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Original Article

In vivo comparative immunotoxic study of histamine receptors (H1R, H2R, H3R and H4R)-agonist

Trivendra Tripathi[a, *], Mohammad Shahid[b], Adil Raza[b], Haris M. Khan[b], Mashiatullah Siddiqui[a], Abida Malik[b], Rahat Ali Khan[c]

[a]*Department of Biochemistry,* [b]*Department of Microbiology and* [c]*Department of Pharmacology, Faculty of Medicine, Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh-202002, U.P., India*

Abstract. Accumulating evidences have highlighted histamine and histamine receptors (HRs)-antagonists' role in immunomodulation. However, the roles of HRs-agonists are still unclear. The present study was therefore designed to delineate the comparative immunotoxic roles of H1-H4-agonist on antibody generation profile in rabbit model. The cohort comprised of seven groups (Group-I negative control, group-II positive control and group-III-VII HRs-agonist-treated) containing 18 (9 male and 9 female) rabbits each. Group-I and group-II received vehicle (sterile distilled water, $1 ml kg^{-1} \times$b.i.d.) intramuscularly. Groups-III-VII (HRs-agonist-treated) received subcutaneous histamine ($100 \mu g kg^{-1}$) and H1-agonist (HTMT), H2-agonist (amthamine), H3-agonist (R-[-]-α-methylhistamine) and H4-agonist (clobenpropit) each in a dose of $10 \mu g kg^{-1}$, respectively, b.i.d. for 10 days (starting from day 1). Groups-II-VII were subsequently immunized with intravenous injection of SRBC at day 3. The estimation of serum immunoglobulins (Ig), IgM and IgG were done by ELISA, and observed at day 0 (pre-immunization) and day 7, 14, 21, 28 and 58 (post-immunization). Results showed that histamine and HRs-agonist could influence a detectable antibody response to SRBC as early as day 7-postimmunization (post-I), which lasted until day 58- post-I. All the results were found statistically significant ($p<0.05$). To conclude, our results provide evidences that HTMT, amthamine and clobenpropit (H1-, H2- and H4-agonist, respectively) have important role in modulation of antibody generation by enhancing production level, in which HTMT have dominant role, while amthamine and clobenpropit play similar role. Conversely, R-[-]-α-methylhistamine (H3-agonist) have dominant inhibitory role on antibody production.

Key words: Histamine, histamine receptors, agonists, immunomodulation, antibody generation, immunotoxic

1. Introduction

Histamine through the activation of its four receptors (H1R, H2R, H3R and H4R) play the imperative role in pathophysiology of several diseases such as allergic, inflammatory, autoimmune, malignancy and diseases of central nervous system (1-3). Histamine receptors (HRs) transduce extracellular signals through different G-proteins: Gq/11 for H1R, Gαs for H2R, Gi/o for H3R and H4R (1). Activation or inhibition of HRs have led to a remarkable increase in the knowledge of histamine effects in the pathophysiology of disease conditions (1,3). Histamine shows agonist property and has different p*K*i 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 (4). Shahid et al. highlighted the important physiological relevance of histamine receptors briefly - H1Rs are responsible for cycle of sleeping and waking, food intake, thermal regulation, emotions and aggressive behavior, locomotion, memory and learning, contraction of smooth muscles; H2Rs are responsible for neuroendocrine and gastric acid secretion; H3Rs are accountable for presynaptic heteroreceptor;

*Correspondence: Dr. Trivendra Tripathi, Ph.D.
Department of Biochemistry
JN Medical College & Hospital
AMU, Aligarh, UP, INDIA
Email: tripathiamu@gmail.com
trivendratripathi@gmail.com

decreased histamine, dopamine, serotonin, noradrenaline and acetylcholine release, sleep and also food intake; and H4Rs are liable for chemotaxis (1).

HRs have been discovered to increase delayed type hypersensitivity (DTH) and antibody mediated immune responses in various pathological processes regulating numerous essential events in allergies and autoimmune diseases in experimental animals, especially in genetically modified mice (5,6). Moreover, H1R-antagonist tripelennamine inhibits histamine binding in T helper (Th) 1 but not in Th2 cells which correlates to predominant H1R expression on Th1 cells. Neither ranitidine (a H2R-antagonist) nor clobenpropit (H4R-agonist/H3R-antagonist) had any impact on histamine binding to Th1 cells, and further showed that the expression of H1R on Th1 cells and H2R on Th2 cells by antibodies generated against the H1R and H2R (7).

