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Combined treatment with H1 and H4 receptor antagonists reduces inflammation in a mouse model of atopic dermatitis

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ABSTRACT

Background: Histamine 4 receptor (H4R) antagonists are considered as new therapeutics for the treatment of atopic dermatitis (AD) and first clinical trials have already shown promising results. Histamine 1 receptor (H1R) antagonists are traditionally used to treat AD although the evidence for the efficacy is weak. The combined blockade of both, H1R and H4R, might provide synergistic anti-inflammatory.

Objective: The study was performed to test the anti-inflammatory potential of a combined treatment with an H1R and an H4R antagonist in a mouse AD model.

Methods: The development of ovalbumin-induced AD-like skin lesions was analysed mice treated with the H1R inverse agonist mepyramine, the H4R antagonist JNJ-39758979 or a combination of both.

Results: Mice treated with mepyramine plus JNJ-39758979 showed less severe skin lesions, with a diminished influx of inflammatory cells, a reduced epidermal thickening and a lower level of IL-33 in lesional skin. Scratching behaviour was ameliorated in mice treated with the combination. Moreover, total numbers of skin-draining lymph node cells and splenocytes were significantly reduced. Both substances given alone did not elicit this strong anti-inflammatory effect.

Conclusion: H1R and H4R antagonists provide synergistic anti-inflammatory effects in a mouse model of AD. The combined therapy with H1R and H4R antagonists might represent a new strategy for the treatment of AD.

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1. Introduction

Histamine 1 receptor (H1R) antagonists belong to one of the largest class of medications used in the treatment of allergic diseases [1]. However in case of atopic dermatitis (AD) evidence for the efficacy of H1R-antihistamines is only weak [1]. The histamine 4 receptor (H4R) is considered to be a new therapeutic target for the treatment of AD [2,3]. In a mouse model of AD, clinical signs are clearly attenuated in H4R knockout mice [4]. Moreover, first H4R antagonists have already entered clinical trials in AD patients. The H4R antagonist JNJ-39758979 relieved pruritus and decreased the

median Eczema Area and Severity Index (EASI) in patients with moderate AD [5]. In addition, the H4R antagonist ZPL-389 significantly reduced clinical symptoms of moderate to severe AD in a phase 2a proof of concept study from Ziarno Pharma (data published June 2016).

In mouse models of hapten-induced allergic dermatitis, the combination of H1R and H4R antagonists showed synergistic inhibition of pruritus [6] and inflammation [7]. The combination was significantly more effective against pruritus than an H1R or an H4R antagonist given alone [6]. In a model of chronic allergic dermatitis (induced by the hapten picryl chloride) established in NC/Nga mice, the anti-inflammatory effect of a combined treatment was similar to that of prednisolone [7]. Thus, the combination of H1R and H4R antihistamines could be a new therapeutic option for chronic allergic skin disease like AD, and might be superior to H4R (and H1R) antagonists given alone. However also the H4R antagonist JNJ-7777120 alone ameliorated picryl chloride skin lesions in NC/Nga mice [7], whereas house dust

Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; H1R, histamine 1 receptor; H4R, histamine 4 receptor; OVA, ovalbumin.

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mite-induced lesions in NC/Nga mice were not significantly affected by H4R antagonists [8]. Thus, there are still studies needed to evaluate the role of a combined treatment with an H1R and an H4R antagonist in mouse models of AD like the ovalbumin (OVA)-induced dermatitis model. The OVA model reflects several important aspects of human AD: Epicutaneously sensitized mice develop skin lesions characterized by epidermal and dermal thickening, infiltration of CD4⁺-T cell and eosinophils. The Th2 cytokines IL-4, IL-5, and IL-13 as well as the chemokines eotaxin and TARC are enhanced in OVA-sensitized skin. Furthermore, serum OVA-specific IgG1, IgE, and IgG2a are elevated and OVA-sensitized mice develop asthma-like symptoms following inhalation of OVA [9]. A recent study evaluated the transcriptomic profiles of six common murine skin inflammation models (NC/Nga, flaky tail, Flg-mutated, OVA-challenged (sensitization and provocation procedure not described in the study), Oxazolone-challenged, and IL-23-injected mice) and determined how they related to human AD skin [10]. In this study, the OVA-challenged model had significant overlap with genes upregulated in human AD but does not capture the downregulated signature of human AD [10]. However, no single murine model fully captured all aspects of the AD profile and each model reflects different immune or barrier disease aspects [10]. Caution should be exercised before translation of murine data to human [10]. Thus, it is important to test new drugs in different animal models. This will help to extrapolate murine data to human and to assess new drugs at the preclinical level.

