

The dual H_{3/4}R antagonist thioperamide does not fully mimic the effects of the 'standard' H₄R antagonist JNJ 7777120 in experimental murine asthma

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Abstract Histamine is detected in high concentrations in the airways during an allergic asthma response. In a murine model of allergic asthma, the histamine H₄ receptor (H₄R)-selective ligand JNJ 7777120 reduces asthma-like symptoms. A sole antagonistic function of JNJ 7777120 at the murine H₄R has, however, been questioned in the literature. Therefore, in the present study, we aimed at analyzing the effects of JNJ 7777120 in comparison to that of the H_{3/4}R-selective antagonist thioperamide. Experimental murine asthma was induced by sensitization and provocation of BALB/c mice with ovalbumine (OVA). JNJ 7777120, thioperamide, or JNJ 5207852, an H₃R-selective antagonist which was used to dissect H₃R- and H₄R-mediated activities of thioperamide, were injected subcutaneously during sensitization and effects were analyzed after provocation. Pharmacokinetic analyses revealed shortest t_{1/2} values in both plasma and lung tissue and lowest maximal concentration in lung tissue for JNJ 7777120 in comparison to thioperamide and JNJ 5207852. Nevertheless, JNJ 7777120 reduced serum titers of allergen-specific (anti-OVA) IgE, inflammatory infiltrations in lung tissue, and eosinophilia in bronchoalveolar lavage fluid. In contrast, thioperamide reduced only eosinophilia in bronchoalveolar lavage fluid, while anti-OVA IgE concentrations and lung infiltrations remained unaffected. JNJ 5207852 had no effect on these parameters. JNJ 7777120

provides beneficial effects in experimental murine asthma, which, however, could only partially be mimicked by thioperamide, despite more favorable pharmacokinetics. Thus, whether these effects of JNJ 7777120 are entirely attributable to an antagonistic activity at the murine H₄R or whether an agonistic activity is also involved has to be reconsidered.

Keywords Histamine H₄ receptor · Mouse · Ovalbumine-induced asthma · JNJ 7777120 · Thioperamide · JNJ 5207852

Introduction

The biogenic amine histamine is an important mediator involved in several (patho-) physiological processes such as type I allergic reactions, e.g., acute bronchial asthma (Westly 2010). Today, four histamine receptor subtypes have been identified, which belong to the family of G protein-coupled 7-transmembrane receptors and are referred to as histamine H₁ receptor (H₁R), H₂R, H₃R, and H₄R (Bakker et al. 2002; Seifert et al. 2013). The H₄R (Oda et al. 2000; Liu et al. 2001; Nguyen et al. 2001) shows substantial sequence homology to H₃R, albeit a different expression spectrum. While the H₃R is found mainly on cells of the nervous system, the H₄R is expressed mainly on cells of hematopoietic origin and is, therefore, supposed to be involved in inflammatory reactions.

A widely accepted model of acute airway inflammation is the murine model of ovalbumine (OVA)-induced experimental asthma (Hartwig et al. 2008; Zosky et al. 2008). In this model, the sensitization phase and the provocation phase of the pathogenesis can be clearly distinguished. The initial sensitization phase results in production of allergen-specific immunoglobulin, primarily of the IgE isotype. Subsequent

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provocation induces an acute allergic reaction in the lung leading to inflammatory infiltrations.

The involvement of histamine (Kozma et al. 2003) and the H₄R in experimental murine asthma in mice have been demonstrated in several studies. These studies rely either on the H₄R-knockout mouse or on the H₄R-selective antagonist JNJ 7777120. In both H₄R-knockout mice and mice treated with JNJ 7777120, symptoms of experimental murine asthma are reduced in comparison to wild type or untreated mice, respectively (Dunford et al. 2006; 2007; Cowden et al. 2010a; Beermann et al. 2012). Currently, the antagonistic activity of JNJ 7777120 is matter of intense debate. While at the human H₄R JNJ 7777120 reduces histamine-induced G protein-dependent signaling, it enhances the β -arrestin pathway in a supposedly G protein-independent manner (Rosethorne and Charlton 2011). Taking also into account the murine H₄R, the situation is even more complicated. When analyzed at recombinant receptors expressed in insect cells in the steady-state GTPase activation assay JNJ 7777120 is a partial inverse agonist at the human H₄R; however, at the murine counterpart, JNJ 7777120 acts partially agonistic (Schnell et al. 2011; Strasser et al. 2013).