Furthermore, H4R shares its highest sequence similarity with the H3R (i.e. 37%), it is not surprising that the H4R is targeted by various imidazole containing H3R ligands. The standard H3R antagonist thioperamide also acts as an antagonist at the human histamine H4 receptor (hH4R). Moreover, H3R agonists' immepip, imetit, (*R*)-α-methylhistamine (Table 1) and imbutamine also act as potent hH4R agonists (8). Also, the H4R is activated by burimamide (H2R/H3R antagonist), clobenpropit (H3R antagonist) (Table 1 & 2), and iodoproxyfan (H3R agonist), indicating that for hH4R agonism considerable structural diversity (piperidine, isothiourea, thiourea, and ether) in the side chain of imidazole ring is allowed, including aromatic substitutions as indicated by the hH4R agonism displayed by clobenpropit [intrinsic activity (α = 0.8)] (8). However, H3R compounds indicate that hH4R efficacy can be modulated by differential hydrophobic substitution on the side chain. Moreover, in the clobenpropit series, a slight change on the isothiourea substituent results in a modulation of H4R efficacy. The clobenpropit analog iodophenpropit (a phenylethyl substituent instead of a benzyl group) retains high H4R affinity (p*K*i = 7.9), but it has lost complete agonistic activity (α = 0) (Table 1 & 2) (8). Clobenpropit has two different pA_2 values i.e. pA_2 = 7.9 (EC_{50} 72 nM) for H4R-agonist and pA_2 = 9.9 for H3R-antagonist (1,11,12). However, Ling et al. had used clobenpropit as H4R-agonist that mimics histamine effect in inducing change of shape of eosinophils (13). Moreover, histopathological and biochemical study of clobenpropit demonstrated its agonist property in rabbit and showed that it causes binucleated hepatocytes and Kupffer cells prominence (14). As far as our knowledge concerned, there is no data available for the agonist property of clobenpropit in immune system; therefore we have decided to investigate its agonist role in immunomodulation.

Table 1. Comparative activities of H1R – H4R ligands at the human histamine H4 receptor (hH4R), for detailed study kindly see Lim et al (8)

Ligands	hH1R			hH2R			hH3R			hH4R		
	p*K*i at H1R	pEC_{50}	α	p*K*i at H2R	pEC_{50}	α	p*K*i at H3R	pEC_{50}	α	p*K*i at H4R	pEC_{50}	α
Histamine	4.2±0.1[a]	—	1	4.3±0.1[b]	—	1	8.0±0.1[c]	8.3±0.1	1	7.8±0.1[d]	7.7±0.1	1
HTMT	—	—	—	—	—	—	—	—	—	—	—	—
Amthamine	—	—	—	5.2±0.1[e]	—	1	—	—	—	5.3±0.1[d]	—	0
(R)-α-Methylhistamine	—	—	—	—	—	—	8.2±0.1[c]	9.5±0.1	1	6.6±0.1[d]	6.2±0.1	1
Clobenpropit	—	—	—	—	—	—	8.6±0.1[c]	9.4±0.1	-1	8.1±0.1[d]	7.7±0.1	0.8
Idophenpropit	—	—	—	—	—	—	8.2±0.1[c]	8.5±0.1	-1	7.9±0.1[d]	—	0
4-Methylhistamine	—	—	—	5.1±0.1[d]	—	1	—	—	—	7.3±0.1[d]	7.4±0.1	1

α = intrinsic activity (1 designated for full agonistic, 0 for neutral antagonist, and -1 for full inverse agonistic activity).
a = p*K*i value was obtained by Bakker et al. with [^{3}H]mepyramine displacement assay (9).
b = p*K*i value was obtained by Leurs et al. with [^{125}I]iodoaminopotentidine displacement assay (10).
c = p*K*i values were determined by Lim et al. with [^{3}H]histamine displacement assay (8).
d = p*K*i values were determined by Lim et al. with [^{3}H]*N*α-methylhistamine displacement assay (8).
e = p*K*i values were determined by Leurs et al. with [^{125}I]iodoaminopotentidine displacement assay (10).
"—" = warranting further investigation.
pEC_{50} = values show the inhibition of 1 μM forskolin-induced CRE-β-galactosidase activity in SK-N-MC/hH4 cells (8).

