

The effect of selective antagonist of H₄ receptor JNJ7777120 on nasal symptoms, cough, airway reactivity and inflammation in guinea pigs

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ABSTRACT

The efficacy of H₄R antagonist JNJ7777120 on nasal symptoms, cough, airway resistance (Raw), inflammatory cell count in bronchoalveolar lavage (BAL) and blood in ovalbumin (OVA) induced allergic rhinitis (AR) was studied in guinea pigs. Animals ($n=8$) were sensitized by i.p. OVA and were repeatedly challenged with nasal OVA to induce rhinitis, seven animals were not sensitized. Animals were pre-treated with JNJ7777120 2.5 and 5 mg/kg i.p. 30 min prior OVA. Cough was induced by inhalation of citric acid, Raw was measured *in vivo* by Pennock's method as baseline, during AR and after JNJ7777120 treatment. Leucocyte count in BAL and blood was analyzed. JNJ7777120 (5 mg/kg) significantly suppressed nasal symptoms and the number of coughs. This compound significantly inhibited airway reactivity to histamine, but not methacholine. Pre-treatment with JNJ7777120 5 mg/kg did not influence significantly the leucocyte count in BAL and blood except for a significant decrease in monocyte count in blood compared to the control group ($p < 0.05$). We conclude that the antitussive action of JNJ7777120 is peripheral. The primary effect of the compound is anti-inflammatory, and the suppression of cough is a consequence of reduced airway inflammation.

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1. Introduction

Chronic cough is a common and frequently debilitating symptom that is often viewed as an intractable problem (Morice et al., 2004). It has been associated with a complex aetiology, and it is frequently present in subjects with gastroesophageal reflux disease, asthma or upper airway diseases (Morice et al., 2011). In multiple adult studies, upper airway cough syndrome (UACS—cough due to a variety of upper respiratory conditions) has been shown to be the most common cause of chronic cough (Pratter, 2006). Animal models and studies in subjects with upper airway diseases showed that cough sensitivity correlates to the presence of nasal inflammation and the magnitude of nasal symptoms. The treatment of inflammation significantly reduced the magnitude of nasal symptoms and also reduced coughing (Plevkova and Song, 2013).

Empiric protocols recommend first-generation H₁ antihistamines for the treatment of UACS, although mechanisms of the

antitussive effect of these inverse agonists of histamine H₁ receptors (H₁R) are not completely understood (Bolser, 2008). The use of antihistamines for cough lacked relevant evidence. Last year, Dicpinigaitis et al. (2014) and co-authors clearly showed that the old generation antihistamine diphenhydramine reduces cough reflex sensitivity in humans with colds. Old generation antihistamines cause adverse effects in multiple body systems, including sedation, caused by a low selectivity for the H₁R and by actions on H₁R in the CNS (Simons and Simons, 2011). In contrast to first-generation compounds, second-generation H₁-antihistamines are relatively free from adverse effects; however, they are ineffective in the treatment of cough in UACS (Bolser, 2008). Discovery of the H₄R and its expression on numerous immune cells has prompted a re-evaluation of the actions of histamine, suggesting a new potential for H₄R blockers and a possible synergy between H₁R and H₄R antagonists in targeting various inflammatory conditions (Thurmond and Gelfand, 2008).

The administration of JNJ7777120, a novel and selective H₄ receptor antagonist, showed significant anti-inflammatory effects on allergic airway inflammation (Thurmond and Gelfand, 2008). JNJ7777120 blocked the functional, histopathological and

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biochemical changes induced by an ovalbumin challenge in a model of allergic asthma in guinea pigs, suggesting that these effects are mediated by the down-regulation of key pro-inflammatory cytokines (Somma et al., 2013). H₄R is expressed on the structural and immune cells of nasal mucosa and its expression increases with inflammation (Yan et al., 2010a,b). In an allergic rhinitis model with mice, JNJ777120 inhibited nasal symptoms and also had immunomodulatory functions (Takahashi et al., 2009). The aim of our study was to determine the effect of the H₄ antagonists JNJ777120 on cough induced by citric acid and on specific airway resistance in conscious ovalbumin-sensitized animals in a model developed by Brozmanova et al. (2008) and validated in our laboratory.