Thus, at the moment, it cannot clearly be defined whether JNJ 7777120 acts exclusively antagonistically or also agonistically in vivo in mice. Therefore, we compared the effects of JNJ 7777120 and thioperamide, a well-established dual antagonist at the murine H₃R and H₄R (Takeshita et al. 2003; 2004). In order to rule out potential H₃R-mediated effects of thioperamide, we also included JNJ 5207852, a selective antagonist at least at the mouse and the rat H₃R (Barbier et al. 2004; Bonaventure et al. 2007) in this study. Interestingly, the effects of JNJ 7777120 were not superimposable to that of thioperamide, indicating that the activity of JNJ 7777120 at the murine H₄R in vivo should be evaluated carefully.

Materials and methods

Mice

A total of 139 female BALB/c mice of 8 weeks of age were obtained from Elevage Janvier (Le Genest-Saint-Isle, France) and housed in community cages on a 12-h light cycle and fed mouse chow and water ad libitum in the animal facility of the Hannover Medical School. In order to enable their acclimatization, mice were kept for at least 2 weeks in the animal facility before starting the experimental procedures. All animal manipulations were performed in accordance to the German animal protection law under a protocol approved by the local governmental authority (Bezirksregierung Hannover; # 08/1550).

Sensitization and allergen challenge of mice

Experimental murine asthma was induced by sensitization with OVA grade VI and provocation with OVA grade V (both from Sigma-Aldrich, Taufenkirchen, Germany). For sensitization, mice were injected intraperitoneally (i.p.) on days 1 and 14 with 10 μ g of OVA, which was absorbed to 1.5 mg of phosphate buffered saline (PBS)-solubilized aluminum hydroxide (Pierce Biotechnology, Rockford, IL). In control experiments, mice were sham sensitized with aluminum hydroxide. For provocation on days 21–24, mice were exposed to 1 % (*m/v*) OVA/PBS for 20 min on four consecutive daily expositions in a custom-made nebulizer box in which the mice could move freely.

Histamine receptor-ligand administration

The H₃R-selective antagonist JNJ 5207852 (1-({4-[3-(piperidin-1-yl)propoxy]phenyl} methyl)piperidine), the H₄R-selective ligand JNJ 7777120 (1-[(5-chloro-1*H*-indol-2-yl) carbonyl]-4-methylpiperazine) (both kindly provided by Dr. Armin Buschauer, University of Regensburg, Germany), and the H₃R and H₄R antagonist thioperamide (Sigma-Aldrich) were dissolved in 20 % (*v/v*) DMSO/PBS to obtain solutions of 10 mM. They were administered in final doses of 20 mg/kg body weight (De Bie et al. 1998; Dunford et al. 2006). The mice were divided into four experimental groups, which were treated either with the solvent 20 % (*v/v*) DMSO/PBS or with one of the histamine receptor ligands. Treatments were performed by subcutaneous (s.c.) injections using a total volume of 100 μ l with each application 30 min before as well as 2 h after sensitizing OVA injections (Beermann et al. 2012). In the pharmacokinetic analyses, mice were treated with histamine receptor-selective ligands as described above, but without induction of experimental asthma.

Animal dissection

Mice were sacrificed with an i.p. overdose of a mixture of xylazine (Rompun; 70 mg/kg body weight; Bayer, Leverkusen, Germany) and ketamine (Ketalar; 230 mg/kg body weight; Albrecht, Aulendorf, Germany). Mice were bled by heart puncture and sera or plasma were prepared and frozen at -80°C . Lungs were excised and weighted and immediately shock frozen in liquid nitrogen for pharmacokinetic analyses or were individually lavaged in situ with 1.0 ml aliquots of sterile PBS (bronchoalveolar lavage, BAL) and afterwards fixed in 4 % (*m/v*) formalin and embedded in paraffin.