Table 2. Lim et al. have demonstrated hH4R affinity for selected H4R compounds as determined with displacement of the binding of (^{3}H)histamine, (^{3}H)JNJ 7777120, or (^{125}I) iodophenpropit (8)

Histaminergic ligands	p*K*i		
	[^{3}H]histamine	[^{3}H]JNJ 7777120	[^{125}I]iodophenpropit
Histamine	7.8±0.1	7.7±0.1	7.6±0.2
4-Methylhistamine	7.3±0.1	7.6±0.1	7.4±0.1
Clobenpropit	8.1±0.1	7.9±0.1	7.8±0.1
Iodophenpropit	7.9±0.1	7.7±0.1	7.7±0.1
Thioperamide	6.9±0.1	7.1±0.1	7.4±0.1
JNJ 7777120	7.8±0.1	7.8±0.2	7.8±0.1

Moreover, several studies of histamine receptors in rabbit model are well documented (15-22). Our recent immunomodulation studies in rabbit model have demonstrated that histamine has a short-term effect on antibody generation (until its presence in the body), and the antibody generation titer in vivo were affected by the concentration of histamine (23,24). The scope of histamine research has been implicated in immune responses. The newly discovered H4R plays an important role in inflammation (1,3) and has opened a new way for the functions of histamine in immune system. The data on the role of H3R and H4R in immune regulation are limited. Due to lack of immunomodulatory researches on H1R – H4R, our present comparative study thus account for exploration of the regulatory mechanisms in the control of immune processes through effector cells derived histamine, exogenous histamine and histamine H1R - H4R agonist in immunomodulation in rabbit model.

2. Materials and methods

2.1. Experimental design

To evaluate the systemic antibody response, 126 (63 Male and 63 Female) New Zealand adult healthy rabbits of either sex weighing 1.36±0.24 kg were randomized equally into seven treatment groups, i.e. 18 rabbits (9 male and 9 female) in each group. Group-I (negative control) remained non-immunized and received only vehicle (sterile distilled water, 1 $mlkg^{-1}$ × b.i.d..). Group II was vehicle (sterile distilled water, 1 $mlkg^{-1}$ × b.i.d.)-treated and immunized as a positive control. Group III was histamine-treated and immunized, group IV was H1R-agonist-treated and immunized, Group V was H2R-agonist-treated and immunized, Group VI was H3R-agonist-treated and immunized and Group VII was H4R-agonist-treated and immunized. The animals were housed in well-maintained animal facility at central animal house, J. N. Medical College & Hospital, A.M.U., Aligarh, in the Bioresources unit under a 12 hr light/dark cycle, temperature (22±2˚C) and were allowed free access to standard laboratory diet including green vegetables and tap water until experimentation. 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 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.

2.3. Drugs and doses

Following drugs were used: histamine dihydrochloride obtained from Himedia laboratories Pvt Limited, India; H1R-agonist [Histamine Trifluoro-Methyl Toluidide (HTMT)-dimaleate], H2R-agonist (amthamine dihydrobromide), H3R-agonist [R-(-)-α-methylhistamine dihydrobromide] and H4R-agonist (clobenpropit dihydrobromide) which were kindly donated by Tocris Bioscience, Tocris Cookson Ltd., United Kingdom.

Histamine dihydrochloride (100 μgkg^{-1}) and other agonists [HTMT-dimaleate, amthamine dihydrobromide, R-(-)-α-methylhistamine

dihydrobromide, clobenpropit dihydrobromide] (10 μgkg^{-1}) were administered subcutaneously (s.c.) twice a day [12 hourly (8 am and 8 pm)] for 10 subsequent days (starting from 3 days prior to immunization until 7 days after immunization). All doses were referred to the weight of the salts used.

2.4. 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% 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 (25). Finally the concentration was adjusted to 5% (1×10^9 cells/ml) in PBS for immunization before use.