2. Materials and methods

The study was conducted on male Dunking Hartley guinea pigs, obtained from an accredited breeding facility (L. Sobota, Městec Králové, Czech Republic). The animals were housed in an approved animal holding facility maintained at a controlled room temperature of 21–22 °C, with humidity 60–70%, ventilation, a 12-h light–dark cycle and with free access to water and standard animal food.

Animal care was provided and the experiments were conducted in agreement with the Animal Welfare Guidelines of the Comenius University and statutes and rules of the Slovak Republic (protocol no 3467/13-221).

The animals were adapted twice to laboratory conditions to reduce future stress. They stayed in a plethysmographic box to familiarize themselves with the environment and the laboratory staff. In the box, they were exposed to an aerosol of buffered saline for 2 min, which corresponds to the experimental procedure. Later, the animals were challenged with 0.4 M citric acid and, based on their responses, were divided into a control group ($n = 7$), and OVA group ($n = 8$)—both groups involved a proportional count of normo-, hypo- and hyper-reactors. The reason for this group assignment pattern is that the cough response to citric acid in conscious animals has a high variability.

2.1. Sensitization

Control group (sham sensitized) ($n = 7$) animals were injected by intraperitoneal saline 1 ml. OVA group ($n = 8$) animals were sensitized by intraperitoneal administration of 10 µg of OVA suspension together with aluminium hydroxide as an adjuvant in 1 ml of saline; OVA sensitivity was confirmed by skin prick tests 21 days later.

2.2. Assessment of nasal symptoms after antigen challenge

To obtain a relevant model of UACS, sensitized animals were repeatedly challenged intranasally by an allergen five times, always once in a week (0.5% OVA, 0.015 ml into both nostrils) to increase the magnitude of local nasal symptoms of allergic rhinitis until the plateau of the response was achieved. The plateau in the nasal responsiveness was associated with a stable up-regulation of the cough reflex. Cough challenges started from this time point.

The intensity of the symptoms was evaluated by trained observers using a nasal symptom score system, which was developed and validated by Brozmanova et al. (2005,2008), matching symptom intensity to numeric values (maximum 6 and minimum 0).

A nasal symptom score was calculated based on the validated scale system. Observed signs and symptoms were the count of sneezes and (i) nasal discharge: no discharge 0, mild/moderate 1, discharge falling in drops from nose 2, (ii) eye/conjunctival reaction: no changes 0, hazy eyes 1, visible lacrimation 2 and (iii) nasal

phenomenon: no crackles 0, audible crackles 1, crackles audible from a distance 2.

2.3. Citric acid-induced cough in alert guinea pigs

The conscious animals ($n = 15$) were individually placed in the plethysmograph (type 855, Hugo Sachs Electronic, March, Germany) which consisted of a head and a body chamber. The opening between the head and body chambers was equipped with a plastic collar lining around the animal's neck to prevent communication between chambers. An appropriate collar size was chosen for each animal to prevent neck compression. The head chamber was connected to a nebulizer (Pari Provokation Test I, Menzel, Germany, manufacturer's specification: output 5 l min⁻¹, particle mass median aerodynamic diameter 1.2 µm). A suction device adjusted to the same input flow (5 l min⁻¹) was connected to the head chamber to maintain constant airflow through the chamber during the aerosol administration. Airflow changes were measured using a pneumotachograph (Godart, Germany) with a Fleisch head connected to the head chamber. These data were recorded with the acquisition system ACQ Knowledge (Biopack, Santa Barbara, CA, USA). Respiratory sounds, including sounds during coughing and sneezing, were recorded with a microphone placed in the roof of the head chamber and connected to a preamplifier and MP3 recorder. The pneumotachograph and microphone output were simultaneously recorded for off-line analysis.