Differential cell count

Total cell counts in BAL fluids and viability were determined by trypan blue exclusion using a Neubauer chamber. Leukocyte

subsets (eosinophils, neutrophils, macrophages, or lymphocytes) were counted in cytospins from BAL fluids stained with Diff-Quik (Medion Diagnostics, Dudningen, Switzerland). A total of 400 cells were counted in each sample.

Histology

Fixed and paraffin-embedded lungs were cut into 4 μm slices and stained with hematoxylin/eosin. From each lung, six sections of both the right and left lobes were evaluated by a pathologist in a blinded fashion. Quantification of the bronchial inflammation was performed according to the scoring index described by Beermann et al. (2012).

Mouse anti-OVA IgE ELISA

Serum levels of OVA-specific IgE were analyzed by ELISA using a mouse-specific anti-IgE antibody. Briefly, OVA-coated microtiter plates (Nunc, Roskilde, Denmark) were incubated with test sera, diluted 1:2,000 in PBS containing 5 % (w/v) BSA, and serial dilutions of a standard serum obtained from a hyperimmunized mouse. Murine IgE was detected by subsequent incubation with a biotinylated rat anti-mouse IgE antibody (BD/Pharmingen, Franklin Lakes, NJ) and peroxidase-coupled streptavidine (Invitrogen-Biosource, Carlsbad, CA). Peroxidase-dependent conversion of tetramethyl benzidine was quantified by reading at 405 nm. Titers were calculated according to the standard serum obtained from a hyperimmunized mouse, which was set at 1×10^6 U/ml.

Pharmacokinetic analyses of histamine receptor-selective ligands

At zero, 15, 30, 60, 120, 240, and 360 min after s.c. injection of the respective ligand, mice were bled and lungs were excised as described above. One hundred fifty to two hundred milligrams of lung tissue was processed to homogenates in 2 ml screw cap tubes, each containing 200 mg Garnet Matrix A, a ceramic bead (all from MP Biomedicals, Illkirch, France) and 800 μl of extraction buffer (ethanol/ H_2O (70/30 [v/v], containing 0.113 $\mu\text{g}/\text{ml}$ of the internal standard JNJ 10191584)), using a FastPrep-24 device (MP Biomedicals). The resulting homogenates were cleared by centrifugation (10 min, 20,800 $\times g$, 4 $^\circ\text{C}$), and 600 μl of the cleared supernatant fluids were transferred into new vials and evaporated at 40 $^\circ\text{C}$ under a nitrogen stream until dryness. Pellets were dissolved in 150 μl methanol/ H_2O (50/50 [v/v]), cleared, and subjected to analysis. Plasma was prepared by protein precipitation. A twofold volume of a mixture of methanol and 0.2 M ZnSO_4 (80/20 [v/v]) containing 0.65 $\mu\text{g}/\text{ml}$ JNJ10191584 (internal standard) was added to the blood samples and centrifuged (10 min, 20,800 $\times g$, 4 $^\circ\text{C}$).

Processed homogenates and plasma were subjected to quantification of JNJ 7777120, thioperamide, and JNJ 5207852 by HPLC (Shimadzu Germany, Duisburg, Germany)-coupled tandem mass spectrometry (API2000, AB SCIEX, Foster City, CA, USA). Samples were separated chromatographically on a Synergy Polar-RP 80A Mercury column (10 $\times$ 2 mm, 4 μm ; Phenomenex, Aschaffenburg, Germany) connected to a Polar-RP Security Guard by a gradient of eluent A (3/97 methanol/water [v/v]; 50 mM ammonium acetate; 0.1 % [v/v] acetic acid; all from Sigma-Aldrich, Taufenkirchen, Germany) and eluent B (97/3 methanol/water [v/v], 50 mM ammonium acetate, and 0.1 % [v/v] acetic acid) at a flow rate of 400 $\mu\text{l}/\text{min}$. Concentrations of histamine receptor-selective ligands were calculated in correlation to organ weights.