2.5. Immunization of rabbits

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

2.6. Sample collection

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. Blood samples were kept at room temperature for 120 minutes and then left for overnight at 4ºC. Blood samples were centrifuged for 10 minutes at 580×g, and serum was separated and heated at 56ºC for 30 minutes to inactivate complement proteins and stored in aliquots containing sodium azide as preservative at -20ºC till tested further (26).

2.7. Enzyme linked immunosorbent assay (ELISA)

To determine the SRBC-specific-immunoglobulins (Ig), SRBC-specific-IgM and SRBC-specific-IgG response, the whole SRBC-enzyme linked immunosorbent assay (ELISA) (23,24,27,28) was carried out on polystryrene plates. Polystryrene MaxiSorp immunoplates were coated with SRBC suspension (5×10^6/100 µL PBS). The plates were held overnight at 4ºC. Each sample was coated in duplicate and half of the plates served as control 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 25ºC 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 37ºC. After incubation, the plates were washed four times with 200 µL of PBS. Each rabbit serum diluted 1:100 in PBS (100 $\mu Lwell^{-1}$) was adsorbed for 90 minutes at 37ºC, and then overnight at 4ºC 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 $\mu Lwell^{-1}$) in respective plates and incubated at 37ºC for 60 minutes. The washing step was repeated as before and 100 $\mu Lwell^{-1}$ TMB substrate was added and the plates were incubated at 25ºC for 60 minutes. The enzymatic reaction was stopped by adding 50 $\mu Lwell^{-1}$ 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 $A_{test- control.}$

2.8. 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 12.0, Inc., Chicago, IL).

3. Results

3.1. Profile of total anti-SRBC-immunoglobulins (Ig) level

The profile of total anti-SRBC-immunoglobulins (Ig) titer was studied by whole SRBC-ELISA method (23,24,27,28) (Fig. 1). 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 total serum Ig titer over the span of 58 days in all the groups and was found statistically significant at each experimental post-I days. The detailed summary of statistically analyzed Ig level by two way analyses of variance (ANOVA) followed by Newman-Keuls post hoc test are shown in Fig. 1. By day 7- post-I, the anti-

SRBC-Ig titer were detected and reached a peak but by days 14-, 21-, 28- and 58- post-I, there was a gradual decrease in all experimental groups (except H1R-agonist-treated and positive control (untreated) groups, where the highest peak was detected at day 14- post-I and then there was a gradual decrease).

More extensive evaluation revealed that anti-SRBC-Ig increased sharply up to 7 days post-I, and there was decrease in histamine-treated group at days 14- and 21- post-I as compared to positive control group, while this group showed similar antibody titer near to positive control at days 28- and 58- post-I. However, H1R-agonist (HTMT)-treated, H2R-agonist (amthamine)-treated and H4R-agonist (clobenpropit)-treated rabbits showed enhancement of total anti-SRBC-Ig level as opposed to histamine-treated, positive control and H3R-agonist [R-(-)-α-methylhistamine]-treated rabbits. H1R-agonist (HTMT)-treated rabbits showed initially inhibition at day 7 post-I and later enhancement of anti-SRBC-Ig level at days 14-, 21-, 28- and 58- post-I as opposed to H2R-agonist (amthamine)-treated and H4R-agonist (clobenpropit)-treated rabbits and demonstrated increase of Ig titer lower than observed in H2R- and H4R-agonists-treated group (Fig. 1). Anti-SRBC-Ig levels at each experimental post-I days was observed similar in H2R-agonist- and H4R-agonist-treated groups.

On the other hand, H3R-agonist (R-(-)-α-methylhistamine)-treated group showed inhibition of anti-SRBC-Ig level at days 7-, 14-, 21-, 28- and 58- post-I as compared to H1R-, H2R- and H4R-agonists while it showed inhibition of total serum anti-SRBC-Ig level as compared to histamine-treated and untreated (positive control) groups over the span of 58 days. No anti-SRBC-Ig response was noticed in group I (negative control) during whole of the study period (Fig. 1).

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

Anti-SRBC-IgM was determined by whole SRBC-ELISA method (23,24,27,28) (Fig. 2). No anti-SRBC-IgM response was observed in all experimental groups (negative control, positive control and drug 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 analyses of variance (ANOVA) followed by Newman-Keuls post hoc test are shown in (Fig. 2).

