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To cite this article: Gomathi Nagarajan & Elden Berla Thangam (2020): Effect of H4R Antagonist N-(2-Aminoethyl)-5-Chloro-1H-Indole-2-Carboxamide (Compound A) in a Mouse Model of Allergic Asthma, Immunological Investigations, DOI: [10.1080/08820139.2020.1712415](https://doi.org/10.1080/08820139.2020.1712415)

To link to this article: <https://doi.org/10.1080/08820139.2020.1712415>



Published online: 27 Jan 2020.



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Effect of H4R Antagonist N-(2-Aminoethyl)-5-Chloro-1H-Indole-2-Carboxamide (Compound A) in a Mouse Model of Allergic Asthma

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ABSTRACT

Context: Allergic asthma is a multifactorial airway disease characterised by chronic lung inflammation and airway remodelling. The histamine H4 receptor involved in the chemotaxis of leukocytes and mast cells to the site of inflammation is suggested to be a potential drug target for allergy and asthma. In this study we examined the effect of Compound A, N-(2-Aminoethyl)-5-chloro-1H-indol-2-carboxamide a H4 receptor antagonist in allergic asthma mice model.

Objective: To investigate the anti-asthmatic effect of compound A in *in vivo*, airway inflammation in ovalbumin (OVA) induced allergic asthma mouse model was used.

Methodology: Allergic asthma was induced in Balb/c mice using ovalbumin. BAL fluid was examined for the level of IgE, IL-4, IL-5, IL-13 and IL-17 using ELISA. Furthermore, infiltration of leucocytes by histopathology and effect of compound A on signalling molecules were examined in lung tissue.

Results: In mice pre-treatment with compound A (10 mg/kg, 20 mg/kg, 30 mg/kg) at different concentrations markedly reduced the levels of IgE, Th2 cytokine IL-4, IL-5, IL-13 and Th17 cytokine IL-17 in BAL fluid. Histopathological examination of lung tissue showed that compound A was able to reduce the level of inflammatory infiltrates. Furthermore, lung tissue from Compound A treated group shown to down-regulate the levels of signalling molecules such as ERK1/2, Akt, SAPK/JNK and NF- κ B compared to OVA treated group.

Discussion and conclusion: Taken together our data demonstrates that compound A has shown to block the H4R-mediated allergic inflammation in this allergic asthma mice model and may be used as a molecule to study the function of H4R.

Abbreviations: Compound A, N-(2-Aminoethyl)-5-chloro-1H-indol-2-carboxamide; JNJ7777120, 1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine; H₄R: Histamine 4 Receptor; AHR: Airway hyper responsiveness

KEYWORDS

H4R antagonist; mast cells; histamine; Th2 cytokines; compound A; allergy and asthma

Introduction

Allergic asthma is a multifactorial airway disease characterized by chronic lung inflammation and airway remodelling affecting people of all age group. It has caused about 4,89,000 deaths in 2013, majority of which occurs in developing countries (Masoli et al. 2004). The recent increase in asthma ranges from allergies to environmental pollution. It is well

established that cross-linking of IgE antibodies on mast cells by antigen triggers the release of chemical mediators such as histamine, eicosanoids, cytokines, chemokines and various inflammatory mediators which causes immediate allergic reaction (Janeway et al. 2001).

Mast cells and basophils are the main source of histamine production in allergic inflammation. These cells primed with IgE release histamine from their preformed stores which acts on vascular smooth muscle cells and endothelial cells, leading to increased vasodilation and vascular permeability which causes broncho-constriction, increased airway hyper-responsiveness (AHR), mucus production and activation of T-helper 2 (Th2) cytokines such as IL-4, IL-5, IL-13 (Panettieri 2003). Histamine has thought to be an important mediator of asthma due to its ability to recapitulate symptoms of asthma in *in vitro* and *in vivo* models (Thurmond et al. 2004; Venable et al. 2005; Annan and Silverman 1993; Engel et al. 2010; Christopher et al. 2012; Audaloussi et al. 2013; Beerman et al. 2012; Somma et al. 2013; Thurmond et al. 2014a, 2014b; Rossbach et al. 2009; Seike et al. 2010; Beaumer et al. 2011; Suwa et al. 2011; Engelhardt et al. 2012; Lim et al. 2005; Ahmad et al. 2015). It acts through its four known receptors, all belonging to GPCR family (60,91,93). The Gαq/11 coupled H1R plays a role in increased vascular permeability and smooth muscle contraction. The Gαs-coupled H2R acts as a key modulator in the secretion of gastric acid; the H3R is expressed predominantly in the central nervous system (CNS). The Gαi/o coupled H4R has its expression in cells of hematopoietic origin. Recent reports indicate that H4R is involved in redistribution and recruitment of mast cells in the mucosal epithelium, control of immune cell trafficking, pro-inflammatory mediator's release, increased expression of adhesion molecules and rearrangement of the actin cytoskeleton leading to immune cell migration from the bloodstream into the sites of inflammation amplifying allergic symptoms and maintaining chronic inflammation (Schroeder et al. 2001; Zampeli and Tiligada 2009).