Statistical analysis

If not stated otherwise, statistical analyses were performed by calculating means $\pm$ SD for each parameter within an experimental group. Analysis of significance was performed using one-way ANOVA with Bonferroni post test or chi-square test. *p* values of <0.05 were considered significant. For pharmacokinetic analysis, curves starting at the maximum concentration value were best fitted by nonlinear regression using a one phase decay equation and half lives were calculated thereof (all calculations were performed using GraphPad Prism 5).

Results

The present study was performed to detect possible differences in the antagonistic profiles of the histamine receptor-selective ligands JNJ 7777120 and thioperamide, interacting with the H_4R and the H_4^- and the H_3R , respectively. The H_3R -selective antagonist JNJ 5207852 was used to assess the H_3R -directed activity of thioperamide. Since potential differences may be due also to differences in the pharmacokinetics of the compounds, we initially analyzed their quantities occurring in plasma and lung after their s.c. injection (Fig. 1). Plasma concentrations of all three compounds rose rapidly, reaching maximum values (c_{max}) between 15 and 30 min after injection (t_{max}). The plasma c_{max} of thioperamide was about 13 $\mu\text{g}/\text{ml}$, being more than threefold higher than the maximum concentrations of JNJ 7777120 and JNJ 5207852. In the lung tissue, c_{max} of JNJ 7777120 and of JNJ 5207852 also occurred between 15 and 30 min, while t_{max} of thioperamide was 30–60 min (Fig. 1). The c_{max} of JNJ 5207852 and thioperamide in lung tissue reached comparable values of about 150 $\text{pg}/\mu\text{g}$ protein, while the c_{max} of JNJ 7777120 was only about half of it. The decline of the concentrations in both plasma and lung tissue occurred with half-life times ($t_{1/2}$) of about 20 min for JNJ 7777120 and about 80 min for JNJ

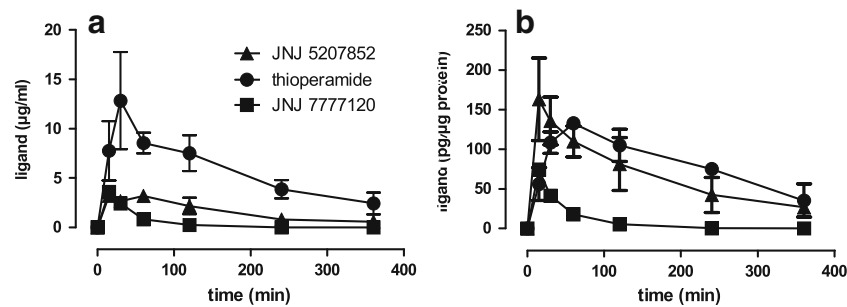


Fig. 1 Pharmacokinetic analysis of JNJ 5207852, thioperamide, and JNJ 777120. Female BALB/c mice ($n = 3$ per group and time point analyzed) were injected s.c. with 20 mg/kg body weight of the compounds indicated in the legend and after different periods of time; as

indicated on the x-axis, the compounds were quantified in plasma (a) and lung homogenates (b) by HPLC-coupled mass spectrometry. Data shown are means $\pm$ SD

5207852. In contrast, $t_{1/2}$ of thioperamide differed between plasma and lung tissue, while in plasma it was similar to that of JNJ 5207852 (about 80 min) and it was enhanced in lung tissue (about 200 min). The relative total drug exposure in the lung was estimated by calculation of the areas under the curves (AUC) for each ligand. Thioperamide gave a maximal AUC (30,000), followed by that of JNJ5207852 (25,000). In contrast, JNJ 777120 resulted in an about 10 times less AUC (3,000).