Anti-SRBC-IgM increased sharply up to 7 days post-I and by days 14-, 21-, 28- and 58- post-I, there was a decrease in all drug treated groups as compared to positive control group. In histamine-treated group, anti-SRBC-IgM increased sharply up to 7- day post-I (enhancement as compared to positive control and H3R-agonist-treated group), while there was decrease at days 14- and 21- post-I as compared to positive control group. While at days 28- and 58- post-I, it showed similarity to positive control's anti-SRBC-IgM level. Moreover, histamine-treated group showed enhanced IgM as compared to H3R-agonist-treated group at days 14-, 21-, 28- and 58- post-I. The total anti-SRBC-IgM titer of H1R-agonist-, H2R-agonist- and H4R-agonist-treated rabbits showed enhancement as opposed to H3R-agonist-treated, histamine-treated and positive control rabbits (Fig. 2). Moreover, H2R-agonist-treated rabbits showed similar anti-SRBC-IgM titer to H4R-agonist-treated rabbits at each experimental post-I days. While H1R-agonist-treated group showed inhibition of anti-SRBC-IgM titer as compared to H2R-agonist- and H4R-agonist-treated groups at days 7-, 14-, 21-, 28- post-I, however this group showed enhancement at day 58- post-I as compared to H2R-agonist- and H4R-agonist-treated rabbits. No anti-SRBC-IgM response was noticed in group I (negative control) during whole of the study period (Fig. 2).

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

Anti-SRBC-IgG was determined by whole SRBC-ELISA method (23,24,27,28) (Fig. 3). The detailed summary of statistically analyzed IgG level by two way analyses of variance (ANOVA) followed by Newman-Keuls post hoc test are shown in Fig. 3. No anti-SRBC-IgG response was observed in all experimental groups (negative control, positive control and HRs-agonist-treated) at day 0 pre-I; however there was an initial increase and then gradual decrease in serum-IgG titer over the time period in all the groups.

Anti-SRBC-IgG profile increased sharply up to 7- days post-I and by days 14-, 21-, 28- and 58- post-I, it was diminished in histamine-treated group as compared to all other experimental groups. In histamine-treated group, anti-SRBC-IgG raised sharply up to 7- day post-I [enhancement as compared to positive control and H3R-agonist-treated group] and also the enhancement of IgG of this group was seen at days 14-, 21-, 28- and 58- post-I as compared to H3R-agonist-treated group, while there was decrease in IgG titer at days 14-, 21-, 28- and 58- post-I as compared to positive control group.

The total anti-SRBC-IgG titer of H1R-agonist-, H2R-agonist- and H4R-agonist-treated rabbits showed enhancement as opposed to positive control, H3R-agonist- and histamine-treated rabbits over the time span of 58 days (Fig. 3). Furthermore, H2R-agonist-treated rabbits showed similar anti-SRBC-IgG titer to that of H4R-agonist-treated rabbits at each experimental post-I days. H1R-agonist-treated group showed inhibition of anti-SRBC-IgM titer at days 7- post-I, while it showed enhancement at 14-, 21-, 28- and 58- post-I as compared to H2R-agonist- and H4R-agonist-treated groups. No anti-SRBC-IgG response was noticed in group I (negative control) during whole study period (Fig. 3).

4. Discussion

Since *in vivo* studies looking for toxicological (immunotoxic and hepatotoxic) impact of HRs-agonists in experimental models are fragmentary. Recently, our histopathological and biochemical studies of livers of rabbits treated with HRs (H1R-H4R)-agonists have demonstrated that short-term treatment by histamine and its receptors-agonist produce differential patterns of hepatotoxicity in terms of hepatic congestion (histamine and H2R-agonist), centrilobular necrosis (H1R-agonist), binucleated (H4R-agonist) and multinucleated hepatocytes (H2R- and H3R-agonist) and prominent Kupffer cells (KCs) (H4R-agonist) as compared to control group, suggested that HRs on induction via their specific-agonist produce differential pattern of hepatotoxicity (14). However, there immunotoxic role on antibody generation are still unclear or incomplete. Therefore, our present study has been designed to observe comparative immunological role of same HRs-agonist used for hepatotoxic study (14).

Lim et al. (8) have demonstrated significant comparative activities of H1R – H4R ligands (used in this study) at the human histamine H4 receptor (hH4R) (Table 1). Moreover, for *in vivo* histaminergic studies, 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 warranting further study.