The objective of this study was to assess the role of a combination therapy with the H1R inverse agonist mepyramine and the H4R antagonist JNJ-39758979 in the OVA-induced allergic dermatitis model.

2. Material and methods

2.1. Mice

Female BALB/c mice (BALB/cAnNCr1) were obtained from Charles River (Sulzfeld, Germany). All animals were healthy and were housed in groups of 6–7 mice per cage at 22 °C with a 12-h light/dark cycle. Water and a standard diet (Altromin) were available ad libitum. The animal experiment has been approved by the LAVES, Oldenburg, Germany (AZ.G33.14-42502-04-13/1311).

2.2. OVA-induced AD model

Mice were sensitized epicutaneously with OVA (Sigma Aldrich) as described in detail previously [11]. In brief, 100 µl OVA solution (1 mg/ml PBS) was placed on a patch of gaze, which was tied to the depilated abdominal skin with an elastic fixation bandage (Peha-haft™, Hartmann) for one week. Each mouse had a total of three 1-week exposures to OVA grade V separated from each other by 2-week intervals (sensitization). Two weeks after the last treatment with OVA grade V, mice received identical patches with OVA grade VI for one week (1st challenge). Two weeks after the first challenge, mice received again patches with OVA grade VI for another week (2nd challenge). Mice were between the ages of 8–9 weeks (around 20 g body weight) at the beginning of the sensitization and sacrificed at the end of the second challenge.

2.3. Treatment and control groups

The following substances were tested in the OVA model: The H1R inverse agonist (referred to as antagonist throughout the article) mepyramine (20 mg/kg, Tocris), the H4R antagonists JNJ-39758979 (20 mg/kg, provided by Janssen Research & Development), or a combination of both (20 mg/kg + 20 mg/kg). Drugs were

compared to vehicle treated mice (aqua ad injectionem). All substances were injected intraperitoneally (i.p.) twice daily during the 1st and 2nd challenge week. One group of mice was treated with PBS instead of OVA. These PBS treated mice are referred to as control mice ($n=6$). The combination of mepyramine and JNJ-39758979 was tested in two independent experiments by two different investigators with a total of 13 mice per group. The effects of mepyramine and JNJ-39758979 alone were each tested in one experimental setting with $n=6$ mice per group.

2.4. Assessment of dermatitis

Severity of dermatitis was assessed 6 h after removal of the OVA patch after both challenge weeks by two blinded investigators using a standardized skin score ranging from 0 (no symptoms) to 4 (extreme symptoms). Clinical measures analysed were scaling, erythema and erosion/excoriation. Total skin score was calculated as sum of scores of scaling, erythema and excoriation (maximum possible score of 12).

2.5. Analyses of spontaneous scratching behaviour

Scratching behaviour was determined 30 min and 5 h after removal of the OVA patch in the challenge phase. To exclude that the removal of the bandage itself increased scratching behaviour and not the allergen, spontaneous scratching behaviour of control mice were compared to OVA treated mice 30 min and 5 h after removal of the bandages during all sensitization and challenge weeks. Mice were recorded on video over 30 min and afterwards scratching bouts with the hind paws were analysed.

2.6. Histological analysis of the skin

Lesional skin sites were separated, fixed and paraffin sections stained with haematoxylin and eosin (HE) and Giemsa. Epidermal thickness of ten randomly selected areas was assessed using Axiovision 4.8 software. Total inflammatory cell influx was determined semiquantitatively by a score system ranging from “–” (none) to “++++”4 (very high cell influx). Mast cells (Giemsa stain) were counted in ten parts of high-power fields (HPF) at 400× magnification.

2.7. Mouse anti-OVA IgE ELISA

OVA-specific IgE was determined in the serum by means of ELISA (OVA specific IgE ELISA from BioLegend).