Eosinophilia in the BAL fluid is a hallmark of experimental murine asthma. In sham-sensitized and OVA-provoked mice, macrophages were the main cell type in the BAL fluid, while eosinophils were virtually absent (not shown). In OVA-sensitized and provoked mice, the total cell number in BAL fluids was dramatically enhanced (100,000 vs. 500,000 cells/mouse). This was mainly due to the rise in numbers of eosinophils, which accounted for about 80 % of total cells (Fig. 2). Neither the application of DMSO nor the treatment with JNJ 5207852 altered eosinophilia in BAL fluids. In contrast, treatment with thioperamide as well as with JNJ 777120 significantly reduced eosinophilia by about 20 % (Fig. 2).

Upon sensitization against the experimental allergen OVA, mice develop OVA-specific antibodies from the IgE isotype. In sham-sensitized and OVA-provoked mice, anti-OVA IgE was virtually absent, while it was readily detectable in sera of OVA-sensitized and provoked mice (not shown). In asthmatic mice, serum anti-OVA IgE concentrations were neither affected by application of the solvent DMSO nor by treatment with JNJ 5207852 or thioperamide. Only the H_4R -selective ligand JNJ 777120 reduced the anti-OVA concentration in sera of asthmatic mice significantly (Fig. 3).

The allergic acute late phase reaction is accompanied by massive inflammatory infiltration in the affected tissue. In mice, sensitization and provocation with OVA lead to peribronchial and perivascular infiltrations of leukocytes. Infiltrations were not affected by treating the mice with DMSO, JNJ 5207852, or thioperamide. Only JNJ 777120

significantly reduced inflammatory infiltrations in the lung tissue of OVA-sensitized and provoked mice (Fig. 4).

Discussion

Recently, the antagonistic activity of JNJ 777120, which is selective for the H_4R in several species including mice (Thurmond et al. 2004), has been questioned (Neumann et al. 2010; Rosethorne and Charlton 2011; Schnell et al. 2011; Seifert et al. 2011). While JNJ 777120 reduces inhibition of forskolin-induced cAMP accumulation by histamine in SK-N-MC neuroblastoma cells transfected with human or murine H_4R and inhibits histamine-mediated increase of intracellular

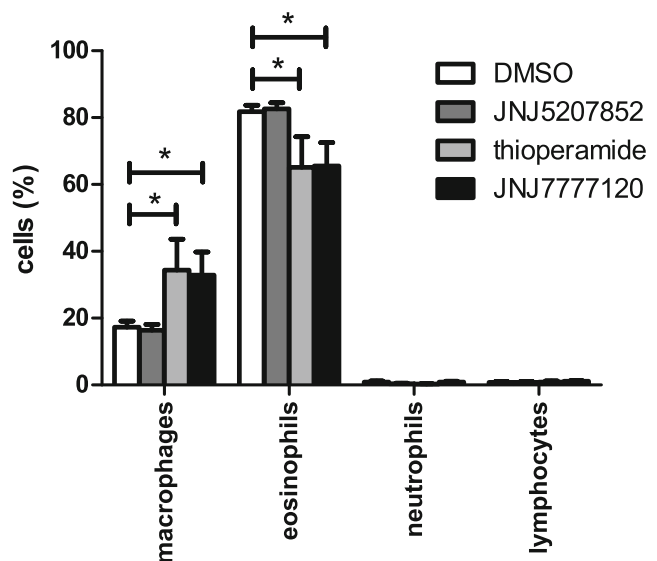


Fig. 2 Effects of JNJ 5207852, thioperamide, and JNJ 777120 on eosinophilia. BALB/c mice ($n = 8$ mice per group) were sensitized to and challenged with OVA and treated with DMSO, JNJ 5207852, thioperamide, or JNJ 777120 during sensitization. Cells in BAL fluids, collected 24 h after the final challenge (total cell numbers: DMSO, $447,500 \pm 165,000$; JNJ 5207852, $587,000 \pm 114,500$; thioperamide, $412,000 \pm 183,000$; JNJ 777120, $434,000 \pm 209,000$), were differentially counted. Data shown are means $\pm$ SD (* $p \leq 0.05$)

