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EXPERIMENTAL AUTOIMMUNE MYOCARDITIS IN RATS AND THERAPEUTIC HISTAMINE H_1 – H_4 RECEPTOR INHIBITION

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Myocarditis, a life threatening disease, is still not adequately treated. Histamine plays an important role in physiology and pathophysiology of cardiovascular system. All four histamine receptors (H_1R – H_4R), are present in the heart. Experimental autoimmune myocarditis (EAM) was used to investigate which histamine receptor had a greater impact on the disease's progression. EAM was evoked in Lewis rats by porcine myosin immunization. Mepyramine, ranitidine and ciproxifan were used to inhibit H_1R, H_2R and H_3R receptors, respectively, and 2,4-diaminopyrimidines: ST994, ST1012, ST1006 were ligands of H_4R. Quinapril, an ACE inhibitor, served as a reference drug. Drugs were administered daily, either from 0 – 2 weeks or from 2 to 4 weeks post EAM induction. Cardiac dysfunction developed with significant decreases in left ventricular ejection fraction and fractional shortening due to dilatation and wall thickening. EAM rats treated with mepyramine and ST994 in weeks 0 – 2 had the lowest decreases. These treated with ST994, ST1012 or quinapril performed much better the following 2 weeks without therapy than did the other groups. On autopsy their hearts were smaller, less fibrotic, histopathological changes in them of a lower grade. When the treatment started with 2 weeks' delay, the ST994-treated EAM rats showed the highest median survival. H_4 receptor antagonism inhibits heart remodelling, preserves heart contractility, improves survival and may be of potent therapeutic relevance in human clinics. The blockade of H_1 receptor inhibits heart dilatation but does not prolong the life.

Key words: *histamine receptors' antagonists, experimental autoimmune myocarditis, dilated cardiomyopathy, angiotensin converting enzyme inhibitor*

INTRODUCTION

Post infectious cardiomyopathy in man often progresses to dilated cardiomyopathy. The latter is a major cause of chronic heart failure, a disorder still causing high morbidity and mortality for being largely unmodified by a current treatment (1, 2). If ongoing inflammation causes functional impairment of the heart, conceivably therapeutic interventions must suppress the continued inflammation. Histamine, which plays an important role in cardiovascular system physiology by participating in: regulation of heart function, peripheral resistance and circulating blood volume (3, 4), is also a well-known mediator of inflammation (5). Histamine functions are mediated by four histamine receptors abbreviated H_1R to H_4R. All of them are G protein coupled receptors (6) and are present in heart (7-11). Experimental studies have provided evidence that, by targeting one of these receptors, histamine may modify leukocyte reactivity, *i.e.* it may suppress: histamine release from basophils and mast cells, release of lysosomal enzyme from neutrophils, production and release of antibodies from lymphocytes, cell proliferation stimulated by antigens or mitogens, cell chemotaxis to the site of inflammation, as well as cytokine production (12). In cardiomyopathy the benefits of H_2R antagonists have been indicated based on retrospective and prospective clinical studies (13). Likewise, in experimental viral myocarditis treatment with the H_1R antagonist, cetirizine, has been reported advantageous (14). There is no data concerning the most recently described histamine H_4 receptors that are present on immune and inflammatory cells, however, the results of studies on other organ inflammation (15, 16, 17, 18). clearly point to the potential benefits of H_4R antagonists.

Here, by using an experimental model of autoimmune myocarditis (EAM) that mimics post infectious cardiomyopathy in man (19), we attempted to investigate which of the four histamine receptors had a greater impact on the disease's progression.

MATERIALS AND METHODS

Experimental animals

All experimental procedures were conducted with respect to the law regulations of the European Community (conformed

Directive 2010/63/EU) and Poland, and had been approved by the Local Ethics Committee for Animal Experiments in Lodz. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (20, 21).

Lewis male rats, 8 weeks of age, were purchased from the Animal House at Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland. The rats were kept in plastic cages (3 – 4 rats per box) in a temperature-controlled animal room, with relative humidity 55%, a 12 hour light/dark cycle (light beginning at 7 a.m.) and had free access to feed and water.

Animals were arbitrarily assigned to control and experimental groups. At the beginning of the experiment, each group consisted of 10 rats. The smaller number of animals in the groups at the end point of the experiment is the result of mortality. The higher rats' number of untreated EAM group results from set of figures from different parts of the experiment; untreated EAM group was included in each stage of the study.

Experimental procedures

EAM was induced in the rats by immunization with porcine heart myosin in complete Freund's adjuvant in a dose 0.33 mg in 135 µl given subcutaneously into each rear footpad, twice with one week apart (19).

Histamine receptor ligands and quinapril (ACE inhibitor) were applied daily either for the first 2 weeks or between 2 to 4 weeks post EAM induction, the experimental end point being 4 weeks.

For the therapy implemented in the second time interval (*i.e.* 2 to 4 weeks post EAM induction) the EAM rats were allocated to different treatment groups based on the similar posterior wall thickness (PW, cm) measured by echocardiography.

The following drugs were used: for H_1R - mepyramine (20 mg/kg, i.g.); H_2R - ranitidine (10 mg/kg, s.c.); H_3R - ciproxifan (1 mg/kg, s.c.), H_4R ligands - ST994 (N4-(4-methylbenzyl)-6-(4-methylpiperazin-1-yl)pyrimidine-2,4-diamine, neutral antagonist), ST1012 (4-(isoindolin-2-yl)-6-(4-methylpiperazin-1-yl)pyrimidin-2-amine, inverse agonist), ST1006 (N4-(2,6-dichlorobenzyl)-6-(4-methylpiperazin-1-yl)pyrimidine-2,4-diamine, partial agonist) (each by Alzet osmotic pump, 1 mg/kg). quinapril (20 mg/kg, i.g.) was employed as a reference drug (22). Control rats were given s.c. 0.25 ml of physiological saline solution (0.9% NaCl). H_4R ligands and ciproxifan were synthesized by a German partner as described previously (23).

Blood collection

Once a week 1 ml of blood was collected from a lateral tail vein after rat immobilization in a plastic tube and the local warming of the animal tail to cause dilation of the vein and promote the bleeding (24). As an anticoagulant 5% sodium citrate was employed and blood plasma separated by centrifugation was used to measure ceruloplasmin activity.

Heart function was examined by transthoracic echocardiography before, and at 2 and 3 weeks of EAM, according to the American Society of Echocardiography (25). An Esaote MyLab 30 gold CardioVascular portable ultrasound machine was used with a bioprobe PA 023 (Phased array transducer 7.5 - 10 mHz) on animals that were anesthetized with intraperitoneal ketamine (60 mg/kg) and xylazine (10 mg/kg) hydrochloride (Biowet Pulawy, Poland). The following parameters were measured from M-mode tracing: left ventricular end-diastolic dimension (LVDd, cm), left ventricular end-systolic dimension (LVDs, cm), fractional shortening (FS, %), end-diastolic posterior wall thickness (PWd, cm), end-systolic posterior wall thickness (PWs, cm), interventricular septum thickness in diastole (IVSd, cm), interventricular septum thickness in systole (IVSs, cm), and left ventricular ejection fraction (EF, %) (25, 26). FS and EF parameters were calculated automatically by the ultrasound system.

Experimental endpoint

Four weeks post myosin, all survivors were euthanized by decapitation. Blood was collected from neck arteries, excised hearts were macroscopically evaluated and their weights recorded. The ratio of the heart weight to body weight ($hw \cdot bw^{-1}$ coefficient) were calculated as an one of indicators of cardiac hypertrophy (19, 26, 27).

In any experimental series for histological examination 1 – 2 representative heart/s from each experimental group were fixed in total in 10% phosphate buffered formalin while the remaining hearts underwent sectioning. Dissected left and right atria as well as ventricles were immediately frozen on dry ice for biochemical and molecular biology analyses, whereas apexes were fixed for histology.

Histology

The tissues were embedded in paraffin and a 3 – 4 micrometer section cut. Paraffin sections were stained with hematoxylin and eosin (H & E), azan trichrome and alcian blue pH 2.5 P.A.S. Other sections were processed for immunohistochemical demonstration of CD3 antigen on reactive T cells and CD68 antigen on macrophages in tissue with lymphoid infiltrates, and troponin C to elucidate heart muscle damage. LSAB+ Universal System-HRP produced by Dako, an Agilent Technologies Company (Santa Clara, CA, USA) was applied for our research.

For immunohistochemical study we applied Rabbit polyclonal CD3 antibody (dilution 1:100), Monoclonal Mouse Anti-Human CD68 clone PG-M1 (dilution 1:100) and Mouse Monoclonal Antibody Troponin C clone 1A2 (dilution 1:50). For immunostaining of these antibodies we performed an antigen unmasking technique (high temperature in Target Solution pH 9.0 for CD3, CD68 and 45 minutes incubation at RT for CD3, CD68, Troponin C. For quantitative analysis of the expression of the investigated protein we applied MultiScanBase v 8.08 Image Analysis System (CSS, Ltd. Warsaw, Poland).