2.8. Cytokine determination in the skin

Lesional skin was shock-frozen in liquid nitrogen and stored at –80 °C until homogenization. For determination of cytokines skin samples were homogenized under liquid nitrogen. The homogenates were transferred in 200 µl RPMI 1640 medium (Biochrom) and 1 mmol/l Pefabloc (Roche). After centrifugation (3000 × g, 10 min, 4 °C) the supernatants were collected and the protein content was determined with a protein assay (Biorad). IL-5, IL-10, IL-13, IL-31, IL-33, TARC and TSLP were measured by ELISA (all R&D Systems, IL-31 ELISA was purchased from eBioscience).

2.9. Single cell preparation of lymph nodes and spleens

Single cell suspensions were prepared from skin draining lymph nodes (Lnn. axillares) and spleens. Cell count was determined in a Neubauer chamber. A part of the lymph node cells was used for flow cytometry analysis and the remaining cells were used for CD4⁺ T cell isolation using the EasySep™ mouse CD4

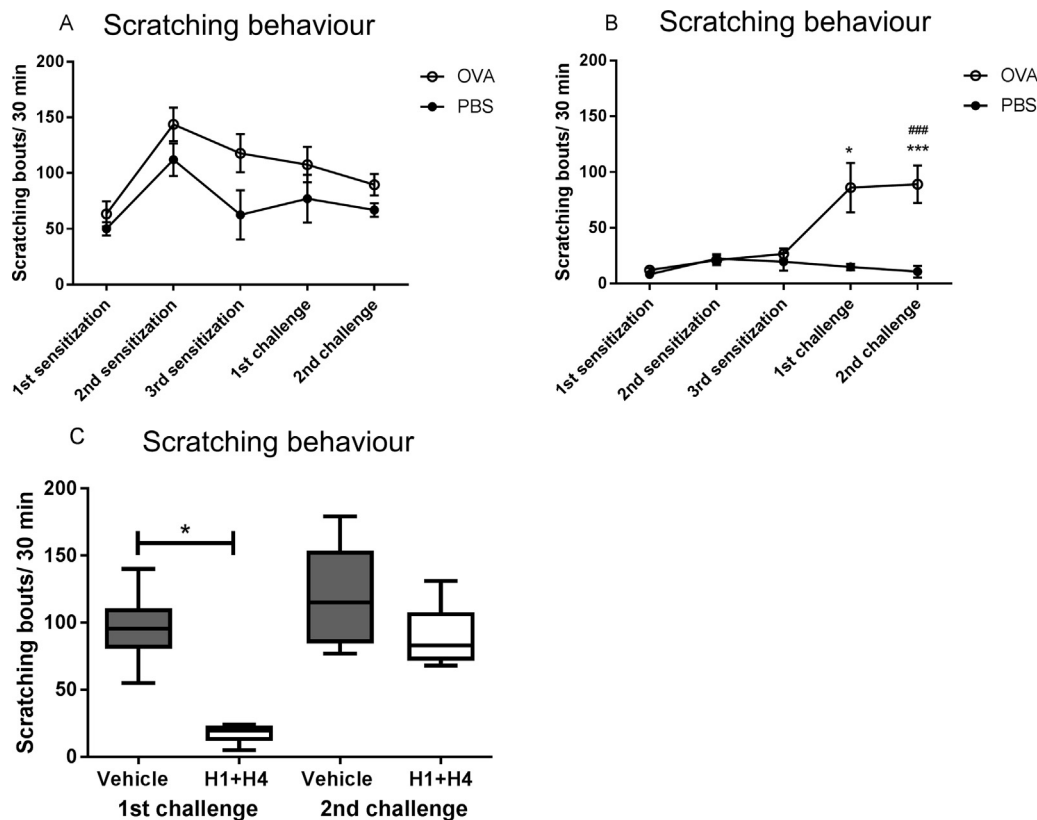


Fig. 2. OVA-induced scratching behaviour. Scratching behaviour of mice sensitized and challenged with OVA in comparison to mice treated with PBS instead of OVA. (A) Scratching behaviour determined 30 min after removal of the OVA or PBS patch. OVA-treated mice showed no increase in scratching behaviour. (B) Scratching behaviour determined 5 h after removal of the OVA or PBS patch. OVA-treated mice showed a significant increase in scratching behaviour in the challenge phase compared to PBS-treated mice. $^{***}p < 0.01$ one-way ANOVA (OVA 2nd challenge vs. PBS 2nd challenge); $^{*}p < 0.05$ one-way ANOVA (OVA 1st challenge vs. OVA 1st sensitization); $^{***}p < 0.001$ one-way ANOVA (OVA 2nd challenge vs. OVA 1st sensitization). (C) Treatment with the combination of mepyramine (20 mg/kg i.p.) plus JNJ-39758979 (20 mg/kg i.p.; H1 + H4) significantly reduced scratching behaviour in the first (1st challenge), and by trend in the second challenge (2nd challenge) with OVA. $^{*}p < 0.05$ one-way ANOVA (Vehicle 1st challenge vs. H1 + H4 1st challenge).