The present study has been investigated the total serum Ig, IgM and IgG generation profile against SRBC (a T cell-dependent test antigen) (27,29) in negative control (untreated) and treated groups [positive control (treated with sterile distilled water)-, H1R-agonist (HTMT)-, H2R-agonist (amthamine)-, H3R-agonist [R-(-)-α-methylhistamine]-, H4R-agonist (clobenpropit)- and histamine treated-experimental groups] in healthy rabbits. Our previous studies have demonstrated that the histamine released from immunological stimuli *in vivo* could influence a detectable antibody response to SRBC (23,24).

It must be emphasized here that to the best of our knowledge, none of the earlier reports have demonstrated the comparative immunotoxic study of anti-SRBC-Ig, IgM and IgG profile modulated by histamine and its four receptors (H1R - H4R)-agonist.

It has been well documented that histamine shows the agonist properties of its receptors (1,3,4,7,8). HRs are distributed in all parts of body and modulate several reactions both *in vivo* and *in vitro* (1). Histamine also modulates immunological reactions and directly affects B-cell antibody production as a co-stimulatory receptor on B-cells (30). It has also been 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 (31), suggesting an important role of H1R signaling in response triggered from B-cell receptors. Jutel et al. showed a different pattern of antibody responses to T-cell dependent antigens like ovalbumin and demonstrated that H1R-deleted mice produced high ovalbumin-specific IgG1 and IgE as compared to wild type mice (7).

Keeping in view the above facts, especially the paucity of literature (i.e., immunomodulatory role of histamine H1Rs - H4Rs), defining the correlation of HRs-agonists in immune regulation and modulation, and the fragmentary literature of HRs describing existing immunomodulatory role *in vivo* system, the present study was planned. This study revealed that the histamine treated-rabbits showed immunopotentiating properties by enhancing the anti-SRBC-antibody (Ig, IgM & IgG) levels as compared to positive control group over a span of study period of 58 days. Furthermore, our comparative H1-, H2-, H3- and H4-agonist study on *in vivo* immunoregulatory processes demonstrated enhanced generation profile of anti-SRBC-Ig, IgM and IgG in H1R-agonist (HTMT)-, H2R-agonist (amthamine)- and H4R-agonist (clobenpropit)-treated rabbits, while it showed inhibition in H3R-agonist (R-[-]-α-methylhistamine)-treated rabbits as compared to positive control rabbits during the whole study period.

The major significance of these findings is the comparative immunotoxic evaluation of