The cough challenge was performed using an inhalation of 0.4 M citric acid for 10 min. Cough was defined as expiratory airflow interrupting the basic respiratory pattern accompanied by a coughing sound. Coughs were analyzed (using cough-related sounds and airflow) by two trained persons who were blind to the treatment. The most important task of the observers was to differentiate coughs from sneezes and artefacts by the software Sonic Visualizer. Their results were compared and (if no statistically significant differences occurred) averaged.

The animals were challenged by citric acid (CA) and the cough response was measured several times, always with a one-week interval between the challenges to prevent tachyphylaxis. In the OVA group, the first measurement of the cough response was performed after intranasal administration of saline solution (0.015 ml per nostril) 10 min prior to the cough challenge. In other measurements, the cough response was measured after an intranasal administration of ovalbumin (0.5% sol. 0.015 ml per nostril) and an induction of symptoms of allergic rhinitis (AR). The cough response was measured 10 min after the intranasal administration of ovalbumin without pre-treatment, then after intraperitoneal pretreatment with JNJ777120 in two different doses (2.5 mg/kg and 5 mg/kg of body weight) and after intraperitoneal pretreatment with diphenhydramine 30 mg/kg (first-generation H₁ antihistamine) 30 min prior to the induction of rhinitis, which is 40 min prior to the cough challenge. The cough response in the control group was detected concurrently with the OVA group.

2.4. Measurement of specific airway resistance in alert guinea pigs

Specific airway resistance (Saw) was measured with a simple non-invasive plethysmographic technique, Pennock's method (Pennock et al., 1979). Conscious OVA-sensitized animals were placed in a double chamber body plethysmograph composed of a head chamber and a body chamber (type 855, Hugo Sachs Electronic, March, Germany). The nasal airflow was measured in the head chamber; the thoracic respiratory airflow was calculated from the pressure changes in the body chamber. Both measurements were made with differential pressure transducers (Simsoft, Martin,

Slovakia) and recorded to a PC for Pulmodyn Pennock W software analysis.

In this system, the specific airway resistance is proportional to the phase difference between nasal airflow and thoracic respiratory flow (Pennock et al., 1979). Specific airway resistance was calculated from the phase difference between the thoracic respiratory flow and nasal airflow based on a Lissajous loop presentation of the thoracic respiratory flow and nasal airflow on the X- and Y-axes. Specific airway resistance was measured after the inhalation of saline, histamine and methacholine aerosols three times overall, in OVA-sensitized animals before the i.n. allergen challenge (before), after an administration of ovalbumin and the development of nasal symptoms of AR (OVA) and after i.p. JNJ777120 pre-treatment (5 mg/kg) and an induction of AR (JNJ777120+OVA).

2.5. Inflammatory cell count in bronchoalveolar lavage fluid and blood

One additional OVA-sensitized group was used for this part of the study ($n=8$), along with the control animals ($n=7$) and previously used OVA-sensitized animals ($n=8$). The groups were designed as controls (controls), OVA-sensitized animals with rhinitis (OVA) and OVA-sensitized animals with rhinitis pre-treated by JNJ777120 (5 mg/kg) 30 min prior to the OVA challenge (JNJ777120+OVA).

The animals were euthanized with an i.p. overdose of urethane (1.5 g/kg i.p.). Guinea pigs were secured supine on a warming pad. A midline incision in the neck exposed the trachea, which was cannulated. After placing a cannula, the lungs were lavaged with a sterile saline solution (0.9% NaCl, 2×10 ml/kg b.w.). The total number of cells in the bronchoalveolar lavage fluid (BALF) was evaluated microscopically using a Bürker's chamber and expressed per $1 \mu\text{l}$ of BAL fluid. The BAL fluid was then centrifuged at 1500 rpm for 15 min. In the sediment of BAL fluid, a differential leucocyte count (monocytes, neutrophils, eosinophils and lymphocytes) was evaluated in May-Grünwald and Giemsa-Romanovsky stainings (evaluated approximately 800–1000 cells).