3.3. Epidermal thickening and inflammatory cell influx is diminished in H1R plus H4R antagonist treated mice

Lesional skin sites of mice treated with mepyramine + JNJ-39758979 and with mepyramine alone showed a reduced number of total inflammatory cells (Table 1) whereas the H4R antagonist did not influence the cell influx. OVA increased the thickness of the epidermis. Epidermis of mice treated with OVA was $48 \pm 8 \mu\text{m}$ thick whereas the epidermis of control mice was only $24 \pm 4 \mu\text{m}$. Skin lesions of mice treated with mepyramine + JNJ-39758979 showed a diminished epidermal thickening compared to vehicle treated mice (Fig. 3). The epidermis was about 46% thinner whereas the epidermis of mepyramine treated mice was only 6% and that of JNJ-39758979 only 12% thinner than the epidermis of

vehicle treated mice. A significantly lower number of mast cells were found in lesional skin sites of mice treated with mepyramine plus JNJ-39758979 (control 1.7 ± 0.6 ; vehicle 5.9 ± 2.1 ; mepyramine + JNJ-39758979 3.7 ± 0.9 cells/HPF; $p < 0.05$ Mann–Whitney test). Also mepyramine alone significantly reduced the number of mast cells (vehicle: 5.4 ± 1.8 ; mepyramine: 3.4 ± 0.8 cells/HPF; $p < 0.05$ Mann–Whitney test). The H4R antagonist did not significantly reduce the number of mast cells at lesional skin sites (vehicle: 5.0 ± 2.1 ; JNJ-39758979: 4.5 ± 0.5 cells/HPF).

3.4. No difference in OVA-specific IgE antibody response

OVA-specific IgE levels in the serum were not influenced by any treatment tested. No OVA-specific IgE could be measured in control mice (data not shown).

3.5. Effects of H1R + H4R antagonist treatment on levels of cytokines in skin tissue

IL-10, IL-31 and TSLP were slightly reduced in mice treated with mepyramine + JNJ-39758979 but not statistically significant; IL-5 did not differ between the two groups (Fig. 4). A significant reduced level of IL-33 was measured in skin of H1R+H4R antagonist treated skin (Fig. 4). The H4R antagonist alone slightly reduced the level of IL-33 in the skin (vehicle: $27 \pm 5 \text{ pg}/\mu\text{g}$; JNJ-39758979: $22 \pm 8 \text{ pg}/\mu\text{g}$), whereas IL-33 was significantly increased in skin of mepyramine treated mice (vehicle: $13 \pm 5 \text{ pg}/\mu\text{g}$;

Table 1
Cell influx into the skin.

	Control	Vehicle	H1	H4	H1 + H4
Cell influx	—	+++	++	+++	++

Inflammatory cell influx in lesional skin of mice challenged with OVA for two weeks. Inflammatory cell influx was determined semiquantitatively by a score system ranging from “—” (none) to “++++” (4) (very high cell influx). Control: mice treated with PBS instead of OVA. Vehicle: OVA sensitized and challenged mice treated with vehicle. H1: OVA sensitized and challenged mice treated with mepyramine (20 mg/kg i.p.) during challenge. H4: OVA sensitized and challenged mice treated with JNJ-39758979 during challenge. H1 + H4: OVA sensitized and challenged mice treated with mepyramine (20 mg/kg i.p.) plus JNJ-39758979 (20 mg/kg i.p.) during challenge.