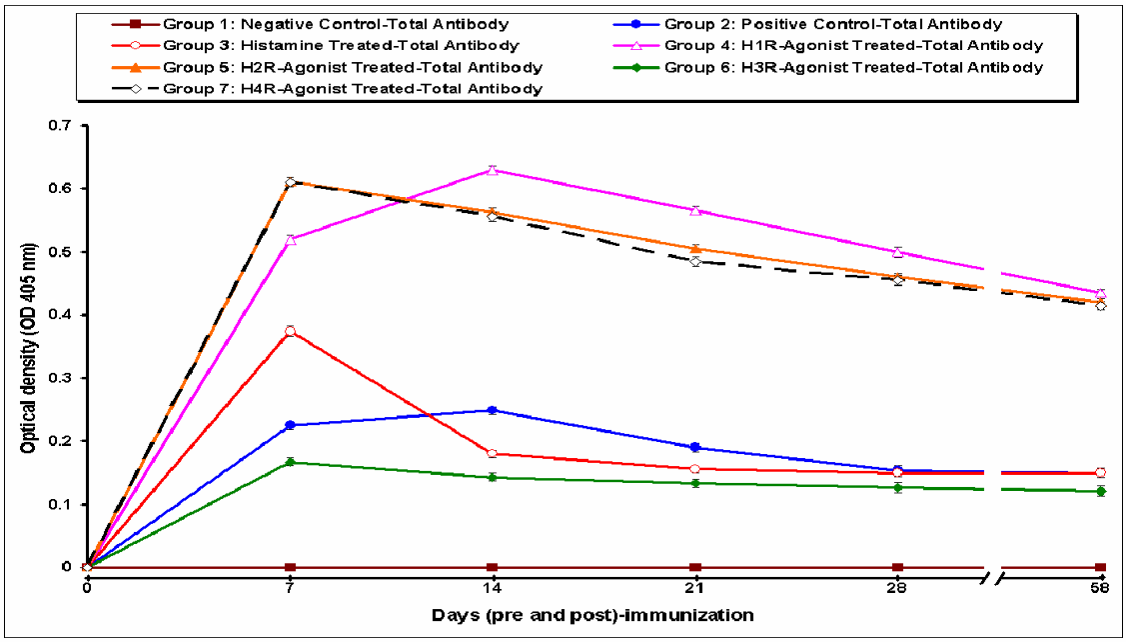

Fig. 1. SRBC-specific Immunoglobulins (Igs) production titers in H1R-, H2R-, H3R- & H4R-agonist-treated rabbits by whole SRBC-ELISA method in duplicate 1:100 diluted sera. The results demonstrate mean ± s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F=40968.340$, $DF=6,119$; $p<0.01$) and days ($F=123866.4$, $DF=5,595$; $p<0.01$) on SRBC were statistically significant. The interaction (treatments × days) effect of ($F=10015.733$, $DF=30,595$; $p<0.01$) these on SRBC were also found to be significant.

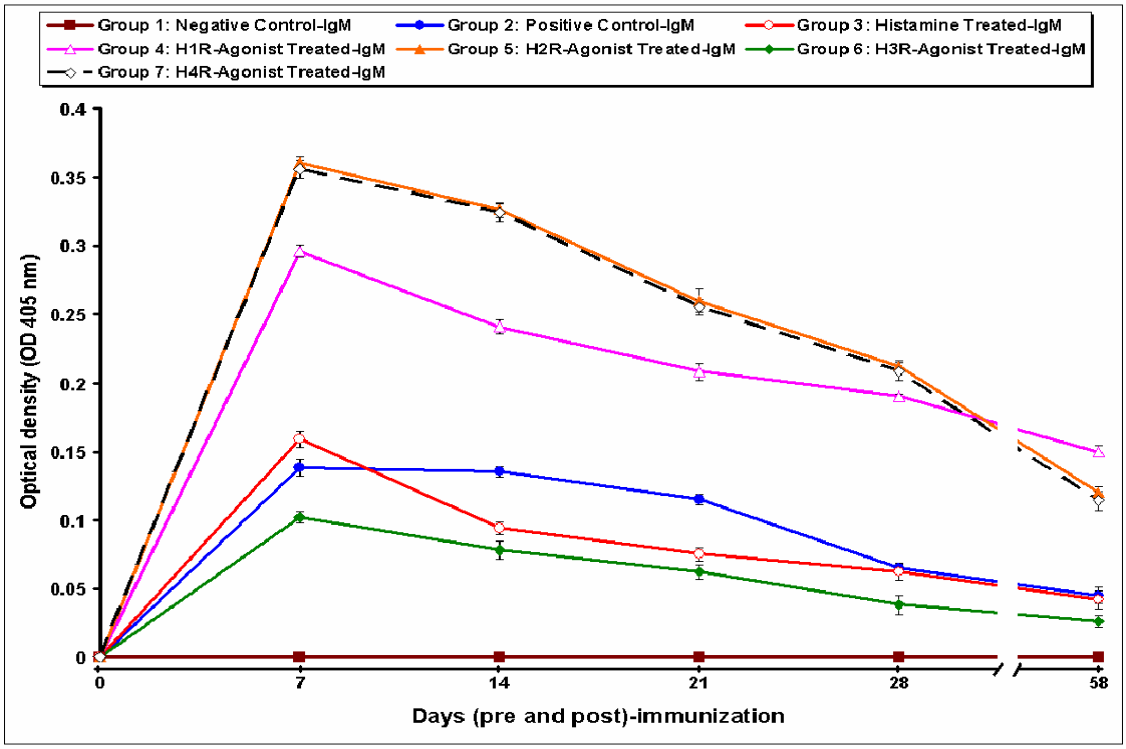

Fig. 2. SRBC-specific Immunoglobulin-M (IgM) production titers in H1R-, H2R-, H3R- & H4R-agonist-treated rabbits by whole SRBC-ELISA method in duplicate 1:100 diluted sera. The results demonstrate mean ± s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F=8975.923$, $DF=6,119$; $p<0.01$) and days ($F=40509.989$, $DF=5,595$; $p<0.01$) on SRBC were statistically significant. The interaction (treatments × days) effect of ($F=3038.703$, $DF=30,595$; $p<0.01$) these on SRBC were also found to be significant.