Samples of blood were taken from the heart with a sterile syringe with a needle to a test tube with K3-EDTA and centrifuged at 3000 rpm for 20 min. The total number of cells in the blood was evaluated microscopically using a Bürker's chamber and expressed per $\times 10^9/\text{L}$ of blood (after staining with a Türk solution). A differential leucocyte count in the blood sediment (lymphocytes, neutrophils, monocytes, eosinophils and basophils) was evaluated microscopically in May-Grünwald and Giemsa-Romanovsky (evaluated approximately 300 cells).

2.6. Reagents

Urethane and citric acid were purchased from Fisher (Slovak Republic). A 50% urethane was administered to the peritoneal cavity in a dose of 1.5 g/kg of body weight. Ovalbumin, $\text{Al}(\text{OH})_3$ and citric acid were purchased from Fisher (Slovak Republic). JNJ777120, diphenhydramine, histamine, methacholine and all other chemicals were purchased from Tocris (Slovak Republic).

2.7. Statistical analysis

For evaluation of the statistical significance of data from the cough studies, non-parametric non-paired tests and multiple comparison tests were used as appropriate. Data for the final cough count are expressed as median $\pm$ interquartile range. Specific airway resistance is expressed as mean value $\pm$ standard error of mean, and multiple comparison tests were used to analyze differences between samples. For inflammatory cell count in bronchoalveolar lavage fluid and blood, one-way analysis of variance

(ANOVA) was used. $p < 0.05$ was considered statistically significant. Data from the tracheal and lung tissue reactivity experiments are shown in grams of smooth muscle tension. All data are shown as mean $\pm$ SE. For statistical analysis, one-way ANOVA was used. $p < 0.05$ was considered statistically significant.

3. Results

3.1. The effect of JNJ777120 on citric acid-induced cough in ovalbumin-sensitized awake guinea pigs

Numbers of coughs in the control group (not-sensitized) and the OVA group (sensitized) are showed in Fig. 1. Data from the control group confirmed the reproducibility of measurements of the cough responses, because numbers of coughs in the control group recorded concurrently with the OVA group did not differ significantly between measurements. In the OVA group, the cough response was increased after a nasal allergen challenge—as expected—and an early phase of allergic rhinitis significantly up-regulated the cough response from 11 ± 2 to 19 ± 3 coughs per provocation, med $\pm$ IQR, $p < 0.05$. Intraperitoneal pretreatment with a lower dose (2.5 mg/kg) of JNJ777120 showed a tendency to reduce coughing and suppress nasal symptoms (data not shown); however, the data did not reach any significance. A higher dose (5 mg/kg) of JNJ777120 significantly suppressed the nasal symptoms and number of coughs obtained during provocation from 19 ± 3 to 10 ± 1 , med $\pm$ IQR, $p < 0.05$. The antitussive effect of a higher dose of JNJ777120 was comparable to the antitussive effect of diphenhydramine (30 mg/kg) that significantly suppressed nasal symptoms and the number of coughs from 19 ± 3 to 11 ± 1 coughs per provocation.

3.2. The effect of JNJ777120 on specific airway resistance in vivo

The nasal allergen challenge (assigned as ovalbumin) significantly enhanced airway reactivity to both histamine and methacholine when compared to control responses of animals prior to OVA administration (assigned as before) ($p < 0.05$). JNJ 777120 pretreatment significantly inhibited airway reactivity to histamine ($p < 0.05$), but not methacholine (Fig. 2) in animals with allergic rhinitis induced by OVA on JNJ 777120 medication.