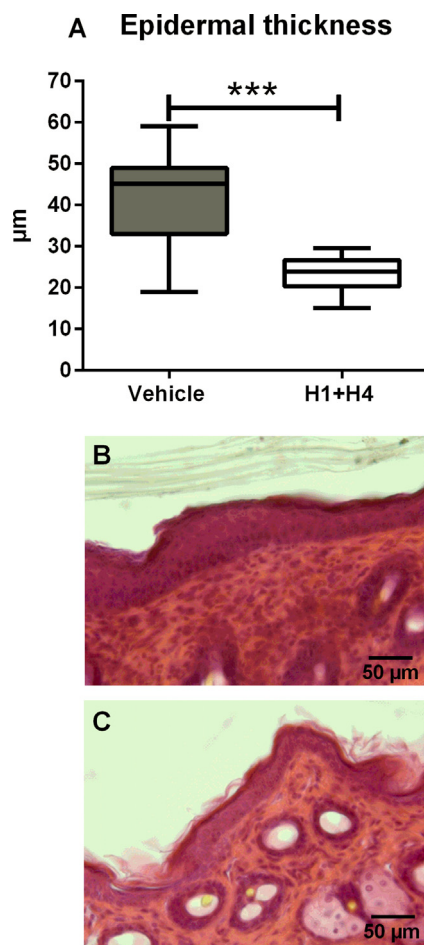


Fig. 3. OVA-induced epidermal thickening. (A) Epidermal thickness of abdominal skin of mice challenged with OVA for two weeks. Treatment with mepyramine (20 mg/kg i.p.) plus JNJ-39758979 (20 mg/kg i.p.; H1 + H4) significantly diminished the OVA-induced epidermal thickening. *** $p < 0.01$ Mann–Whitney test. $n = 13$ mice per group. Epidermis of control mice is 24 ± 4 µm (mean $\pm$ SD). (B and C) HE-stain of lesional skin sites of mice challenged with OVA for two weeks. OVA induced a massive cell influx and an epidermal thickening. Inflammatory cell influx and increase in epidermal thickness was diminished in mice treated with mepyramine + JNJ-39758979 (C) compared to vehicle-treated mice (B).

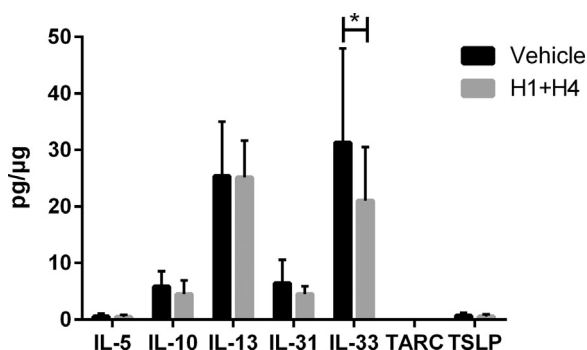


Fig. 4. Levels of cytokines measured in lesional skin sites. Data show pg cytokine per 1 µg protein. Mice treated with a combination of mepyramine and JNJ-39758979 (H1 + H4) produced significantly less IL-33. * $p < 0.05$ Mann–Whitney test. $n = 13$ mice per group.

mepyramine: 20 ± 3 pg/µg). All other cytokines tested were not influenced by mepyramine of JNJ-39758979 alone. The level of TARC in lesional skin sites was below the limit of detection.

3.6. Treatment with the combination of H1R and H4R antagonists reduces the number of lymph node cells

Mice treated with the combination mepyramine + JNJ-39758979 as well as mice treated with mepyramine alone had a significantly reduced number of cells in the skin draining lymph node compared to vehicle treated mice (Table 2). Thereby the absolute cell count of CD19 + B cells, CD4 + T cells, CD8 + T cells, CD11c + dendritic cells and F4/80 + macrophages were lower in mice treated with the combination H1R + H4R antagonist (Table 2). However, no alteration in the cellular profile could be observed. Treatment with the H4R antagonist alone neither did reduce the cell count, nor altered the cellular profile in the lymph node (Table 2). In supernatants of lymph node cells as well as CD4 + T cells re-stimulated with OVA hardly any IL-4, IL-10, INF-γ, IL-6, TNF-α or IL-13 could be measured.

3.7. Treatment with H1R + H4R antagonists modulates cytokine secretion of splenocytes

H1R + H4R antagonist-treated mice had a significantly reduced number of splenocytes, compared to vehicle-treated mice (Fig. 5A). Mepyramine (vehicle: 34 ± 9 , mepyramine: 32 ± 19 mio. cells) and JNJ-39758979 (vehicle: 20 ± 6 , JNJ-39758979: 21 ± 14 mio. cells) alone did not influence the total cell count. Splenocytes re-stimulated with OVA produced detectable amounts of all cytokines tested (Fig. 5B). Splenocytes of mice treated with the combination mepyramine + JNJ-39758979 produced significantly more IL-10 and IL-5 (Fig. 5B), all other cytokines did not differ compared to vehicle treated mice. Splenocytes of mice treated with mepyramine alone produced significantly less IL-6 (vehicle 320 ± 63 ; mepyramine: 166 ± 68 pg/ml; $p < 0.05$ Mann–Whitney test), whereas in mice treated with the combination mepyramine + JNJ-39758979 IL-6 was only slightly reduced. All other cytokines measured did not differ between groups.

