatments \times days) ($F=284.557$, $DF=20,425$; $p<0.01$) on SRBC were found to be significant; [†]the effect of treatments ($F=81.660$, $DF=4,85$; $p<0.01$), days ($F=1114.784$, $DF=5,425$; $p<0.01$) and the interaction (treatments \times days) ($F=81.555$, $DF=20,425$; $p<0.01$) on SRBC were found to be significant; [‡]the effect of treatments ($F=105.174$, $DF=4,85$; $p<0.01$), days ($F=1142.434$, $DF=5,425$; $p<0.01$) and the interaction (treatments \times days) ($F=127.024$, $DF=20,425$; $p<0.01$) on SRBC were found to be significant.

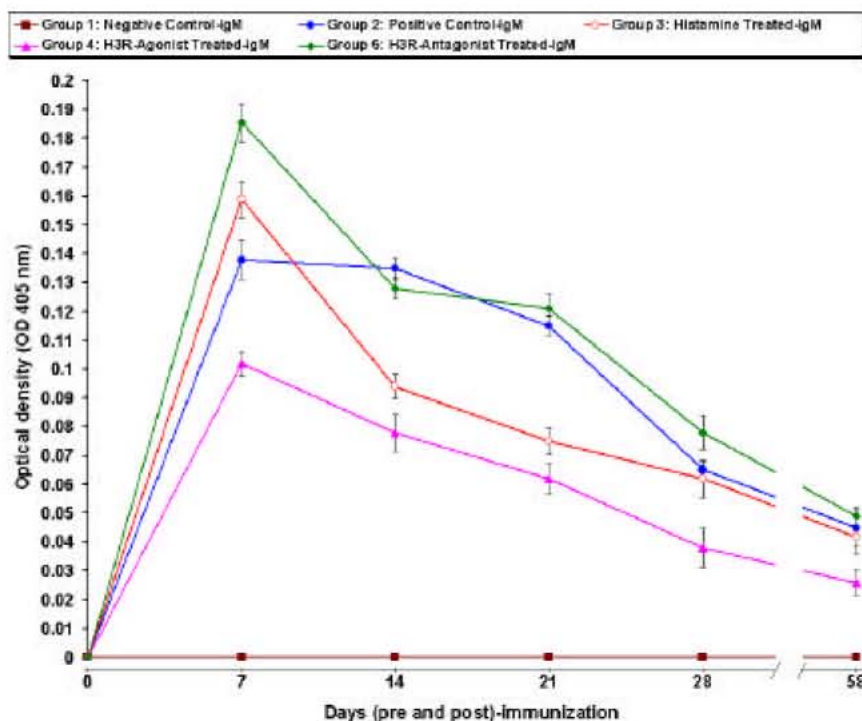


Fig. 2: SRBC-specific Immunoglobulin-M (IgM) production titers in H3R-agonist/-antagonist-treated rabbits by whole SRBC-ELISA method in duplicate 1:100 diluted sera. The results demonstrate mean \pm s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F=3143.902$, $DF=4,85$; $p<0.01$) and days ($F=9652.705$, $DF=5,425$; $p<0.01$) on SRBC were statistically significant. The interaction (treatments \times days) effect of ($F=803.867$, $DF=20,425$; $p<0.01$) these on SRBC were also found to be significant.

a plateau in the positive control group as compared to all drug-treated groups. H3R-agonist (R-[α -methylhistamine dihydrobromide)-treated rabbits showed significant ($p<0.01$) suppression of anti-SRBC-Ig production as opposed to histamine-treated, H3R-antagonist-treated and positive control rabbits over a time span of 58 days. However, histamine and H3R-antagonist studies showed different patterns; histamine showed initial enhancement and later suppression of anti-SRBC-Ig production profile during the whole study as compared to control groups only (significant increase of serum anti-SRBC-Ig level was noticed at day 7-post-I ($p<0.01$) and suppression of serum anti-SRBC-Ig levels at days 14-and 21-post-I ($p<0.01$), while the results were found to be similar to control group at days 28-and 58-post-I. H3R-antagonist (iodophenpropit dihydrobromide)-treated rabbits showed enhancement of anti-SRBC-Ig level at days 7-and 14-post-I in comparison to histamine-treated group (significant ($p<0.01$) at day 7-and 14-post-I) and positive control group (significant ($p<0.01$) at days 7-and 14-post-I), whereas this group demonstrated enhancement

(as compared to histamine-treated) and suppression (as compared to positive control) of serum anti-SRBC-Ig levels at day 21-post-I which were found statistically significant ($p<0.01$). Moreover, anti-SRBC-Ig levels at days 28-and 58-post-I were found to be similar in H3R-antagonist, histamine and positive control groups. No antibody response was noticed in group I (negative control) during whole of the study period (Fig. 1 and Table 2).