The preliminary screening histological study was done using 100 × magnification on a minimum of 3 cross sections of left ventricles, primarily because of the focal and patchy distribution of observed myocardial changes. Qualitative analysis of cardiac muscle was performed using a final light microscopic magnification of × 400. The photomicrographs were taken at 100, 200, 400, and 800 × magnifications.

Biochemical and molecular biology assays

For the plasma ceruloplasmin activity the o-dianisidine method (28) was used. Each plasma duplicate sample (0.05 ml) was incubated for 5 min and 15 min at 37°C in a sodium acetate buffer, pH 5.5 with a final o-dianisidine concentration 10^{-8} M, in a final volume of 1 ml. The reaction was stopped with 9 M H_2SO_4 and the resulting purple-red product measured at 540 nm. Plasma protein content was determined by the method of Lowry *et al*. (29) and ceruloplasmin activity was expressed in µmol o-dianisidine oxidized/min/mg protein.

The collected tissues were processed to measure hydroxyproline (spectrophotometry), histamine (ELISA), and transcripts for MCP-1 and TNF-α (RT-PCR).

Hydroxyproline was assayed as a marker of fibrosis by Woessner's method (30) with slight modifications, based on the reaction of oxidized hydroxyproline with p-dimethylaminobenzal-

dehyde (DMBA, Ehrlich's reagent). The homogenized tissues (in 5 volumes of distilled water) were hydrolysed with 6 N HCl (1 ml HCl per 0.1 ml homogenate) at 105°C for 24 hours. The hydrolysates were then filtered, neutralized by 1 M NaOH to pH 5 – 7 and diluted to 3.33 ml with distilled water. 0.5 ml of the sample, made up with distilled water to 1 ml of the final volume, was taken for further analysis. Hydroxyproline was oxidized to pyrrole by 0.5 ml of chloramine-T in a citrate buffer (pH = 6.0) and methylene glycol, then mixed and incubated at room temperature for 20 min. In order to remove the excess chloramine-T, 0.5 ml of 3.15 M perchloric acid was added. After 5 min, the samples were treated with 0.5 ml of 20% DMBA and incubated at 60°C in a water bath for 20 min. After cooling the samples, the absorbance was measured at 560 nm. Histamine was assayed by a Histamine Research ELISA, which was used according to the manufacturer's recommendations.

RNA preparation and real-time polymerase chain reaction

1. RNA extraction

Total RNA was extracted with the use of TRIzol®-reagent (Invitrogen Life Technologies, CA, USA) according to the producer's instructions. RNA extracts were purified with the use of DNase I and RNeasy Mini Kit (Qiagen, Hilden, Germany), the integrity of RNA was assessed by 0.8% agarose gel electrophoresis and ethidium bromide staining. RNA was measured with the use of a GeneQuant II spectrophotometer (Pharmacia Biotech, Cambridge, UK).

2. Real-time RT-qPCR.

The quantitative analysis of *TNF*, *MCP1* and *GAPDH* transcripts was carried out using an Opticon™ DNA Engine Sequence Detector (MJ Research Inc., MA, USA) and SYBR Green I chemistry (SYBR Green Quantitect RT-PCR Kit, Qiagen). Amplification was performed using previously described oligonucleotide primers (*Table 1*). The standard curve was appointed for commercially available standards of *β-actin* gene (Applied Biosystems, CA, USA). The reverse transcription was carried out at 50°C for 30 minutes, activation of HotStarTaq DNA Polimerase was carried out at 95°C for 15 minutes, and three-step cycles were: 94°C for 15 seconds, 60°C for 45 seconds and 72°C for 30 seconds (40 cycles). The RT-qPCR products were separated on 8% polyacrylamide gel stained with silver salts. The identification of product length was made by analysis with GelScan v.1.45 software (Kucharczyk TE, Warsaw, Poland) and with regard to the molecular weight marker pBR 322/Hae III (Fermentas International Inc., ON, Canada). T_m (melting temperature) of RT-qPCR products was appointed using DissociationCurve Software (Applied Biosystems).

Materials

Myosin from porcine heart, Freund's Complete Adjuvant, mepyramine maleate, ranitidine hydrochloride, quinapril hydrochloride, o-Dianisidine, 4-(dimethylamino)benzaldehyde (Ehrlich's reagent) were purchased form Sigma-Aldrich, St. Louis, MO, USA. Natrium chloratum 0.9% was obtained from Baxter Polska, Warsaw, Poland.

Histological staining reagent, *i.e*. Mayer's hematoxylin and Eosin Y were produced by Aqua-Med, Lodz, Poland, whereas Trichrome Stain Kit and Alcian Blue pH 2.5 P.A.S. Kit by Bio-Optica, Milano, Italy. Rabbit polyclonal CD3 antibody and Monoclonal Mouse Anti-Human CD68 clone PG-M1 were purchased form DakoCytomation, Glostrup, Denmark; Monoclonal Antibody Troponin C clone 1A2 was bought from Leica Biosystems Newcastle Ltd., UK.

Histamine Research ELISA kit was purchased from Demeditec Diagnostics GmbH, Germany.

Data analysis

Data were analysed with GraphPad Prism 6 (San Diego, CA, USA). Means ± SEM were calculated. The Shapiro-Wilk's test was performed to determine whether the data distribution is normal. Differences between groups were tested by one-way ANOVA followed by Dunnett's or Tukey's multiple comparison test, respectively. Comparisons of survival curves were done with Long-rank (Mantel-Cox) test. Post hoc multiple comparison for each treatment against EAM was carried out and Bonferroni correction for multiple comparisons used to set the threshold for declaration of significance. The criterion for significance for all the comparisons was $P < 0.05$. Pearson's correlation coefficient (r) was used to measure of the strength of the association between the indicators reflecting myocardial hypertrophy. Only correlations significant at $P < 0.05$ were considered.

RESULTS

Time course of inflammation as assessed by ceruloplasmin activity measurement; the effect of the applied drugs

Plasma ceruloplasmin activity was increased by myosin immunization, its peak at 2 weeks coincided with either the

Table 1. Oligonucleotide primers used for detection of mRNA of genes TNF-α, MCP-1, GAPDH and β-actin gene. MCP-1, monocyte chemoattractant protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; F, forward primer; R, reverse primer; r, rat; Tm, melting temperature; bp, base pairs.

Symbol of the primer	Sequence	Gene symbol	T_m of the product	Product lenght	References
rTNF-α F	5'- CGTCGTAGCAAACCACCAAGC -3'	rTNF-α	84.0	296 bp	31
rTNF-α R	5'- ACCAGGGCTTGAGCTCAGCTC -3'				
rMCP-1 F	5'- CTCAGCCAGATGCAGTTAATGC -3'	rMCP-1	79.6	82 bp	32
rMCP-1 R	5'- TTCTCCAGCCGACTCATTGG -3'				
rGAPDH F	5'- AACTCCCTCAAGATTGTCAGCAA -3'	rGAPDH	81.5	107 bp	32
rGAPDH R	5'- GTGGTCATGAGCCCTTCCA -3'				
βF	5'- TCACCCACACTGTGCCCATCTACGA-3'	β-actin	85.6	295 bp	33
βR	5'- CAGCGGAACCGCTCATTGCCAATGG-3'				