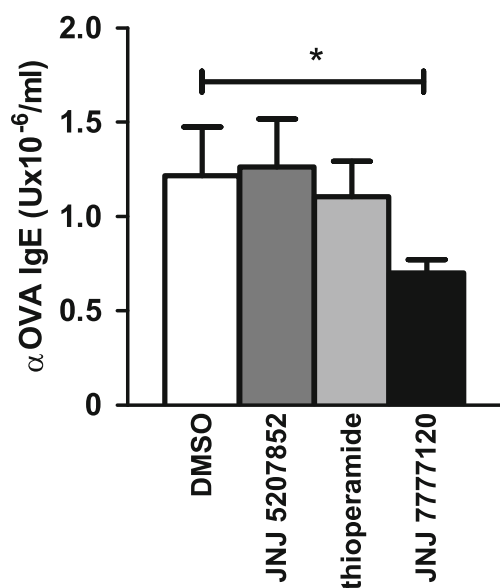


Fig. 3 Effects of JNJ 5207852, thioperamide, and JNJ 777120 on anti-OVA IgE concentrations in sera. BALB/c mice ($n = 8$ mice per group) were sensitized to and challenged with OVA and treated with DMSO, JNJ 5207852, thioperamide, or JNJ 777120 during sensitization. In sera, collected 24 h after the final challenge, anti-OVA IgE titers were determined by ELISA. Data shown are means $\pm$ SD ($*p \leq 0.05$)

calcium concentration and chemotaxis in murine mast cells (Thurmond et al. 2004), JNJ 777120 also acts as partial agonist at the mouse H_4R when expressed in Sf9 cells and analyzed for steady-state GTPase activity catalyzed by G_i proteins (Schnell et al. 2011). Moreover, another recent study supposes that JNJ 777120 activates β -arrestin signaling in a G protein independent manner (Rosethorne and Charlton 2011). Consequently, the beneficial effect of JNJ 777120 in vivo such as in the murine model of OVA-induced asthma cannot be definitively attributed to an inhibitory effect of JNJ 777120 at the murine H_4R .

Therefore, in the present study, we compared the effects of JNJ 777120 to those of the dual $H_{3/4}R$ antagonist thioperamide on typical symptoms of murine experimentally induced asthma. In order to rule out H_3R -specific effects of thioperamide, the H_3R -selective antagonist JNJ 5207852 (Bonaventure et al. 2007) was included in this study. Figure 5 demonstrates the structures and the receptor selectivity of the compounds studied, and comprehensively summarizes the data of this study: the compounds pharmacokinetic data and their effects on various parameters of murine bronchial asthma.

In the literature, a very limited number of data about the pharmacokinetics of JNJ 777120, thioperamide, and JNJ 5207852 exist (Silva et al. 1997; Barbier et al. 2004; Sindelar et al. 2004; Venable et al. 2005) and none of these studies analyzed the compound kinetics in the lung. However, since at least one of the basic parameters species, route of application and dosages applied, differed to our study, also a comparison to

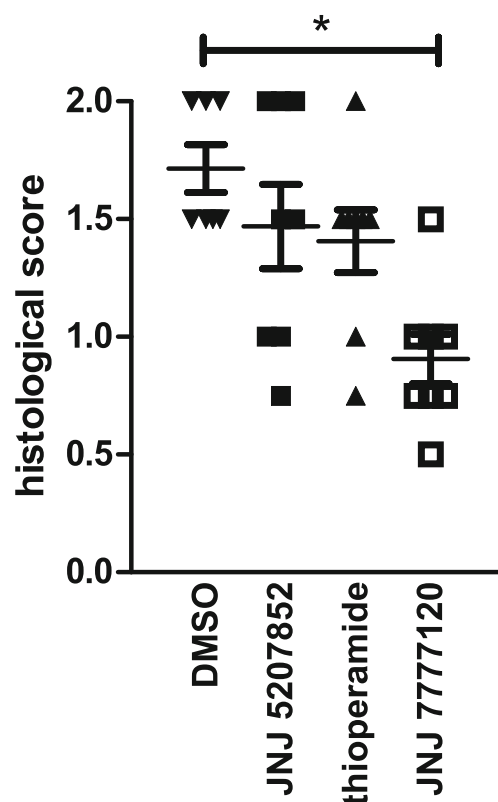
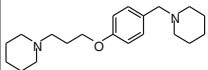
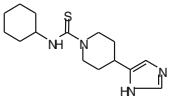
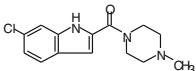