Profile of anti-SRBC-immunoglobulin-M (IgM) production: Anti-SRBC-IgM was determined by whole SRBC-ELISA method [5,6,10-12,26] (Fig. 2) and direct HA [10,11,25] (Table 2). No IgM response was observed in all experimental groups at day 0-pre-I, however there was an initial increase and then gradual decrease in serum-IgM titer over time in positive control and drug-treated groups. The detailed summary of statistically analyzed IgM production by two way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test are shown in Fig. 2 and Table 2. By day 7-post-I, the IgM titer increased

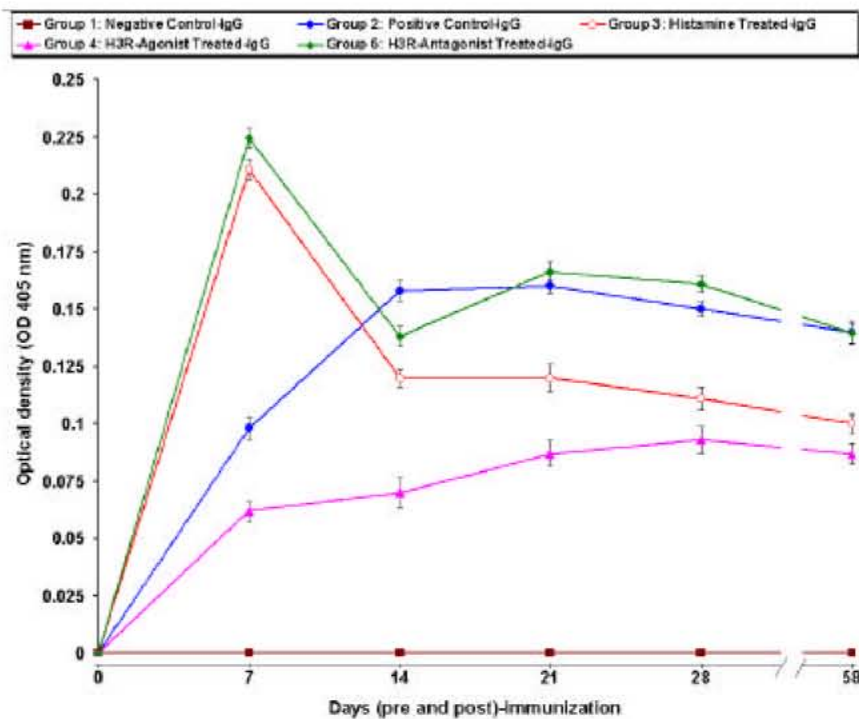


Fig. 3: SRBC-specific Immunoglobulin-G (IgG) production titers in H3R-agonist/-antagonist-treated rabbits by whole SRBC-ELISA method in duplicate 1:100 diluted sera. The results demonstrate mean \pm s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F=3140.182$, $DF=4,85$; $p<0.01$) and days ($F=2831.942$, $DF=5,425$; $p<0.01$) on SRBC were statistically significant. The interaction (treatments \times days) effect of ($F=439.006$, $DF=20,425$; $p<0.01$) these on SRBC were also found to be significant.

and obtained highest peak, but by days 14-, 21-, 28- and 58-post-I there was a gradual decrease in all experimental groups. In histamine-treated group, anti-SRBC-IgM increased steeply up to 7-day post-I (significant ($p<0.01$) enhancement as compared to positive control and suppression as compared to H3R-antagonist-treated group) and there was a significant decrease from days 14- to 58-post-I as compared to positive control and H3R-antagonist groups. H3R-agonist-treated rabbits showed significant ($p<0.01$) suppression of anti-SRBC-IgM as opposed to all experimental groups. Nonetheless H3R-antagonist-treated rabbits showed significant ($p<0.01$) enhancement of anti-SRBC-IgM as compared to histamine-treated (significant ($p<0.01$) at days 7-, 14- and 21-post-I) and positive control (significant ($p<0.01$) at day 7-post-I), whereas this group demonstrated significant ($p<0.01$) suppression at day 14-post-I and showed further enhancement at days 21-, 28- and 58-post-I as opposed to positive control. However, it demonstrated similar anti-SRBC-IgM level to histamine-treated group at days 28- and 58-post-I. No anti-SRBC-IgM response was noticed

in group I (negative control) during whole of the study period (refer to Fig. 2 and Table 2).