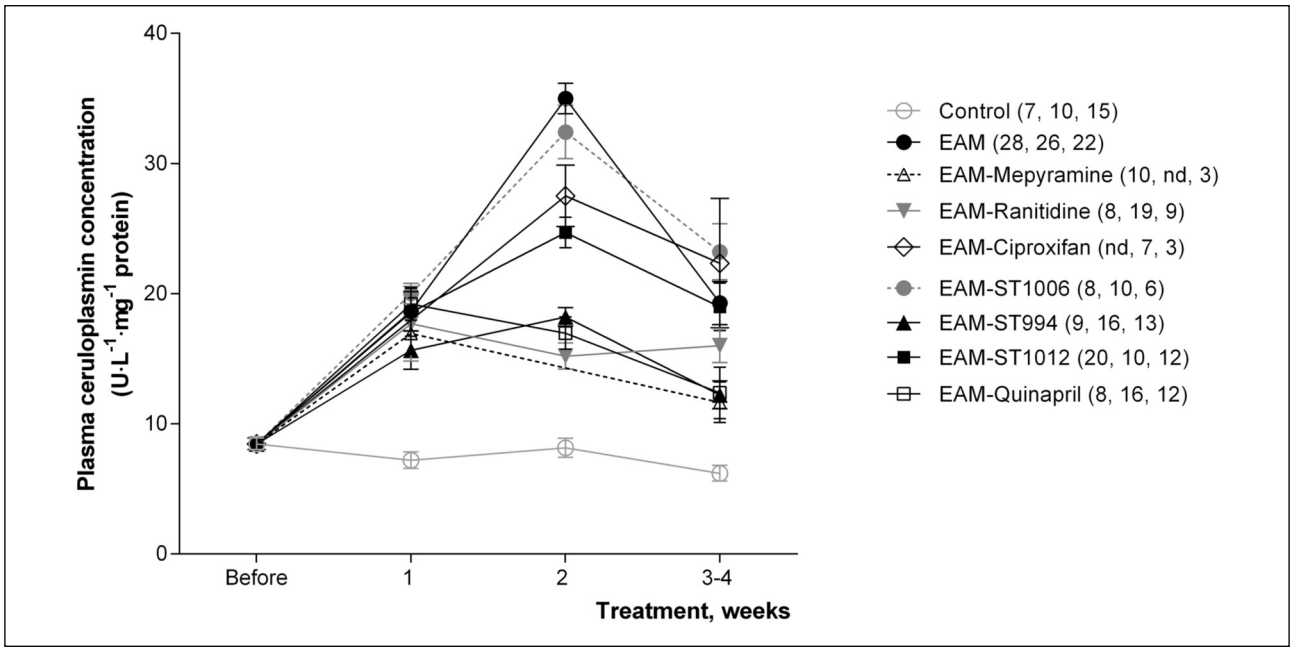

Fig. 1. Plasma ceruloplasmin activity in EAM rats, untreated and treated with histamine H_1 – H_4 receptors' ligands. Quinapril was used as a reference compound. The values are means ± SEM. Numbers in brackets refer to number of experimental animals for each group in the following weeks of the experiment, *i.e.* 1st, 2nd or 3rd 4th, respectively. At the beginning of the experiment (Before), plasma concentrations of ceruloplasmin were measured in 45 rats. EAM, experimental autoimmune myocarditis; nd, not determined.

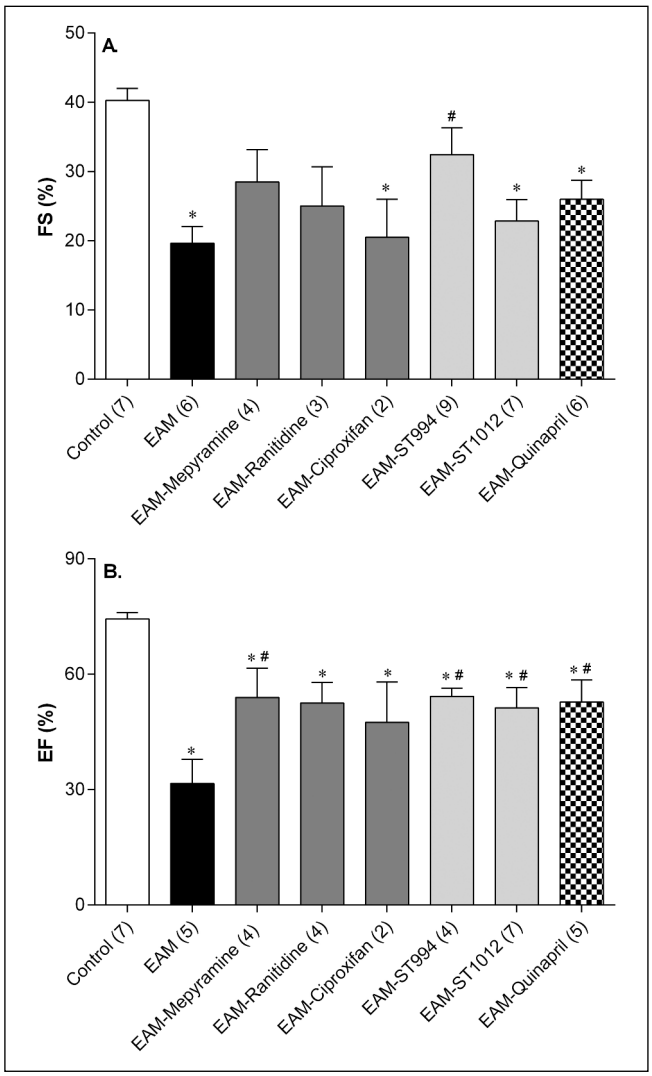

Fig. 2. Left ventricular fractional shortening (FS) and ejection fraction (EF) of male Lewis rats with autoimmune myocarditis (EAM) treated with histamine H_1 – H_4 receptor ligands measured one week after completion of treatment. The histamine H_1 – H_4 receptor ligands were administered for 2 weeks following first immunization as described in Methods. Quinapril, an ACE inhibitor, was used as a reference compound. The values are means ± SEM. Numbers in brackets refer to number of experimental animals for each group. One-way ANOVA and Dunnett's multiple comparison test: * versus the corresponding control group, # versus the corresponding EAM group.

Table 2. Selected echocardiographic parameters of male Lewis rats with autoimmune myocarditis treated with histamine H1 – H4 receptors ligands and control animals. The histamine H1 – H4 receptors ligands were administered for 2 weeks following first immunization as described in Materials and Methods. Quinapril, an ACE inhibitor, was used as a reference compound. The values are means ± SEM for number of experimental animals given in brackets. One-way ANOVA and Dunnett's multiple comparison test: * versus the corresponding control group, # versus the corresponding EAM group. EAM, experimental autoimmune myocarditis; 2 wk, 2 weeks post immunization; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; IVSd, end-diastolic ventricular septum thickness; IVSs, end-systolic ventricular septum thickness; PWd, end-diastolic posterior wall thickness; PWs, end-systolic posterior wall thickness; EF, left ventricular ejection fraction

Parameter	Control	EAM	Treated EAM					
			EAM-Mepyramine	EAM-Ranitidine	EAM-Ciproxifan	EAM-ST994	EAM-ST1012	EAM-Quinapril
LVDd (cm)								
Before experiment	0.35 ± 0.03 (7)	0.36 ± 0.01 (19)	0.34 ± 0.01 (10)	0.34 ± 0.01 (10)	0.38 ± 0.01 (9)	0.33 ± 0.02 (8)	0.32 ± 0.01 (10)	0.35 ± 0.03 (9)
2 wk	0.37 ± 0.02 (7)	0.42 ± 0.02 (18)	0.42 ± 0.02 (9)	0.42 ± 0.01 (10)	0.45 ± 0.02 (9)	0.42 ± 0.01 (8)	0.39 ± 0.01 (9)	0.41 ± 0.03 (8)
LVDs (cm)								
Before experiment	0.21 ± 0.01 (7)	0.22 ± 0.01 (19)	0.19 ± 0.01 (10)	0.20 ± 0.01 (10)	0.26 ± 0.01 (9)	0.21 ± 0.01 (9)	0.20 ± 0.01 (10)	0.26 ± 0.02 (9)
2 wk	0.22 ± 0.01 (7)	0.27 ± 0.01* (18)	0.25 ± 0.02 (9)	0.25 ± 0.01 (10)	0.29 ± 0.01* (9)	0.26 ± 0.01 (9)	0.22 ± 0.01$^{\#}$ (9)	0.25 ± 0.02 (9)
FS (%)								
Before experiment	37.71 ± 2.11 (7)	37.61 ± 0.83 (18)	42.20 ± 1.54 (10)	40.22 ± 1.28 (9)	31.67 ± 3.43 (9)	36.11 ± 3.41 (9)	39.40 ± 1.44 (10)	39.33 ± 2.37 (9)
2 wk	40.29 ± 1.73 (7)	31.93 ± 1.67 (15)	38.00 ± 2.56 (8)	38.33 ± 2.95 (9)	34.11 ± 2.25 (9)	33.67 ± 2.35 (9)	43.11 ± 2.37$^{\#}$ (9)	33.67 ± 2.65 (8)
IVSd (cm)								
Before experiment	0.16 ± 0.01 (7)	0.16 ± 0.01 (19)	0.17 ± 0.01 (10)	0.17 ± 0.01 (10)	0.16 ± 0.01 (9)	0.17± 0.01 (9)	0.16 ± 0.01 (9)	0.17 ± 0.01 (9)
2 wk	0.17 ± 0.01 (7)	0.21 ± 0.01* (18)	0.21 ± 0.01 (9)	0.26 ± 0.01*$^{\#}$ (9)	0.21 ± 0.01 (9)	0.19 ± 0.01 (9)	0.22 ± 0.02* (9)	0.17 ± 0.01$^{\#}$ (8)
IVSs (cm)								
Before experiment	0.20 ± 0.01 (7)	0.20 ± 0.01 (19)	0.22 ± 0.01 (10)	0.21 ± 0.01 (10)	0.20 ± 0.01 (9)	0.22 ± 0.02 (9)	0.20 ± 0.01 (9)	0.22 ± 0.01 (9)
2 wk	0.22 ± 0.01 (7)	0.27 ± 0.01* (18)	0.28 ± 0.02* (9)	0.32 ± 0.01*$^{\#}$ (10)	0.28 ± 0.01 (9)	0.23 ± 0.01 (9)	0.26 ± 0.02 (9)	0.22 ± 0.01$^{\#}$ (8)
PWd (cm)								
Before experiment	0.19 ± 0.01 (7)	0.18 ± 0.01 (19)	0.17 ± 0.01 (10)	0.18 ± 0.01 (10)	0.18 ± 0.01 (9)	0.20 ± 0.01 (9)	0.19 ± 0.01 (9)	0.19 ± 0.01 (8)
2 wk	0.21 ± 0.01 (7)	0.29 ± 0.02* (18)	0.31 ± 0.03* (9)	0.33± 0.01* (9)	0.37 ± 0.04*$^{\#}$ (9)	0.23 ± 0.01 (9)	0.26 ± 0.02 (8)	0.22 ± 0.01 (6)
PWs (cm)								
Before experiment	0.26 ± 0.01 (7)	0.25 ± 0.01 (19)	0.23 ± 0.01 (10)	0.25 ± 0.01 (10)	0.25 ± 0.01 (9)	0.26 ± 0.01 (9)	0.24 ± 0.01 (9)	0.28 ± 0.01 (9)
2 wk	0.28 ± 0.01 (7)	0.36 ± 0.02 (18)	0.39 ± 0.02* (9)	0.35 ± 0.02 (9)	0.44 ± 0.04*$^{\#}$ (9)	0.32 ± 0.01 (9)	0.34 ± 0.03 (9)	0.32 ± 0.03 (6)
EF (%)								
Before experiment	77.29 ± 2.54 (7)	75.39 ± 1.22 (18)	77.30 ± 2.60 (10)	75.90 ± 2.63 (10)	73.50 ± 2.51 (6)	75.75 ± 2.41 (8)	76.50 ± 1.78 (10)	76.89 ± 2.60 (9)
2 wk	76.71 ± 2.32 (7)	59.29 ± 2.45* (14)	71.33 ± 2.99$^{\#}$ (9)	66.22 ± 3.13 (9)	73.78 ± 3.96$^{\#}$ (9)	69.38 ± 2.88 (8)	78.00 ± 2.41$^{\#}$ (9)	72.67 ± 2.13$^{\#}$ (9)