4. Discussion

This study demonstrated that the combination of an H1R and an H4R antagonist shows synergistic anti-inflammatory action in a mouse model of AD.

H1R antagonists are already widely used for the treatment of allergic diseases, but in case of AD their efficacy is insufficient [1]. In line with this, mepyramine alone could not prevent the OVA-induced skin inflammation in this study, which is in accordance with other studies in mice [4,7]. The H4R was brought into focus as a new target for the treatment of chronic inflammatory diseases like AD [2,12]. In this study, JNJ-39758979 could not improve dermatitis severity and also a dose enhancement up to 50 mg/kg (per os) did not modulate the clinical signs (data not shown). In H4R^{-/-} mice in contrast OVA-induced skin lesions are clearly ameliorated [4]. Anti-inflammatory effects of H4R antagonists were demonstrated in hapten-induced dermatitis models [7,13], but not in house dust mite extract induced lesions in NC/Nga mice or in the OVA model [4,8]. A reason for the contradictory results in the mouse models might be the pharmacokinetic and pharmacodynamic properties of the H4R antagonist used. The half-life in mice of JNJ-39758979 (20 mg/kg per os) is 4.7 h, and a dosing twice daily was assumed to be sufficient [14,15]. However, for clinical efficacy the substance most likely has to reach effective drug levels in the skin [16], which has not been tested in the different mouse models yet.

Table 2
Cell number per lymph and flow cytometric analysis of lymph node cells.

Total number	Vehicle	H1	Vehicle	H4	Vehicle	H1 + H4
Lymph node cells (mio)	11.6 ± 3.2	7 ± 2 [*]	12.3 ± 3.8	11.9 ± 5.7	7.5 ± 3.5	4.5 ± 4.2 [*]
CD4+ T cells (mio)	–	–	–	–	2.2 ± 1.1	1.1 ± 0.2
Flow cytometry	Vehicle	H1	Vehicle	H4	Vehicle	H1 + H4
CD4+ T cells (mio)	5.1 ± 1.6	3.1 ± 0.8	4.6 ± 2.4	5.1 ± 2.2	3.7 ± 1.2	2.9 ± 2
CD8+ T cells (mio)	2.2 ± 0.7	1.4 ± 0.4 [*]	2.2 ± 1	2.9 ± 1.3	1.6 ± 0.4	1.2 ± 0.8
B cells (mio)	2.7 ± 1	1.6 ± 0.6 [*]	1.7 ± 1.1	2 ± 1.6	2.5 ± 0.9	1.7 ± 1.2
Dendritic cells	196k ± 37k	140k ± 57k	155k ± 98k	164k ± 65k	141k ± 45k	99k ± 66k
Macrophages	15k ± 4k	14k ± 14k	21k ± 19k	12k ± 16k	12k ± 45k	7k ± 4k

Total numbers of lymph node cells (counted in Neubauer chamber) and number of CD4+ T cells isolated from the skin draining lymph node (Lnn. axillares). In addition absolute numbers of CD4+ T cells, CD8+ T cells, B cells, dendritic cells and macrophages analysed by flow cytometry in single-cell suspensions from lymph nodes (Lnn. axillares) are shown. H1: OVA sensitized and challenged mice treated with mepyramine (20 mg/kg) during challenge. H4: OVA sensitized and challenged mice treated with JNJ-39758979 (20 mg/kg) during challenge. H1 + H4: OVA sensitized and challenged mice treated with mepyramine (20 mg/kg) plus JNJ-39758979 (20 mg/kg) during challenge. Control mice had a total number of 4 ± 1 (mean ± SD) mio lymph node cells (cellular profile was not determined in control mice).

^{*} $p < 0.05$ Mann–Whitney test (treatment group vs. vehicle group).