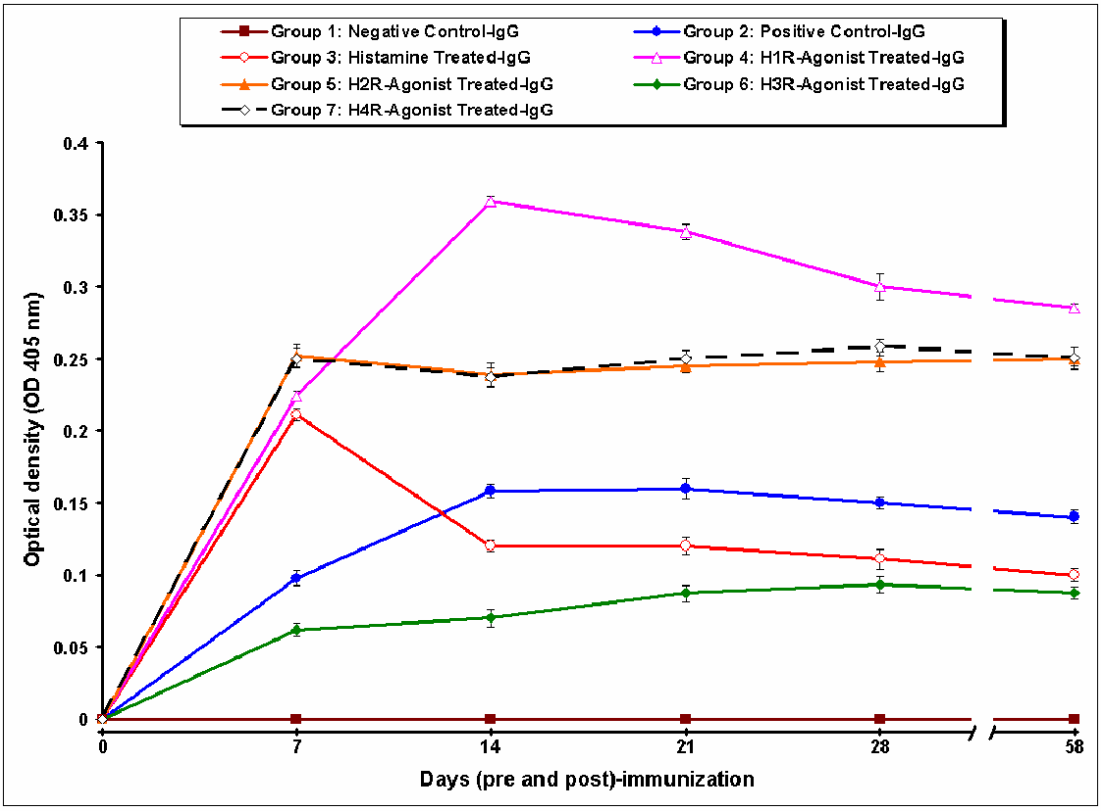

Fig. 3. SRBC-specific Immunoglobulin-G (IgG) production titers in H1R-, H2R-, H3R- & H4R-agonist-treated rabbits by whole SRBC-ELISA method in duplicate 1:100 diluted sera. The results demonstrate mean ± s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F=8363.643$, $DF=6,119$; $p<0.01$) and days ($F=11101.804$, $DF=5,595$; $p<0.01$) on SRBC were statistically significant. The interaction (treatments × days) effect of ($F=903.632$, $DF=30,595$; $p<0.01$) these on SRBC were also found to be significant.

histaminergic ligands (HTMT, amthamine, clobenpropit and R-[-]-α-methylhistamine) needs further investigation.

5. Conclusion

Our results provide evidences that HTMT, amthamine and clobenpropit have important role in modulation of antibody generation, among which HTMT have dominant role, while amthamine and clobenpropit play similar role in immunomodulation. On the other hand, R-[-]-α-methylhistamine have dominant inhibitory role in immunomodulation.

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