completion of treatment or its beginning. Except for rats from the ST1006 group, rats that were treated immediately post immunization with histamine receptor ligands showed, like those treated with quinapril, significantly lower plasma ceruloplasmin values than their untreated counterparts. Within the 2 next weeks the enzyme activity decreased in all animals except for the EAM-ranitidine group. However, the rats from EAM, EAM-Ciproxifan and EAM-ST1006 groups all had still over 3 fold the normal while EAM-mepyramine, EAM-quinapril and EAM-ST994 had roughly 2 fold (*Fig. 1*).

Echocardiography examination performed at the end of 2 weeks' therapy revealed that, in comparison with cardiac parameters measured before EAM induction, the untreated EAM-rats exhibited an increase in the diastolic and systolic dimensions of LVD, IVS and PW with a parallel decrease of LVEF (21%). On the other hand, although none of the administered histamine H_1 – H_4 antagonists prevented left ventricle dilation, all treated rats, with the exception of EAM-ranitidine group that had ejection fraction decreased by 13%, kept the initial cardiac function. The rats treated with ranitidine exhibited significantly thicker septa while the animal group treated with ciproxifan, H_3R antagonist, showed the highest posterior wall thickening (*Table 2*).

The following week's examination, however, clearly showed that when there was no further treatment the heart function was dramatically reduced in all rat groups. It is worth noting that the rats from EAM-ST994, and EAM-mepyramine groups had the lowest decrement of fractional shortening, *i.e.* 10% and 25%, respectively, while their left ventricular ejection fraction was reduced by roughly 22% and 25%. At the same time in the untreated EAM rats the FS was decreased by 38% and the LVEF by 47% (*Fig. 2*).

Macroscopic evaluation at autopsy done 4 weeks after the first myosin immunization showed that cardiac hypertrophy and fibrosis was present in all EAM rats, either untreated or treated by any drug. The hearts from untreated EAMs were usually bigger and not soft; the inflammatory changes were diffuse, infiltrating the heart wall causing its induration and deformation, while in the hearts of rats treated with quinapril and some histamine receptor ligands, with the exception of ciproxifan and ST1006, the changes were focal, scattered and concerned mostly the heart surface.

The heart weight to body weight ratio was increased 2-3-folds in diseased rats, with the lowest values found in the ST994-treated EAM group (*Fig. 3A*). Accordingly, the diseased hearts, enriched in tough fibrous tissue, differ in their hydroxyproline and histamine concentrations. The left ventricular hydroxyproline concentration showed a positive correlation with hypertrophy while that of histamine a negative one (*Fig. 3B*, *3C*).

In most rats with immune myocarditis, but not in those treated early with ST994 and quinapril, infiltration of myocardium with macrophages and monocytes (*Fig. 4A*, *4B*) could be documented /inferred by high levels of transcripts of proinflammatory cytokine tumor necrosis factor-α (TNF-α) as well as monocyte chemoattractant protein-1 (MCP-1), EAM- the ranitidine group having them the highest.

Histologically inflammation can imitate acute rejection and the autoimmunity model of myocarditis can be considered as acute rejection. Therefore the International Society for Heart and Lung Transplantation working formulation (ISHLT 2004) of cardiac allograft pathology was used for the assessment of cellular infiltration and the severity of myocardial damage (34) (*Table 3*).

Table 3. Severity of cellular infiltration and myocardial damage. The International Society for Heart and Lung Transplantation working formulation (ISHLT 2004) of cardiac allograft pathology was used for assessment of cellular infiltration and the severity of myocardial damage. The data show the number of rats with a definite degree of myocardial injury in relation to all analyses carried out in the group.

Group	Grade			
	0	**1R, mild**	**2R, moderate**	**3R, severe**
Control	9 / 9	–	–	–
EAM	–	1 / 10	2 / 10	7 / 10
EAM-mepyramine	–	1 / 10	5 /10	4 / 10
EAM-ranitidine	–	1 / 8	2 /8	5 / 8
EAM-ciproxifan	–	2 / 9	1 /9	6 / 9
EAM-ST1006	–	1 / 9	1 / 9	7 / 9
EAM-ST994	3 / 11	7 / 11	1 / 11	–
EAM-ST1012	1 / 10	2 / 10	5 / 10	2 / 10
EAM-quinapril	–	7 / 9	2 / 9	–

Rats from groups EAM-ST1006 > EAM > EAM-ciproxifan > EAM-ranitidine presented the highest grade of heart damage while the lowest EAM-quinapril and EAM-ST994. Myocardial specimens were analysed for myocyte damage, microvasculopathy (CMV), and fibrosis. For this first, myocytolysis, coagulation necrosis, and healing ischemic lesions were assessed. Histologic features of cardiac CMV include unspecific changes, such as concentric intimal thickening with or without foamy macrophages and sub-endothelial accumulation of lymphocytes (endothelialitis) while for fibrosis perivascular, interstitial, and replacement ones were examined (*Table 4*).

As presented in *Table 3* and *4* and as illustrated in *Fig. 5*, treatment with the ligands blocking histamine signalling, especially *via* H_4R attenuated EAM; the inflammation, myocardial damage and fibrosis were reduced and survival rate (*Fig. 6A*) was increased as compared to untreated counterparts. Median survival which was undefined for EAM-ST994, EAM-ST1012 and EAM-quinapril was 22.5 days for EAM and even less – 20 days for EAM-ranitidine.

When the drug treatment started at the peak of inflammation (after 2 weeks EAM) the animal mortality was very high. Of the groups consisting of 9 – 10 rats, only 1/10 EAM and 1/9 mepyramine-treated, compared with 5/10 treated with EAM-ST994, survived till the end of the fourth week median survival being respectively 18.5, 22 and 25.5 days (*Fig. 6B*).

These data indicate a greater benefit of H4R antagonists in experimental autoimmune myocarditis.