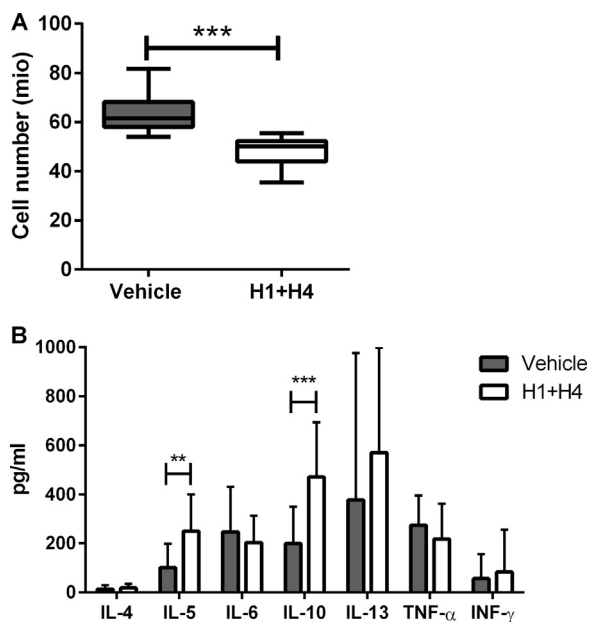


Fig. 5. Splenocyte stimulation with OVA. (A) Total numbers of splenocytes after two challenges with OVA. Mice treated with a combination of mepyramine and JNJ-39758979 (H1 + H4) had a significantly reduced number of splenocytes compared to vehicle-treated mice. $n = 13$ mice per group. Number of splenocytes of control mice is 19 ± 5 mio (mean ± SD). (B) Levels of cytokines measured in supernatants of splenocytes re-stimulated with OVA (100 µg/ml) for 96 h. Splenocytes of mice treated with a combination of mepyramine and JNJ-39758979 (H1 + H4) produced significantly more IL-5 and IL-10. $**p < 0.01$, $***p < 0.001$ Mann–Whitney test. $n = 13$ mice per group.

First studies in mouse models of allergic dermatitis indicate, that the combined treatment with H1R and H4R antagonists is more effective than H1R or H4R antagonist alone [6,7].

The combined treatment of the H1R antagonist olopatadine and the H4R antagonist JNJ-7777120 decreased picryl chloride-induced dermatitis in NC/Nga mice as effective as prednisolone [7]. Accordingly, in our study the dermatitis severity was significantly reduced in mice treated with mepyramine plus JNJ-39758979, but not in mice treated with the H1R or H4R antagonist alone. Lesional skin sites of OVA-treated skin show a thickening and inflammation of the dermis and epidermis [17]. Furthermore, a higher number of epidermal layers can be found in OVA sensitized skin [17]. Epidermal hyperplasia is a hallmark of

AD. Histamine modulates the proliferation capacity of keratinocytes, which might represent a mechanism that contributes to the epidermal hyperplasia observed in AD [18]. In the OVA model, the epidermal thickening is less intense in H4R knockout mice, but not in mice treated with an H4R antagonist alone [10]. The lack of effect of an H4R antagonist was confirmed in this study, and also the H1R antagonist alone did not affect the thickness of the epidermis. The combined treatment though clearly abolished the epidermal thickening. The total number of inflammatory cells was also reduced in the skin of H1R + H4R antagonist treated mice, again indicating that the combination of H1R and H4R antagonists abolishes the local cutaneous inflammatory response.