Fig. 4 Effects of JNJ 5207852, thioperamide, and JNJ 777120 on airway inflammation. BALB/c mice ($n = 8$ mice per group) were sensitized to and challenged with OVA and treated with DMSO, JNJ 5207852, thioperamide, or JNJ 777120 during sensitization. Twenty-four hours after the final challenge, lungs were analyzed histologically and the grade of inflammation was scored in a blinded fashion. Data shown are scoring values of individual mice plus means $\pm$ SD ($*p \leq 0.05$)

the data found in plasma is critical. Nevertheless, in mice JNJ 777120 after p.o. administration is reported to disappear from the plasma with a $t_{1/2}$ value of about an hour (Venable et al. 2005), thus quite similar to our finding of 20 min. In contrast, for JNJ 5207852 a $t_{1/2}$ of about 15–20 h has been reported (Barbier et al. 2004), which dramatically differs from our data (80 min). This difference could be attributed to the use of differing species (mouse vs. rat) and application routes (s.c. vs. oral). For thioperamide, $t_{1/2}$ values depend on the doses applied and are described to range from 2–8 h at comparable dosages (Silva et al. 1997; Sindelar et al. 2004). Although these data were generated in rats after oral or i.p. application, they fit quite well to our observation of about 3 h. Thus, with the exception of JNJ 5207852, our plasma $t_{1/2}$ data are in good accordance to those already published.

Our pharmacokinetic analyses revealed differences of the three compounds, as well in their kinetics of appearance in the lung tissue as in the maximum concentration achieved and their kinetics of decline. JNJ 777120 appears in the lung quite rapidly but gives rise to only a relatively low peak concentration and disappears completely within approximately

Fig. 5 Summary of the study. Listed are histamine receptor selectivity, structures, and the observed pharmacokinetic values and effects of JNJ 7777120, thioperamide, and JNJ 5207852 on experimental murine asthma in the present study

compound	H ₃ R-selectivity	structure	t _{1/2} (min)		t _{max} (min)		C _{max}		effect on eosinophilia	effect on anti-OVA IgE	effect on lung infiltration
			plasma	lung	plasma	lung	plasma (μg/ml)	lung (pg/μg)			
JNJ 5207852	H ₃ R		85	81	15	15	3.2	163	→	→	→
thioperamide	H ₃ R & H ₄ R		83	189	30	60	12.8	133	↓	→	→
JNJ 7777120	H ₄ R		23	20	15	15	3.6	74	↓	↓	↓

120 min. The appearance of JNJ 5207852 is also fast while that of thioperamide is rather delayed; however, both give rise to maximum concentrations in the lung twice as high as JNJ 7777120. In addition, their decline is slower in comparison to JNJ 7777120 and does not lead to a complete disappearance within the observed period of time, i.e., 360 min. The estimated total drug exposure, analyzed by calculation of the AUC, of thioperamide and JNJ 5207852 is comparable, but 10 times enhanced in comparison to JNJ 7777120. Thus, concerning the pharmacokinetic parameters of the compounds, the effects of JNJ 5207852 and thioperamide can be compared directly; however, the affinity of thioperamide to the H₃R, at least the human one, is about 100-fold lower (pK_i 7.3; (Seifert et al. 2013)) than that of JNJ5207852 (pK_i 9.2; (Barbier et al. 2004)). The reduced effect of thioperamide in comparison to JNJ 7777120 cannot be attributed to a worse pharmacokinetic of thioperamide, since its 10 times higher estimated total drug exposure is not overcompensated by a dramatically reduced affinity to the mH₄R, which is only about 10 times less in comparison to JNJ 7777120 (pK_i(thioperamide) 7.6; pK_i(JNJ777120) 8.4; (Lim et al. 2005). Thus, concerning pharmacokinetics, thioperamide and JNJ 7777120 are well equilibrated, while JNJ5207852 should be more active in comparison to thioperamide.