DISCUSSION

This is the first study disclosing the effects of *in vivo* treatment of rats with experimental myocarditis with compounds directed each against each of the four histamine receptors (H_1R - H_4R). The obtained results suggest that among the ligands used, these blocking histamine H_4 receptors express the highest therapeutic potential against chronic heart failure. We could observe, like the others (19, 26, 27, 35), that EAM evoked by cardiac myosin immunization was characterized by prominent enlargement of the heart, dilatation of ventricles, diffuse and extensive myocardial fibrosis and an increased level of proinflammatory cytokine and chemokine in cardiac tissue, as measured here by TNF-α and MCP-1 transcripts. Transthoracic echocardiography has shown that these changes were associated with a dramatic fall in left ventricular ejection fraction and shortening fraction. There is compelling evidence that the renin-angiotensin-aldosterone system regulates the fibrotic response in myocardial remodelling triggered by extensive myocarditis, and that the cardioprotective effects of ACE inhibitors may be partly due to a suppression of the fibrotic response through inhibition of TGF-β1 and extracellular matrix components (35-38). Accordingly, quinapril, the angiotensin converting enzyme inhibitor, used in our experiments as a reference drug, significantly reduced the myosin-induced inflammation and remodelling when given as preventive measure. In the cardiac tissue of quinapril treated rats the mRNA expressions of TNF-α and MCP-1 were significantly reduced. The fibrosis (perivascular and interstitial but not the replacement one) was present in 20% and 30% of these rats while among untreated EAM rats' hearts as many as 50% showed perivascular, 70% interstitial and 40% replacement fibrosis. Quinapril also improved myocardial function reflected by ejection and fractional shortening fractions (EF, FS) as well as survival rates. Comparable results were obtained for rats from the EAM-ST994 group. However, one must take into account that the daily dose of the H_4R ligand was twenty time lower (1 mg/kg/day versus 20 mg/kg/day); possibly, the results would be more spectacular if it were bigger. Such high effectiveness of the compound ST994 is related to a preponderant distribution of H_4 receptors on cells and in organs involved in immunological and inflammatory responses, *i.e.* mast cells, basophils, eosinophils, monocytes, dendritic cells, T cells, spleen, bone marrow and lungs. A vast body of literature indicates that H_4Rs mediate chemotaxis of mast cells, leukocytes and dendritic cells, influence the production of various cytokines and chemokines, *e.g.* stimulate proinflammatory cytokine TNF-α production by mast cells, anti-inflammatory IL-10 production by Treg cell, interferon gamma by Th1/Th2 cells, they also suppress production of proinflammatory IL-12 or chemokine CCL-2 production by monocytes and dendritic cells (5, 15, 16, 18, 39-41). However, in addition to H_4R, hematopoietic and immune cells also bear H_1 and H_2 receptors coupled to different transduction pathways, so by varying the expression of these three proteins the resulting effects of histamine could sometimes be different than assumed.

Promising results of experimental studies (42-45) made H_4R an attractive target for inflammatory and autoimmune diseases in human treatments. So far clinical studies have been reported in pruritus, atopic dermatitis, rheumatoid arthritis, asthma, allergic rhinitis and psoriasis. The H_4R antagonist JNJ

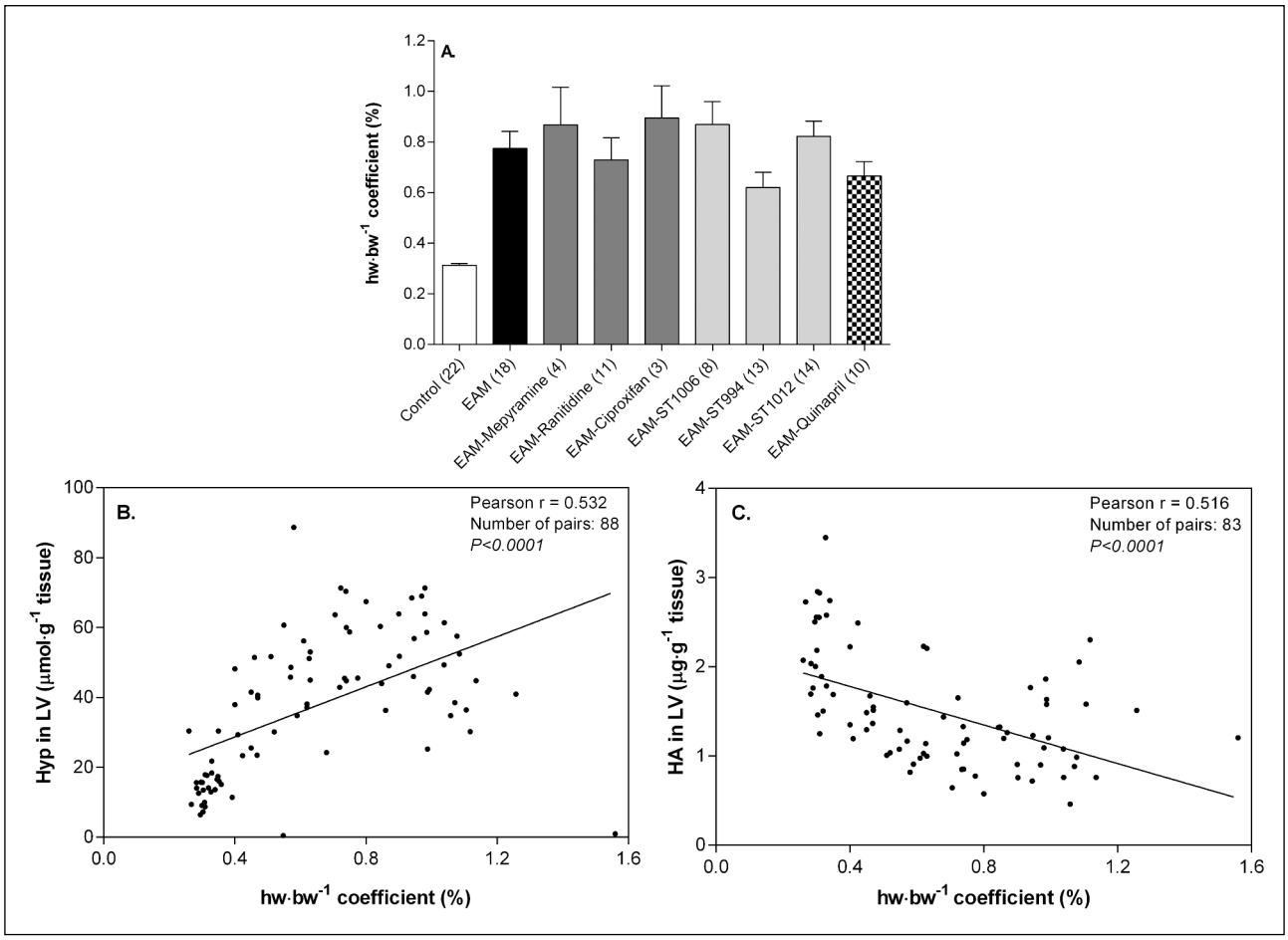

Fig. 3. The heart weight to body weight ratio of EAM rats, untreated and treated with histamine H_1 – H_4 receptors' ligands (A) and statistically significant correlations between indicators reflecting myocardial hypertrophy (B, C). (A): The ratio of heart weight (hw) to body weight (bw) (hw·bw^{-1} coefficient) was calculated as follows: [hw (g) divided by bw (g) × 100]. The values are means ± SEM. Numbers in brackets refer to number of experimental animals for each group. Quinapril was used as a reference compound. The hw·bw^{-1} coefficient in all EAM groups is statistically significantly different from the Control (one-way ANOVA and Dunnett's multiple comparisons test). (B, C): Pearson's correlation coefficient (r) was used to measure of the strength of the association between the indicators reflecting myocardial hypertrophy. EAM, experimental autoimmune myocarditis, hw, heart weight; bw, body weight; LV, left ventricle; Hyp, hydroxyproline; HA, histamine.

Table 4. Histological evaluation of the rats' hearts with autoimmune myocarditis; frequency of myocyte damage, microvasculopathy and fibrosis occurrence in each of the studied group. The data show the number of rats with a definite degree of myocardial injury in relation to all analyses carried out in the group.

Parameter /Group	Control	EAM	EAM &						
			Mepyramine	Ranitidine	Ciproxifan	ST-1006	ST-994	ST-1012	Quinapril
Myocyte damage									
Myocytolysis	0 / 9	10 / 10	4 / 10	6 / 8	3 / 10	9 / 9	2 / 11	4 / 10	6 / 9
Coagulation necrosis	0 / 9	7 / 10	6 / 10	4 / 8	4 / 9	7 / 9	1 / 11	1 / 10	3 / 9
Healing lesions	0 / 9	5 / 10	8 / 10	3 / 8	6 / 9	5 / 9	0 / 11	0 / 10	4 / 9
Cardiac microvasculopathy									
Concentric intimal thickening without foamy macrophages	0 / 9	7 / 10	8 / 10	5 / 8	4 / 9	6 / 9	2 / 11	1 / 10	4 / 9
Concentric intimal thickening with foamy macrophages	0 / 9	5 / 10	6 / 10	6 / 8	5 / 9	7 / 9	0 / 11	0 / 10	4 / 9
Sub-endothelial accumulation of lymphocytes (*endothelialitis*)	0 / 9	4 / 10	4 / 10	7 / 8	5 / 9	8 / 9	0 / 11	0 / 10	4 / 9
Fibrosis									
Perivascular	0 / 9	5 / 10	3 / 10	3 / 8	4 / 9	6 / 9	2 / 11	4 / 10	2 / 9
Interstitial	0 / 9	7 / 10	5 / 10	3 / 8	7 / 9	7 / 9	4 / 11	2 / 10	3 / 9
Replacement	0 / 9	4 / 10	5 / 10	2 / 8	3 / 9	6 / 9	2 / 11	0 / 10	0 / 9