The OVA model shows important aspects of AD [19]. However, it is criticized that no pruritic behaviour is seen [9]. We could clearly demonstrate that OVA sensitized mice develop a strong scratching behaviour during the provocation phase. However, scratching behaviour has to be monitored a few hours after removal of the OVA patch/bandage because just the discomfort directly after removal of the patch/bandage most likely increases the scratching behaviour. The combined treatment with H1R + H4R antagonists significantly reduced the scratching behaviour after the first challenge with OVA, and by trend after the second challenge. This might be related to sedative effect of mepyramine, and mice might have developed a tolerance to the sedative effects after the second treatment week with mepyramine. However, mepyramine alone did not reduce the scratching behaviour, indicating that the anti-pruritic effect of mepyramine + JNJ-39758979 is not mainly due to the sedative properties of the H1R antagonist. In line with this, in the study of Ohasawa et al., 2012 the anti-pruritic efficacy of the combined treatment with the non-sedative olopatadine and JNJ-7777120 was as potent as that of prednisolone [7]. In another hapten-induced dermatitis model, the combination of the non-sedative H1R antagonist cetirizine and JNJ-7777120 reduced the scratching response about 90% [6]. H1R and H4R are both expressed on sensory neurons, and H1R and H4R antagonist might inhibit directly the transmission of pruritus from the peripheral to central nervous system [20]. However, beside histamine, a wide range of substances has been implicated as mediators of pruritus in AD, among them TSLP and IL-31 [21]. The anti-inflammatory action of histamine receptor antagonists might be due to an inhibition of other itch-inducing substances. A recent study has shown that the H4R regulates the TSLP production in murine and human keratinocytes [22]. However, modulation of TSLP or IL-31 concentration in the skin is according to this study not the reason for the antipruritic efficacy of the H1R + H4R antagonist treatment in the OVA model, because this cytokines were not reduced by H1R and H4R

antagonists. However, cytokines in the skin were analysed after the second challenge, and not after the first challenge with OVA, where the antipruritic effect of the H1R+H4R antagonists was more pronounced. Histamine might predominantly contribute to the pruritus at the beginning of skin inflammation, but not in the chronic stage. In line with this, it was shown in a canine AD model, that increased concentrations of dermal histamine were detected only during the initiation (1–3 h) of the allergic reaction [23].

In the skin of mice treated with the combination H1R+H4R antagonist, the level of IL-33 was decreased. IL-33 is upregulated in keratinocytes and endothelial cells of AD patients, and seems to play an important role in the pathogenesis of this disease [24,25]. In general, IL-33 is known to promote a Th2 immune response and also histamine is known to trigger a Th2 immune response [26,27]. An enhanced expression of IL-33 can be found in skin of OVA-sensitized mice [28]. Interestingly, IL-33 deficient mice epicutaneously sensitized with OVA do not develop skin inflammation [25]. The modulation of the IL-33 level in the skin might be one mechanism by which the combined treatment with the combination of H1R and H4R antagonist exerts their anti-inflammatory action in the OVA model. Other cytokines in the skin that were analysed in this study (TSLP, IL-5, IL-10, IL-13, IL-31 and TARC) were not influenced by the histamine receptor antagonists. In contrast, the study of Ohsawa et al. showed that olopatadine + JNJ-7777120 significantly inhibited the increases of IL-4, IL-5 and TARC in the skin of picryl chloride sensitized NC/Nga mice [7].

Beside the local inflammatory response, mepyramine plus JNJ-39758979 also modulated the systemic immune response. The total number of splenocytes was reduced, and in response to in vitro re-stimulation with OVA, splenocytes of mice treated with mepyramine plus JNJ-39758979 produced significantly more IL-10 and IL-5 compared to vehicle-treated mice. Studies with IL-10^{-/-} mice have shown that IL-10 promotes the development of a Th2 response to OVA. IL-10^{-/-} mice showed a lower expression of IL-4 and IL-5 in the skin. In addition, splenocytes of IL-10^{-/-} mice produced no measurable amounts of the Th2 cytokine IL-4, but significantly more of the Th1 cytokine IFN- γ than wildtype splenocytes after re-stimulation with OVA [29]. Thus, the enhanced production of IL-10 might have influenced the IL-5 secretion, which was also increased in H1R+H4R antagonist treated mice. However, these results warrant further investigations because, in general, histamine is known to regulate the development of an allergic state by enhancing the secretion of Th2 cytokines and by inhibiting the production of Th1 cytokines [26]. Moreover, the in vitro situation represents most likely only the profile of OVA-specific T cells which developed during the sensitization and provocation with OVA and does not reflect the situation in vivo.

Taken together, our results indicate that H1R and H4R antagonists provide synergistic anti-inflammatory effects in a mouse model of AD. Indeed, H4R antagonists have already entered clinical trials with patients suffering from AD [5]. JNJ-39758979 decreased the EASI in AD patients compared to placebo treated subjects and in a more recent phase 2a proof of concept study from Ziarco Pharma, the H4R antagonist ZPL-389 significantly reduced clinical symptoms in AD patients. Since H1R antagonist are in general well tolerated the combined therapy with H1R and H4R antagonists might represent a new strategy for the treatment of AD.

Conflict of interest

The authors have no conflict of interest to declare.

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