Furthermore, the H4R activation causes the phosphorylation of ERK1/2, PI3 kinase and NF-κB to induce a variety of cellular responses including chemotaxis leading to excessive infiltration of leukocytes into the lung. Hence, H4R is now considered as a new drug target for the treatment of various inflammatory diseases including allergy and asthma (Seifert et al. 2011).

Recent reports have shown that drugs from various herbal extracts and its active compounds such as AST2017-01, astalbic acid are developed to treat allergic asthma and inflammation (Yuk et al. 2011; Yun Kim et al. 2019). In addition to this antagonist for different cytokines, leukotrienes, etc., were also reported. Based on the involvement of H4R in inflammatory and immunomodulatory functions, several small molecules such as JNJ7777120 which was considered as the first selective H4R antagonist, A-940894, A-943931, A-987306, INCB38579, VUF6007, VUF1489, were tested against H4R and has shown distinct effect on different cell types tested and various animal models of allergy and inflammation studied (Strakhova et al. 2009; Jablonowski et al. 2003; Reher et al. 2012; Shin et al. 2012). Recent reports suggest that H4R antagonist PF-3893787 (NCT00856687), UR-63325 (NCT01260753), KD1157, JNJ39758979 (Thurmond, Chen, et al. 2014a) to treat persistent asthma (NCT00946569), JNJ38518168 for the treatment of rheumatoid arthritis (David et al. 2019) (NCT01679951, NCT00941707, NCT01862224), asthma and psoriasis (NCT01823016 and NCT02295865) are also in various stages of clinical trials. NCT01679951 has been terminated due to issues in efficacy problem (Thurmond et al. 2015). Only a limited number of studies are available on these compounds and its therapeutic potential is not fully understood. Therefore, further

investigations on the development of novel lead compounds to explore their therapeutic potentials in treating allergic diseases are required.

Based on the previous study from our lab using bio-informatics tools and molecular docking we have selected Compound A, the analogue structure of JNJ777120 which lacks the methyl piperazine moiety which is present in JNJ777120 (Christopher et al. 2012). Previously, we reported that Compound A was able to inhibit the intracellular calcium release and Th2 cytokine IL-13 release induced by histamine and 4-methyl histamine in human mast cells (Nagarajan et al. 2016). In the present study, we examined the effect of compound A in an allergic asthma mice model established by sensitisation and challenged with ovalbumin (Scheme 1).

Materials and methods

Materials

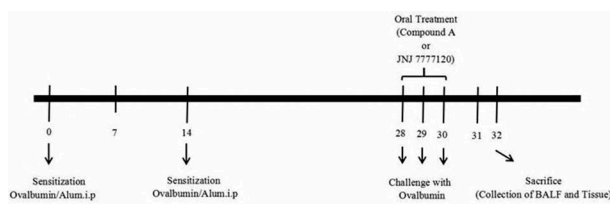
JNJ777120 was purchased from Sigma–Aldrich (St. Louis, MO), IL-13 and IL-17 ELISA kit from R&D Systems (Minneapolis, MN). IgE ELISA kit from affymetrix (Vienna, Austria),

IL-4 and IL-5 ELISA kits from ebiosciences (San Diego, CA), phospho-Akt antibody, phospho-ERK1/2 (Thr 177/Tyr160) and phospho-NF-κB antibody were purchased from Santa Cruz Biotechnology (Dallas, TX). Total ERK 1/2 antibody and total Akt antibody were purchased from Cell Signaling (Burlington, NC). Super Signal West Pico chemiluminescent substrate was purchased from Thermo Scientific and all the other basic chemicals were from Sigma (St. Louis, MO). IMDM, FBS, glutamine, penicillin and streptomycin were purchased from Gibco, Invitrogen, Carlsbad, CA.

Methods

Animals

Female BALB/c mice of age group 6–8 weeks were housed in animal facility under environmental condition of temperature $25 \pm 2^\circ\text{C}$ and 12 h light and dark regimens with water and standard food ad libitum. All the experimental procedures were done according to the approved Institutional Animal Ethical committee protocol (086/835/IAEC-2014).



Scheme 1. A mouse model of allergic airway inflammation and treatment with compound A.

N-(2-aminoethyl)-5-chloro-1H-indol-2-carboxamide (compound A) synthesis

Compound A was synthesised chemically as reported in our previous paper (Nagarajan et al. 2016). In brief, 100 mg of 5-chloroindol-2-carboxylic acid was dissolved in 10.0 ml of benzene and refluxed with 1 ml of SOCl_2 for 5 h at room temperature at 100°C . The excess of SOCl_2 was removed by distillation. The acid chloride was then added to 20 ml of ethylene-diamine and stirred at room temperature. The precipitated solid was removed by centrifugation and the supernatant was removed. The precipitate was dissolved in 10 ml of ethyl acetate, washed with 5 ml of water for two times and the organic layer was evaporated to get a solid. The solid was then dried over anhydrous sodium sulphate. The purity was checked using TLC. The structure of the compound was configured by NMR spectroscopy and molecular weight by mass spectroscopy.