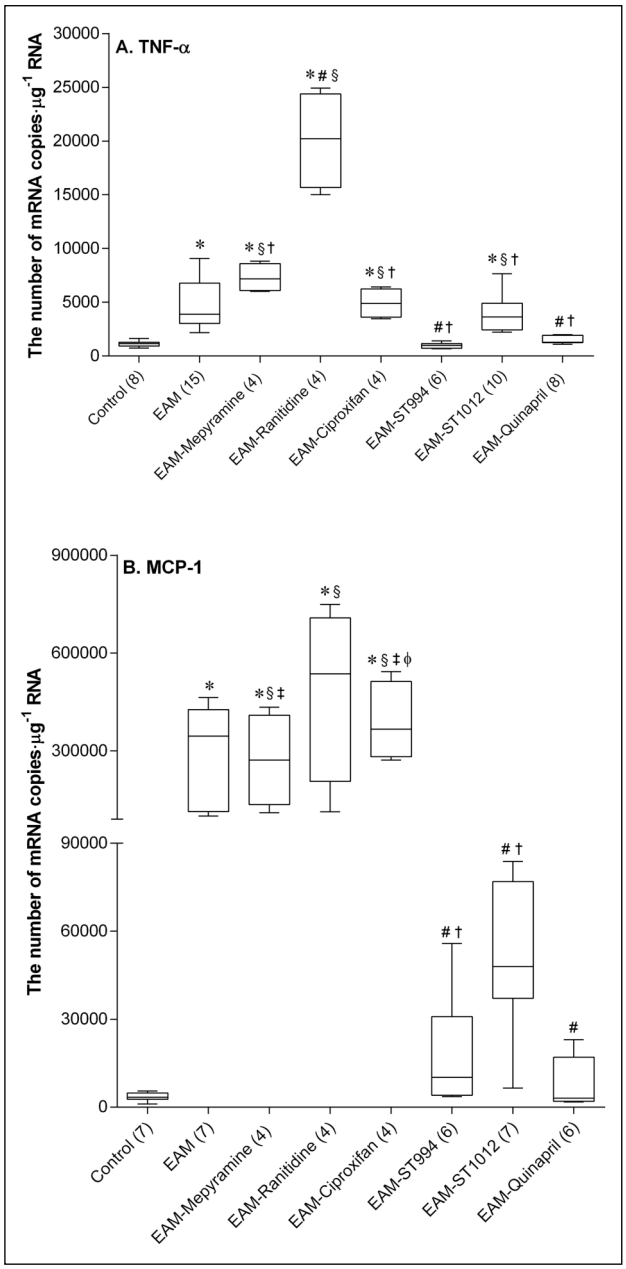

Fig. 4. Myocardial mRNA expression of TNF-α (A) and MCP-1 (B) in left ventricle in rats with autoimmune myocarditis; the effect of treatment with histamine H_1 – H_4 receptors ligands and quinapril, ACE inhibitor. Median (the line in the middle of box) and the range of values (whiskers) correspond for number of rats given in brackets. One-way ANOVA and Tukey's Multiple Comparisons Test: * versus Control (intact animals), # versus EAM, † versus EAM-Ranitidine, ‡ versus EAM-ST994, ϕ versus EAM-ST1012, § versus EAM-Quinapril. EAM, experimental autoimmune myocarditis; MCP-1, monocyte chemoattractant protein-1.

39758979 has exhibited efficacy in atopic dermatitis and in blocking histamine-induced itch (15, 16, 18, 46). Our results reported here on experimental autoimmune myocarditis in rats, which resembles the human disorder, suggest an H_4R antagonist would be of benefit in myocarditis and dilated cardiomyopathy. It inhibited heart remodelling and preserved heart contractions by limiting inflammatory processes. Not only did its positive effects last beyond the 2-week treatment – the decrease of EF and FS between the 2nd and 3rd week was the lowest in the EAM-ST994 group, moreover, the animal survival rate was the highest, which was clearly seen in the 2nd scheme of treatment, where the drug administration begins at the peak of inflammation (*i.e.* 2 weeks after myosin immunization).

As has already been mentioned histamine H_1R and H_2R are co-expressed along with H_4R on hematopoietic and immune cells. Mostly, H_1R activation facilitates proinflammatory effects and Th1 cell proliferation whereas H_2R stimulation by histamine facilitates mostly anti-inflammatory ones – it inhibits chemotaxis of neutrophils and eosinophils, leukotriene synthesis stimulates IL-10 production (15, 17).

In our study mepyramine, an H_1R antagonist, was more efficient than the other drugs in inhibiting heart dilatation; echocardiography showed the left ventricular dimensions changed the least within the week after treatment completion. However, histopathology revealed the hearts were more fibrotic than in H_4R antagonist or quinapril treated rats; in half of the animals interstitial and replacement fibrosis could be found. Additionally, in most these rats cardiac microvasculopathy was present. In 50% of the rats cellular infiltration and myocardial damage was moderate, and in 40% of the rats severe. Accordingly, these rats also showed a higher number of copies of TNF-α mRNA and MCP-1 mRNA in left ventricle than non-treated EAM rats. Likewise, left ventricle from the EAM-ranitidine group showed a higher expression of TNF-α and MCP-1 transcripts. It was the highest of them all. The severity of cellular infiltration and myocardial damage was estimated as grade 3 in over 60% of rats of this group. It should be mentioned that ranitidine treated EAM showed higher ventricular septum thickness (than non-treated EAM) and their survival was not better than non-treated EAM rats. These data do not match recently reported observations in humans of the reduced risk of heart failure and changes in left ventricular morphology in H_2 receptor antagonist users (47). Newly published results of meta-analyses on cardioprotective effect of histamine H_2R antagonists in human congestive heart failure (CHF) showed that H_2R antagonists reduced heart rate, cardiac output and fractional shortening and their beneficial effect on CHF symptoms was suggested to be due to the decrease in myocardial oxygen demands (48). Our study revealed similar effects of H_2 blocker on cardiac parameters in rats with autoimmune myocarditis treated with ranitidine, however, the possible benefits were counterbalanced by parallel stimulation and maintenance of inflammatory processes. In the hearts of mammals H_3 receptors are present on postganglionic sympathetic nerve terminals and may control the release of noradrenaline (11). Ciproxifan which was employed to block H_3R significantly increased posterior wall thickness causing cardiac hypertrophy and stiffening. A high level of monocyte chemoattractant protein-1, which was detected in cardiac tissue, could make an important contribution to heart failure.

Summing up, the obtained data leave no doubt that to prevent inflammation and myocardial fibrosis H_4R is the most appropriate of the four possible receptor targets mediated by histamine. Alternatively, H_4R and H_1R antagonists could be used in combination.

Abbreviations: CD3, cluster of differentiation 3; CD68, cluster of differentiation 68; CHF, congestive heart failure; DMBA, p-dimethylaminobenzaldehyde; EAM, experimental autoimmune myocarditis; EF, left ventricular ejection fraction; FS, fractional shortening; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HA, histamine; HR, histamine receptor; hw, heart weight; Hyp, hydroxyproline; i.g., intragastrically; IVSd, interventricular septum thickness in diastole; IVSs, interventricular septum thickness in