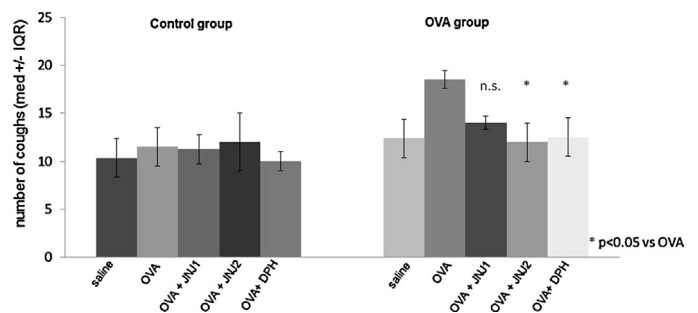


Fig. 1. The number of citric acid-induced coughs per provocation; data are expressed as median and interquartile range. The sensitized group received OVA intranasally, and this challenge induced an early phase of allergic rhinitis during which the cough study was performed. The control group completed the same protocol; however, OVA does not induce any response in unsensitized animals. Trials were assigned as follows: saline—animals received i.n. saline 0.015 ml per nostril approx. 10 min prior to the cough challenge; OVA—animals received i.n. OVA 0.5% sol. 0.015 ml per nostril to induce nasal symptoms prior to the cough challenge; OVA+JNJ1—rhinitis with i.p. pretreatment with JNJ777120 2.5 mg/kg of b.w.; OVA+JNJ2—rhinitis with i.p. pretreatment with JNJ777120 5 mg/kg of b.w.; OVA+DPH—rhinitis with i.p. pretreatment with diphenhydramine 30 mg/kg. n.s.—not significant; * $p < 0.05$.

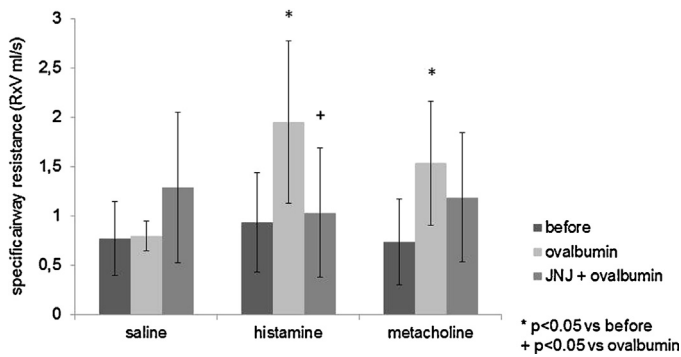


Fig. 2. Data of specific airway resistance measured *in vivo* by Pennock's method in sensitized animals. Resistance was measured three times, baseline (assigned as "before", then during OVA-induced rhinitis (ovalbumin) and finally in OVA-induced rhinitis after 5 mg/kg body weight of JNJ777120 i.p. administration. Inhalation of saline did not influence airway resistance. Inhalation of bronchoactive substances (histamine and methacholine) increased the airway resistance mainly after the allergen challenge. * $p < 0.05$ vs ovalbumin; * $p < 0.05$ vs before.