Eosinophilia in alveolar spaces is a hallmark of experimental murine asthma. Migration of eosinophils has already been shown to be regulated via H₄R, both in humans and in mice (Ling et al. 2004; Cowden et al. 2010b; Reher et al. 2012). In our study, we corroborate this conclusion by showing that JNJ 7777120 and thioperamide, but not JNJ 5207852, reduced eosinophilia, however, only modestly in comparison to steroids such as budesonide (Shen et al. 2002). Thus, for the parameter eosinophilia in lungs of mice with experimental asthma, we can definitively ascribe an

antagonistic function to JNJ 7777120, as already proposed previously (Dunford et al. 2006; Deml et al. 2009; Beermann et al. 2012).

A different picture arises upon analyses of inflammatory cellular infiltrations in the lung tissue and the occurrence of allergen-specific IgE in sera. The production of IgE specific for T cell-dependent allergens, such as OVA, is regulated by histamine and has been attributed to H₁R, H₂R, (Jutel et al. 2001), and to H₄R (Dunford et al. 2006; Deml et al. 2009; Beermann et al. 2012). While the former study relies on genetic knockout models only, where compensatory mechanisms, e.g., via other histamine receptors cannot be excluded, the latter ones also applied a pharmacological model using JNJ 7777120 in mice. The present study confirms the finding of Dunford et al. (2006) concerning the reduced anti-OVA IgE levels in sera of mice with experimental asthma following JNJ 7777120 application and extends the inhibitory effect of JNJ 7777120 to histological analysis of lung tissues. However, also regarding these parameters, their reduction is only modest in comparison to the therapy with steroids (Zhao et al. 2007).

Intriguingly, because we used additional pharmacological controls, our study leads to a different interpretation. Since application of the H₃R antagonist JNJ 5207852 was without effect, a possible effect of thioperamide can be ascribed to the H₄R only. Thioperamide, however, was without effect too. Thus, antagonism at the H₄R is not able to reduce anti-OVA IgE and histological signs of inflammation. JNJ 7777120, in contrast, reduces both inflammatory infiltrations in lung tissue and sera concentrations of anti-OVA IgE. Thus, since differences between JNJ 7777120 and thioperamide are not due to their pharmacokinetics as discussed above, the effect of JNJ 7777120 could be explained, e.g., by agonism at the murine H₄R, which may be mediated either via activation of the β-

arrestin 2 pathway (Rosethorne and Charlton 2011) or by a G protein-dependent pathway (Schnell et al. 2011). The first of these hypotheses, the activation of β -arrestin 2, however, seems to be unlikely since the β -arrestin 2 pathway in allergic airway inflammation has been shown to be pro-inflammatory (Nichols et al. 2012).

In summary, in the mouse model of OVA-induced asthma, the H_4 R-ligand JNJ 7777120 appears to exhibit different functionalities, being either antagonistic or agonistic. In addition to the above discussed agonists function of JNJ 7777120, this may also be explained by the fact that JNJ 7777120 at the murine H_4 R is not a neutral antagonist but a partial agonist (Schnell et al. 2011). Therefore, in the absence or in the presence of only minor concentrations of histamine, JNJ 7777120 acts agonistically, while at high concentrations of histamine, it acts antagonistically. Thus, the pharmacological effects of JNJ 7777120 are much more complex than originally assumed, rendering it necessary for in vivo studies of H_4 R function to apply different H_4 R-selective ligands in addition to the 'standard H_4 R antagonist' JNJ 7777120.

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