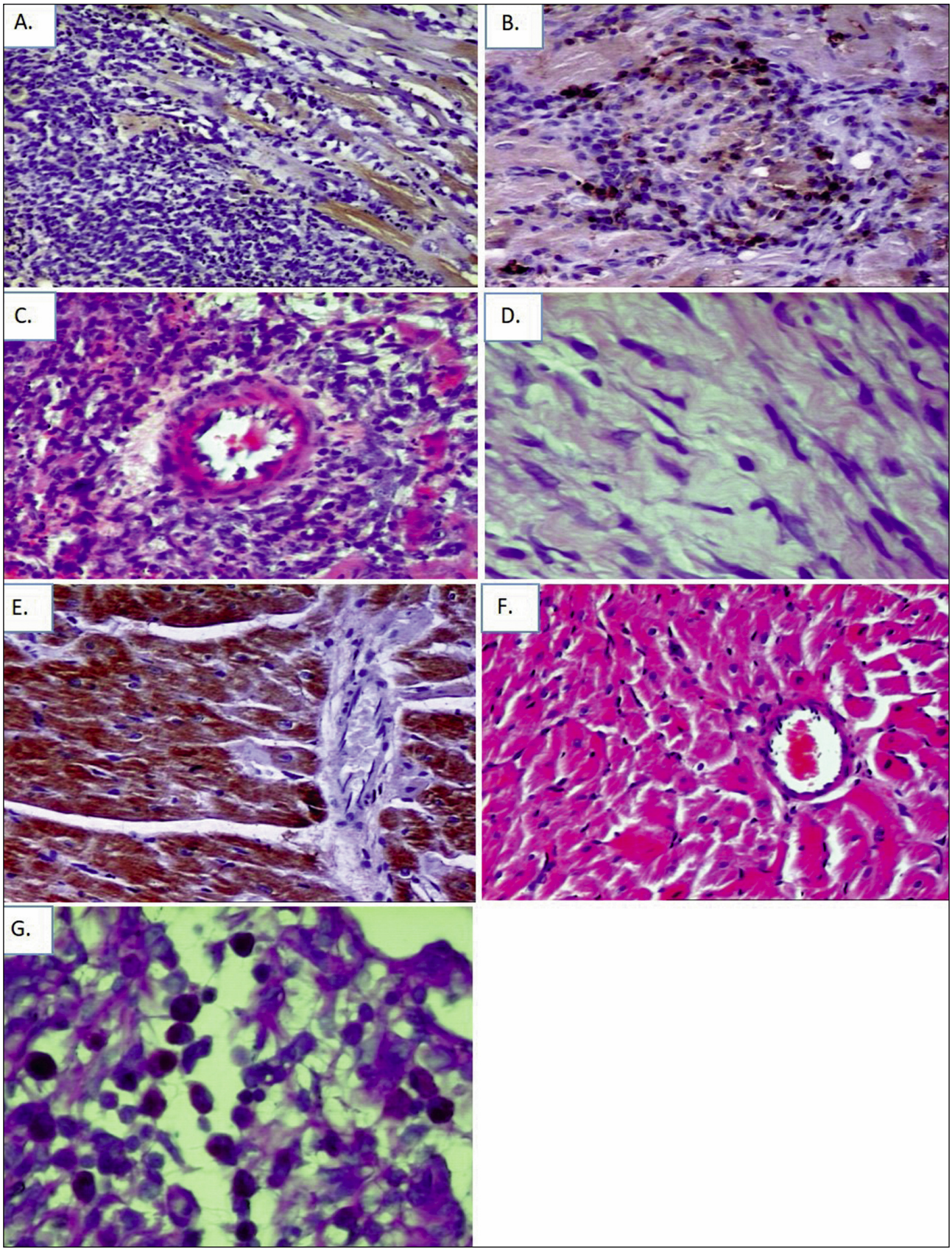

Fig. 5. Representative sections of rat's myocardium showing pathological features and the effect of treatment with H_4R ligand. EAM group: (A): Necrosis area filled with granulation tissue (stained with Anti-Troponin C), (B): CD3 positive staining of T-cells, (C): A blood vessel with thickened wall, swollen and deformed endothelial cells, (D): Area of fibrosis (fibroblasts and young collagen fibers). EAM-ST1012 group: (E): Rather regular expression of troponin C in myocardial fibers in non-affected area (stained with Anti-Troponin C), (F): Unchanged blood vessel among normal myocardial structure, (G): Mast cells with no visible degranulation (stained with Alcian Blue). Magnification: × 200 (A, B, C, E, F); × 400 (D, G).

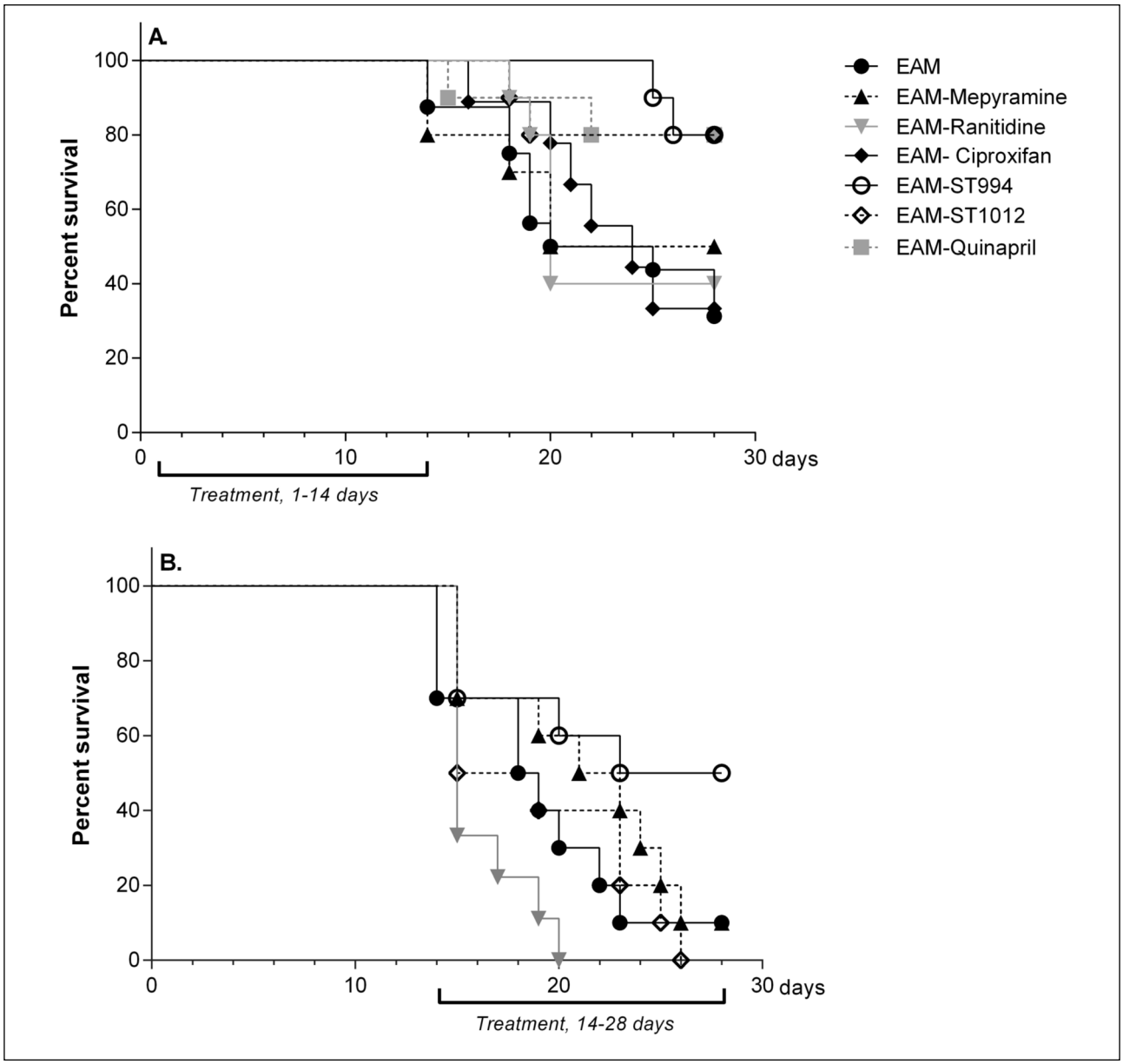

Fig. 6. Kaplan-Meier survival curves of EAM rats untreated and treated with histamine receptors' ligands in two periods of experiment: 1 – 14 days (A) and 14 – 28 days (B). At the beginning of the experiment, each group consisted of 9 – 10 animals. Long-rank (Mantel-Cox) test has shown that the survival curves significantly differ for (A) and (B) with $P < 0.05$. *Post hoc* multiple comparison for each treatment against EAM was carried out and Bonferroni correction for multiple comparisons used to set the threshold for declaration of significance. The differences were statistically significant for (A): EAM versus EAM-ST994; EAM versus EAM-ST1012 and EAM versus EAM-Quinapril; while for (B): EAM-ST994 versus EAM-Ranitidine. EAM versus EAM-ST994, $P = 0.0565$ (NS).

systole; LV, left ventricle; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; MCP-1, monocyte chemoattractant protein-1; PWd, end-diastolic posterior wall thickness; PWs, end-systolic posterior wall thickness; s.c., subcutaneously; ST1006, N4-(2,6-dichlorobenzyl)-6-(4-methylpiperazin-1-yl)pyrimidine-2,4-diamine; ST1012, 4-(isoindolin-2-yl)-6-(4-methylpiperazin-1-yl)pyrimidin-2-amine; ST994, N4-(4-methylbenzyl)-6-(4-methylpiperazin-1-yl)pyrimidine-2,4-diamine.

Author's contributions: Conception and design of the experiments, collection, analysis and interpretation of data were carried out by W.A.F., A.S. and H.S.; A.S. and W.A.F were involved in all experiments execution and manuscript preparation. Molecular biology analyses were done by J.G. and U.M.; K.K. performed rat transthoracic echocardiography, J.K. was responsible for morphological and immunohistochemical analyses, M.M. interpreted the cardiac results. All authors discussed the results, commented on the manuscript and approved its final version.