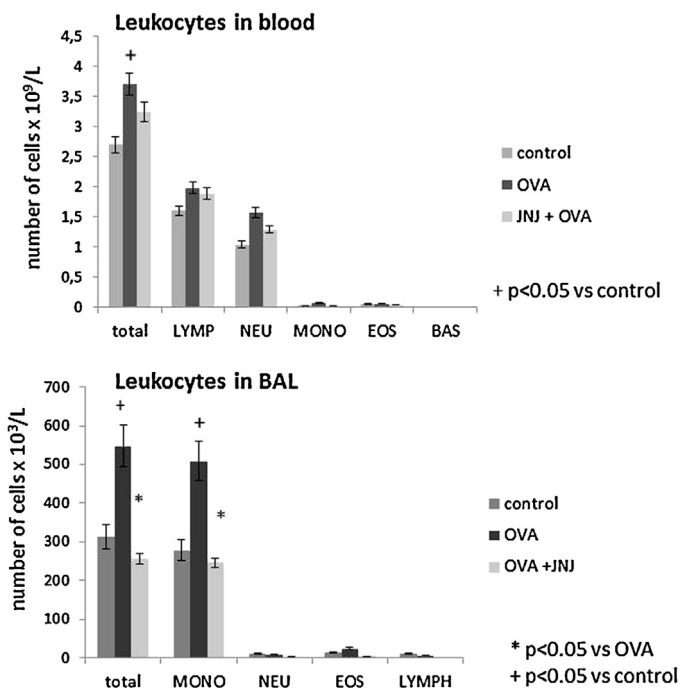


Fig. 3. The total and differential count of leukocytes in peripheral blood and BAL fluid in the control unsensitized animals, then in animals with OVA-induced rhinitis and finally JNJ777120 in animals pretreated with OVA-induced rhinitis. The upper panel shows that early phase increases the total count of leukocytes in blood; differential counts of blood cells did not show significant differences. The total leukocytes count in the BAL fluid in OVA-sensitized animals increased significantly after the OVA challenge. * $p < 0.05$. JNJ777120 pre-treatment significantly reduced the count of cells in BAL (* $p < 0.05$). The only significant difference was found for monocytes/macrophages cells.

3.3. Influence of the H₄R antagonist JNJ777120 on inflammatory cell count in bronchoalveolar lavage fluid and blood

The blood total and differential cell count did not differ significantly between groups (Fig. 3). Significant differences were found in BAL cell counts. The total cell count in the OVA group is significantly increased from control animals and this count is significantly reduced in animals treated with JNJ777120 5 mg/kg. Further analysis of the cell count showed that significant differences were found only for monocytes/macrophages in the BAL of OVA sensitized and then OVA sensitized and JNJ777120-treated animals (Fig. 3).

4. Discussion

Our study showed that an antagonist of H₄ receptor JNJ777120 in the dose of 5 mg/kg of body weight significantly reduced the magnitude of nasal symptoms, the number of coughs, airway resistance and the total count of leukocytes in BAL fluid in a guinea-pig model of upper airway cough syndrome (UACS). Upper airway disorders are one of the most common causes of chronic cough in humans. The pathogenesis of UACS is complex and involves multiple mechanisms, including the mechanical action of secretions on laryngeal and pharyngeal afferents, the enhanced excitability of sensory afferents causing increased cough responsiveness and/or an enhanced excitability of central pathways that produce cough (Bolser, 2008). Other mechanisms are altered breathing patterns, pulmonary aspiration of nasal contents, the nasal-bronchial reflex and an uptake of inflammatory mediators into the systemic circulation (Braunstaal and Fokkens, 2003).

Somma et al. (2013) published an excellent study of effects of JNJ 777120 on allergen-induced airway inflammation, focusing on a broad spectrum of parameters including the cytokines, the oxidative/nitrosative stress and many more; however, the cough reflex was not the main objective of that study. Our study focuses primarily on the cough and its analysis in laboratory conditions based on the recommendations of ERS Cough Task Force. The cough response should be studied in the defined conditions (as a response to capsaicin or citric acid). Somma et al. challenged the animals with the OVA aerosol (which is not a recommended procedure to study cough) and they did not define criteria for differentiation of cough from other behaviours. The guinea pigs are obligatory nasal breathers, and in this aspect, variety of airway defensive reflexes could be induced (Poliacek et al., 2009). The problem of differentiation of the cough from the sneeze in guinea pig is frequently discussed. Our primary focus was to assess the cough response and we differentiated coughs, sneezes and artefacts based on (triple control) pressure changes, observation of the specific cough pressure patterns and finally the cough sound analysis.

Histamine is a mediator that plays a role in the majority of inflammatory processes, not only allergies; therefore histamine receptors are promising targets in the management of inflammation in general. Empiric protocols recommend first-generation H₁ antihistamines for the treatment of cough in UACS, although the mechanism(s) of the antitussive effects of these substances are not completely understood. Potential actions of these drugs on histamine H₁ and/or nonhistaminergic receptors in the central nervous system remain a possible explanation for their effects on cough due to UACS. The effects of older H₁ antihistamines may also be molecule specific, rather than attributable to the entire class of compounds (Bolser, 2008).