Profile of anti-SRBC-immunoglobulin-G (IgG) production: The profile of anti-SRBC-IgG titer was also studied by whole SRBC-ELISA method [5,6,10-12,26] (Fig. 3) and direct HA [10,11,25] (Table 2). The observed profiles were similar by ELISA and HA assays. The detailed summary of statistically analyzed IgG production by two way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test are shown in Fig. 3 and Table 2. No antibody response was detected in all the groups at day 0 (pre-I). More extensive evaluation revealed that anti-SRBC-IgG in histamine-treated and H3R-antagonist-treated groups increased steeply up to 7-days post-I as compared to positive control and H3R-agonist groups ($p<0.01$) (however, H3R-antagonist-treated group showed significantly increased IgG level opposed to histamine-treated group). But by days 14-, 21-, 28- and 58-post-I there was a decrease in anti-SRBC-IgG levels in histamine-treated group as compared to H3R-

antagonist-treated group (significant ($p < 0.01$) decrease at days 14-, 21-, 28- and 58-post-I) and positive control group (significant ($p < 0.01$) decrease from days 14-to 58-post-I). H3R-agonist-treated rabbits showed significant ($p < 0.01$) suppression of anti-SRBC-IgG as opposed to all other experimental groups over a time span of 58 days. H3R-antagonist enhanced significantly ($p < 0.01$) the anti-SRBC-IgG at day 7-post-I and suppressed ($p < 0.01$) at day 14-post-I in comparison to positive control group, however, it further showed significant ($p < 0.01$) enhancement of anti-SRBC-IgG level at days 21-and 28-post-I in comparison to positive control group, while IgG pattern was found to be similar to positive control at day 58-post-I. No anti-SRBC-IgG response was noticed in group I (negative control) during whole of the study period.

DISCUSSION

Histamine is a potent agonist of all four of its receptors (H1, H2, H3 and H4) [2, 4, 10], which modulate different biochemical, pharmacological and immunological reactions both *in vivo* and *in vitro* [4]. It is well documented that histamine directly affects B-cell antibody production as a co-stimulatory receptor on B-cells [5-7, 27-29]. Therefore, in the present study, we investigated total serum antibody (Ig), IgM and IgG generation profile against SRBC, a T lymphocyte-dependent test antigen [26, 30], modulated by endogenous histamine in control (untreated)-, H3R-agonist (R-[-]- α -methylhistamine dihydrobromide) treated-, H3R-antagonist (iodophenpropit dihydrobromide) treated-and histamine treated-experimental groups.

To provide exact evidence relating our investigations to *in vivo* immunoregulatory processes, we used healthy rabbits with and without histamine, H3R-agonist/-antagonist treatments. We demonstrate that H3R-specific agonist/-specific antagonist-treated rabbits were showed a marked deviation of the immune response as compared to control rabbits (untreated). Based on our results, histamine, released after immunological stimuli from effector cells (mast cells and basophils) *in vivo* [4], could influence a detectable antibody response to SRBC [5,6]. Results from *in vivo* immunomodulatory experiments showed suppression of total anti-SRBC-Ig, anti-SRBC-IgM and anti-SRBC-IgG in H3R-agonist-treated group. In contrast, H3R-antagonist-treated group demonstrated increase in anti-SRBC-Ig, IgM and IgG during the entire study period. These results are contrary to our previous study of histamine H1-and H2-receptors-

agonists/antagonists [10,11] and demonstrate that H3-receptor inhibition by H3R-antagonist showed enhancement of antibody production. On the other hand H3-receptor on stimulation by H3R-agonist showed suppression of antibody production. These data provide evidence that histamine H3-receptors in biological system serve as a regulator perhaps by balancing the effects mediated by H1 and H2 receptors.

In this preliminary study, we noticed that exogenously added histamine *in vivo* (until its presence in the body) enhanced the antibody generation profile against SRBC whereas; on further metabolism its effect disappeared on the antibody generation profile bringing it in the range of control antibody level. We observed that H3R-antagonist (until its presence) increased the antibody profile, which came to normal level (control range) in later phase due to its metabolism. This leads us to conclude that the effects of histamine and its antagonist were of short duration which disappeared in latter stage due to their clearance. Thus, both histamine-treated-and H3-antagonist-treated-groups (after metabolism of drugs) showed Ig levels similar to that of controls.

To our knowledge, this is the first report describing the potential immunoregulatory role of H3-receptor-agonist/-antagonist in rabbits over a study period of 58 days pre-and post-immunization. Human disease processes mediated by histamine release may potentially be modified by the use of selective histamine receptor (H3) agonists / antagonists.

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