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REFERENCES

1. Rose NR. Myocarditis: infection versus autoimmunity. *J Clin Immunol* 2009; 29: 730-737.
2. Jensen LD, Marchant DJ. Emerging pharmacologic targets and treatments for myocarditis. *Pharmacol Ther* 2016; 161: 40-51.
3. Jochem J, Zwirska-Korczala KW. Histamine and the cardiovascular system. In: Histamine: Biology and Medical Aspects, A Falus, N Grosman, Z Darvas (eds.). Basel, Karger; Budapest, SpringMed Publishing Ltd. 2004, pp. 303-316.
4. Jochem J, Altinbas B, Yalcin M, *et al*. Involvement of the histaminergic system in the resuscitating effect of centrally acting leptin in haemorrhagic shock in rats. *J Physiol Pharmacol* 2016; 67: 67-74.
5. Tanaka S, Ichikawa A. Recent advances in molecular pharmacology of the histamine systems: immune regulatory roles of histamine produced by leukocytes. *J Pharmacol Sci* 2006; 101: 19-23.
6. Stark H. Histamine Receptors. *Biotrend Reviews* 2007; 1: 1-7.
7. Wolff AA, Levi R. Histamine and cardiac arrhythmias. *Circ Res* 1986; 58: 1-16.
8. Malinowska B, Schlicker E. Identification of endothelial H1, vascular H2 and cardiac presynaptic H3 receptors in the pithed rat. *Naunyn Schmiedebergs Arch Pharmacol* 1993; 347: 55-60.
9. Yamamoto S, Tamai I, Takaoka M, Matsumura Y. Role of histamine H3 receptors during ischemia/reperfusion in isolated rat hearts. *J Cardiovasc Pharmacol* 2004; 43: 353-357.
10. Aldi S, Takano K, Tomita K, *et al*. Histamine H4-receptors inhibit mast cell renin release in ischemia/reperfusion via protein kinase Cε-dependent aldehyde dehydrogenase type-2 activation. *J Pharmacol Exp Ther* 2014; 349: 508-517.
11. Hattori Y, Hattori K, Matsuda N. Regulation of the cardiovascular system by histamine. In: Histamine and Histamine Receptors in Health and Disease. Handbook of Experimental Pharmacology, Y. Hattori, R. Seifert (eds.). Cham, Switzerland, Springer Int. Publ. 2016, pp. 239-258.
12. Schneider E, Leite-de-Moraes M, Dy M. Histamine, immune cells and autoimmunity. *Adv Exp Med Biol* 2010; 709: 81-94.
13. Kim J, Ogai A, Nakatani S, *et al*. Impact of blockade of histamine H2 receptors on chronic heart failure revealed by retrospective and prospective randomized studies. *J Am Coll Cardiol* 2006; 48: 1378-1384.
14. Matsumori A, Yamamoto K, Shimada M. Cetirizine a histamine H1 receptor antagonist improves viral myocarditis. *J Inflamm* (*Lond*) 2010; 7: 39. doi: 10.1186/1476-9255-7-39.
15. Tiligada E, Zampeli E, Sander K, Stark H. Histamine H3 and H4 receptors as novel drug targets. *Expert Opin Investig Drugs* 2009; 18: 1519-1531.
16. Zampeli E, Tiligada E. The role of histamine H4 receptor in immune and inflammatory disorders. *Br J Pharmacol* 2009; 157: 24-33.
17. Baumer W, Rossbach K. Histamine as an immunomodulatory [in German]. *J Dtsch Dermatol Ges* 2010; 8: 495-504.
18. Walter M, Kottke T, Stark H. The histamine H4 receptor: targeting inflammatory disorders. *Eur J Pharmacol* 2011; 668: 1-5.
19. Kodama M, Matsumoto Y, Fujiwara M, Masani F, Izumi T, Shibata A. A novel experimental model of giant cell myocarditis induced in rats by immunization with cardiac myosin fraction. *Clin Immunol Immunopathol* 1990; 57: 250-262.
20. McGrath JC, Drummond GB, McLachlan EM, Kilkenny C, Wainwright CL. Guidelines for reporting experiments involving animals: the ARRIVE guidelines. *Br J Pharmacol* 2010; 160: 1573-15736.
21. McGrath JC, McLachlan EM, Zeller R. Transparency in research involving animals: The Basel Declaration and new principles for reporting research in BJP manuscripts. *Br J Pharmacol* 2015; 172: 2427-2432.
22. Culy CR, Jarvis B. Quinapril: a further update of its pharmacology and therapeutic use in cardiovascular disorders. *Drugs* 2002; 62: 339-385.
23. Sander K, Kottke T, Tanrikulu Y, *et al*. 2,4-Diaminopyrimidines as histamine H4 receptor ligands - Scaffold optimization and pharmacological characterization. *Bioorg Med Chem* 2009; 17: 7186-7196.
24. Diehl KH, Hull R, Morton D, *et al*. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol* 2001; 21: 15-23.
25. Schiller NB, Shah PM, Crawford M, *et al*. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 1989; 2: 358-367.
26. Kumral ZN, Sener G, Ozgur S, *et al*. Regular exercise alleviates renovascular hypertension-induced cardiac/endothelial dysfunction and oxidative injury in rats. *J Physiol Pharmacol* 2016; 67: 45-55.
27. Kamal FA, Watanabe K, Ma M, *et al*. A novel phenylpyridazinone T-3999, reduces the progression of autoimmune myocarditis to dilated cardiomyopathy. *Heart Vessels* 2011; 26: 81-90.
28. Lehmann HP, Schosinsky KH, Beeler MF. Standardization of serum ceruloplasmin concentrations in international enzyme units with o-dianisidine dihydrochloride as substrate. *Clin Chem* 1974; 20: 1564-1567.
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265-275.
30. Woessner JF. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch Biochem Biophys* 1961; 93: 440-447.
31. Wong LY, Cheung BM, Li YY, Tang F. Adrenomedullin is both proinflammatory and antiinflammatory: its effects on gene expression and secretion of cytokines and macrophage migration inhibitory factor in NR8383 macrophage cell line. *Endocrinology* 2005; 146: 1321-1327.
32. Kohno T, Anzai T, Naito K, *et al*. Angiotensin-receptor blockade reduces border zone myocardial monocyte chemoattractant protein-1 expression and macrophage infiltration in post-infarction ventricular remodeling. *Circ J* 2008; 72: 1685-1692.
33. Nakajima-Iijima S, Hamada H, Reddy P, Kakunaga T. Molecular structure of the human cytoplasmic beta-actin gene; interspecies homology of sequences in the introns. *Proc Nat Acad Sci* 1985; 82: 6133-6137.
34. Stewart S, Winters GL, Fishbein MC, *et al*. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant* 2005; 24: 1710-1720.
35. Juan W, Nakazawa M, Watanabe K, *et al*. Quinapril inhibits progression of heart failure and fibrosis in rats with dilated cardiomyopathy after myocarditis. *Mol Cell Biochem* 2003; 251: 77-82.
36. Boos CJ. Cardiovascular protection with ace inhibitors - more HOPE for EUROPA? *Med Sci Monit* 2004; 10: SR23-SR28.

37. Biscotte SM, Levick SP, Bertling LM, Morgan LG, Janicki JS, Brower GL. Angiotensin II mediated activation of cardiac mast cells. *FASEB J* 2007; 21: A1253-A1253.
38. Watanabe K, Sukumaran V, Veeraveedu PT, *et al*. Regulation of inflammation and myocardial fibrosis in experimental autoimmune myocarditis. *Inflamm Allergy Drug Targets* 2011; 10: 218-225.
39. Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung WP. Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. *J Pharmacol Exp Ther* 2003; 305: 1212-1221.
40. Ling P, Ngo K, Nguyen S, *et al*. Histamine H4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br J Pharmacol* 2004; 142: 161-171.
41. Gutzmer R, Diestel C, Mommert S, *et al*. Histamine H4 receptor stimulation suppresses IL-12p70 production and mediates chemotaxis in human monocyte-derived dendritic cells. *J Immunol* 2005; 174: 5224-5232.
42. Takahashi Y, Kagawa Y, Izawa K, Ono R, Akagi M, Kamei C. Effect of histamine H4 receptor antagonist on allergic rhinitis in mice. *Int Immunopharmacol* 2009; 9: 734-738.
43. Dunford PJ, O'Donnell N, Riley JP, Williams KN, Karlsson L, Thurmond RL. The histamine H4 receptor mediates allergic airway inflammation by regulating the activation of $CD4^+$ T cells. *J Immunol* 2006; 176: 7062-7070.
44. Cowden JM, Riley JP, Ma JY, Thurmond RL, Dunford PJ. Histamine H4 receptor antagonism diminishes existing airway inflammation and dysfunction via modulation of Th2 cytokines. *Respir Res* 2010; 11: 86. https://doi.org/10.1186/1465-9921-11-86
45. Cowden JM, Yu F, Banie H, *et al*. The histamine H4 receptor mediates inflammation and Th17 responses in preclinical models of arthritis. *Ann Rheum Dis* 2014; 73: 600-608.
46. Thurmond RL, Chen B, Dunford PJ, *et al*. Pharmacology and clinical activity of toreforant, a histamine H4 receptor antagonist. *Ann Pharmacol Pharm* 2017; 2: 1013.
47. Leary PJ, Tedford RJ, Bluemke DA, *et al*. Histamine H2 receptor antagonists, left ventricular morphology, and heart failure risk. *J Am Coll Cardiol* 2016; 67: 1544-1552.
48. Zhang J, Cai WK, Zhang Z, *et al*. Cardioprotective effect of histamine H2 antagonists in congestive heart failure: a systematic review and meta-analysis. *Medicine* (*Baltimore*) 2018; 97: e0409. doi: 10.1097/MD.0000000000010409.

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