JNJ777120 suppressed cough induced by inhalation of citric acid in the conscious guinea-pig model, which is the best animal model to study cough responses. The reason is that the neurophysiology and neuropharmacology of the guinea-pig vagus nerve is closest to human (Plevkova and Song, 2013). Diphenhydramine – an old generation H₁ antihistamine – was used as a positive control in our cough study. This substance is known to suppress cough and has been used as an ingredient in cough remedies for almost six decades (Lilienfield et al., 1976; Packman et al., 1991) although the evidence supporting that use seems weak (Björnsdóttir et al., 2007). Inhibition of cough by JNJ777120 (5 mg/kg b.w.) was as effective as inhibition obtained after 30 mg/kg of diphenhydramine. How do we explain the reduction of coughing, which was our most important question?

Nasal afferents play an important role in the modulation of the cough reflex and histamine is a mediator that directly stimulates a subset of nasal sensory nerves (Taylor-Clark et al., 2005). Although intranasal administration of histamine did not trigger cough, it

sensitized cough reflex in subjects with allergic rhinitis (Plevkova et al., 2005). The same effect was documented in healthy subjects (Plevkova et al., 2006). Since the effect of the histamine molecule is mediated not only by H₁R, but also by other receptor subtypes (H₂, H₃ and H₄), we can assume that these subtypes of receptors can play a role in the pathogenesis of nasal inflammation and the up-regulation of cough reflex in subjects with nasal inflammation via neuronal mechanisms.

Nakaya et al. (2004) documented H₄R expression on nasal nerves. The study of Yan et al. (2010a,b) confirmed the expression of H₄R in normal nasal mucosa and a significant enhancement of its expression in subjects with allergies and subjects with respiratory syncytial virus infection (Jung et al., 2013). The up-regulation of expression and/or function of H₄R can considerably contribute to airway nerves hyperresponsiveness by sensitization and desensitization of the cough reflex by nasal trigeminal afferents (Buday et al., 2012).

H₄R is predominantly expressed on cells of the immune system, including mast cells, monocytes, eosinophils, dendritic cells, T cells and natural killer cells (Zampeli and Tiligada, 2009). In addition to inflammatory cells, H₄R is expressed on structural cells of the nasal mucosa and upper airway diseases increase its expression. Antagonism of the H₄R receptor had an anti-inflammatory action in studies using *in vitro* and *in vivo* (Thurmond and Gelfand, 2008) and significantly decreased the magnitude of nasal symptoms in animal models of allergic rhinitis. The study of Takahashi et al. (2009) showed that H₄R antagonist JNJ7777120 caused a dose-dependent inhibition of nasal symptoms by single and repeated intranasal and peroral administrations in a model of allergic rhinitis in mice. However, mice are not a suitable model to study cough, because mice lack relevant airway afferents. Therefore, our study provides additional data which support H₄R as an important signalling pathway with potential clinical significance.

Similarly, Yan et al. (2010a,b) found that JNJ7777120 relieves symptoms and inflammatory conditions in a rat model of ovalbumin-induced allergic rhinitis. We obtained similar results in our study. The intraperitoneal administration of JNJ7777120 significantly suppressed the magnitude of nasal symptoms in an ovalbumin-sensitized guinea-pig model of allergic rhinitis. Cough sensitivity is heightened in subjects with upper airways diseases, and it correlates to the presence and magnitude of nasal symptoms (Plevkova and Song, 2013). Reduction of nasal inflammation leads to a reduction of coughing and *vice versa*, which was also verified in our study. Intraperitoneal pre-treatment with JNJ7777120 shows a significant antitussive effect in a guinea-pig model of upper airway cough syndrome. This result is in accordance with the findings of Somma et al. (2013), who found that sensitized guinea pigs subcutaneously pre-treated with JNJ7777120 before inhalation exposure to ovalbumin showed a significant reduction in respiratory alterations compared to the vehicle-treated animals and dose-dependent decreases in cough severity.

In animal models, ovalbumin sensitization followed by ovalbumin inhalation increases leucocyte infiltration and cytokine production in lungs and increases the total cell number in BAL fluids, mainly due to a rise in eosinophils counts. Allergic inflammation in the nasal mucosa initiates infiltration of inflammatory cells in both the nasal mucosa and lung as well as ciliary destruction of bronchial epithelial cells and changes of cytokines and mucin (Han et al., 2005). JNJ7777120 showed an ability to block the functional, histopathological and biochemical changes induced by the ovalbumin challenge *in vivo* due to a down-regulation of pro-inflammatory cytokines (Somma et al., 2013; Neumann et al., 2013). Our data are in agreement with these previously reported findings. The anti-inflammatory effect of the compound used in our model was documented by a significant reduction of the number of leukocytes in the BAL fluid. Further analysis of the cells revealed that

only the monocytes/macrophage count was reduced significantly. The other cell types were not influenced by JNJ7777120. H₄R is expressed on a spectrum of immune cells, including monocytes. We assumed that the inhibition of histamine signalling by JNJ7777120 influenced mainly monocytes in our model, but the reason that eosinophils or other cell types were not reduced remains to be elucidated. The majority of inflammatory mediators either directly activate or sensitize airway afferents responsible for the mediation and modulation of cough (Mazzone and Canning, 2002).

The antitussive effect of JNJ7777120 can be mediated by peripheral mechanisms, but it is not possible to exclude central mechanisms since JNJ7777120 crosses the blood–brain barrier (Dunford et al., 2007) and H₄R is functionally expressed within human and rodent neurons in numerous areas of the central nervous system (Galeotti et al., 2013). JNJ7777120 (unlike first-generation antihistamines) does not cause sedation in rodents (Dunford et al., 2007), so the antitussive effect of JNJ7777120 cannot be attributed to a sedative effect.

Upper airway diseases are frequently associated with functional disturbances of the lower airways, mainly the presence of bronchial hyperresponsiveness. Systemic cross-talk between the nose and lungs has been reviewed many times with clear anatomical and functional bi-directional relationships (Braunstaal and Fokkens, 2003). Allergic rhinitis is characterized by the presence of bronchial hyperresponsiveness, which was also documented in our study. Ovalbumin-sensitized animals developed an early phase of allergic rhinitis after a topical intranasal administration of an allergen, and during this stage, the airway responsiveness to histamine was significantly heightened when compared to the control group both *in vitro* and *in vivo*. JNJ7777120 reduced this hyperresponsiveness significantly, suggesting the role of H₄R in the pathogenesis of airway hyperresponsiveness in inflammation. Whether this effect is a consequence of the direct action of JNJ7777120 on nerves and muscles or an indirect effect mediated by anti-inflammatory action cannot be confirmed. The number of cells in BAL was reduced; therefore, we can assume that the process of inflammation was less intense.

Bronchoconstriction and cough are separate processes; however, they can potentiate each other *via* peripheral and central mechanisms (Mazzone and Canning, 2002). The complex action of JNJ7777120 may contribute to the reduction of coughing also by normalizing the airway bronchomotor response.

In conclusion, JNJ7777120 in the dose of 5 mg/kg of body weight considerably interacts with multiple mechanisms responsible for excessive coughing in subjects with upper airway diseases. It reduces inflammation, nasal discharge, nasal obstruction and it also modulates the responsiveness of lower airways. The data we obtained in our study are in agreement with previously published effects of JNJ7777120 *in vitro* and *in vivo*, suggesting that this line of drugs would have significant clinical application in the near future.

Authors contribution

E.H.-K., T.B. and S.G. performed nasal challenges and cough challenges on conscious animals. S.G. (biomedical engineer) analyzed the data. J.P. performed the blind analysis of cough counts. J.M., D.M., P.K. and A.U.-F. performed analysis of cells in BAL and blood. J.P. supervised the manuscript work and she is the leader of this multidisciplinary team. All authors proofread the manuscript and met the criteria for authorship.

Conflict of interest

The authors declare no conflicts of interest.

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