Sensitization and airway challenge

Animals were divided into six groups [control, OVA, Compound A (10, 20, 30 mg/kg) + OVA and JNJ7777120 (10 mg/kg) + OVA] each group consisting of 6 animals. Ovalbumin was used to induce airway inflammation for different groups, using the method described by Ji-Eun Yuk et al. (24). Briefly, 20 μg chicken OVA along with 2 mg of aluminum hydroxide in 200 μL PBS buffer at the pH 7.4 was used to immunize mice via intraperitoneal (i.p.) injection, on days 0, and booster injections on day 7 and 14. After initial sensitization, on days 28, 29, and 30 the animals were exposed 20 min. to a 1% (w/v) of OVA solution prepared in PBS. The animals in treatment group were pre-treated with compound A or JNJ7777120 orally 20 min before OVA treatment. To characterize the inhibitory effects of compound A, animals were sacrificed on day 32 after the challenge (Scheme 1). In the present work, JNJ7777120 a standard H4R antagonist was used as a standard H4R antagonist.

Collection of bronchoalveolar lavage (BAL) fluid

Mice were sacrificed on day 32, and tracheotomy was done. BAL fluid was collected by injecting 0.5 mL of ice-cold PBS into each lung, by three continuous aspirations via tracheal cannulation. Centrifugation of BAL fluid was done at 250 g, 4°C , for 5 min and each supernatant was collected and stored at -70°C until further use.

Histopathology analysis

Lung tissues were fixed with 10% formalin and were embedded in paraffin which was cut into 4 μm thickness sections for light microscopic examination after staining with hematoxylin and eosin solution.

Analysis of bronchoalveolar lavage (BAL) fluid

Quantitative determination of total IgE in BAL fluid was estimated using a sandwich enzyme-linked immunosorbent assay (ELISA) using manufacturer's instruction. In brief, ELISA plates were coated with IgE in PBS at a concentration of 100 μL /well. Antibodies in

the BAL fluid were estimated using anti-mouse IgE secondary antibodies followed by HRP and then 200 μ L of Tetramethylbenzidine (TMB) was added to all the wells. The plates were then kept for 10 min incubation in dark, and the reactions were read at 450 nm (Biotek Synergy HT multimode reader).

Measurement of Th2 cytokine levels in the BAL fluid

The levels of Th2 cytokine (IL-4, IL-5, IL-13) and Th17 cytokine IL-17 in the BAL fluid were estimated using ELISA kits from R&D for IL-13 and IL-17, IL-4 and IL-5 kits from ebiosciences. The experiments were carried out according to the manufacturer's instructions. The reactions were measured at 450 nm (Biotek Synergy HT multimode reader).

Immunoblotting

We also investigated whether compound A was able to down-regulate the phosphorylation of ERK1/2, SAPK/JNK, Akt, and NF- κ B p65 in the lung tissue of mice. To check for tissue contamination while extraction, β -actin was used as a control. Lung tissue was homogenized in lysis buffer containing protease inhibitors and centrifuged at 12,000 g at 4°C for 25 min. The protein concentrations in the supernatants were estimated using Bradford assay. Protein sample (50 μ g/well) were loaded into each well of SDS-polyacrylamide gel and the separated proteins were transferred to PVDF membranes and were blocked with 5% (w/v) non-fat dry milk dissolved in TBST buffer and incubated overnight at 4°C. The blots were probed with either of anti- NF- κ B (1:50 dilution), ERK1/2, Akt, SAPK/JNK. The blots were then reprobed with anti- β -actin (1:200 dilution) antibodies, followed by anti-mouse-igG-HRP antibodies (santa cruz). The membranes were next washed again with TBST buffer, and the bands were visualized by SuperSignal West Pico chemiluminescent substrate (Thermo scientific) and the image was captured using multi-imaging system (Cell Biosciences). Images from multiple gels (at least three determinations) were quantitated using the fluorchem Q software.

Statistical analysis

Results were analysed using prism 6.0,7.0, San Diego California USA. All data were expressed as mean $\pm$ SEM. The treatment effect was analyzed using one-way ANOVA with Dunnett's multiple comparison tests. P -value <0.05 was considered statistically significant. For dendrogram analysis, the parameters were analysed at 95% confidence intervals and p values of <0.05 was considered to be statistically significant. In all cases, the experiments were repeated two to three times with similar results and representative data are shown.

Results

Effect of compound A on inflammatory cells infiltrates into lung tissue s

To evaluate the anti-inflammatory and anti-asthmatic effect of compound A, histopathological analysis of lung tissue was performed. The animals treated with OVA alone we observed blood vessel congestion along with the presence of dense inflammatory infiltrates

consisting mainly of lymphocytes and neutrophils in the interstitium compared with the normal tissue. Moreover, treatment with either JNJ777120, the standard H4R antagonist or compound A showed to lower the levels of inflammatory infiltrates in the lung tissue compared to OVA treated group (Figure 1). These results indicate that the compound A at different concentrations was able to reduce the level of inflammation in lung tissues of OVA challenged mice.

Effect of compound A on the levels of total IgE in BAL fluid

Asthma and allergic patients exhibit high levels of IgE. Therefore, we examined the inhibitory effect of compound A on IgE production in BAL fluid in OVA-mediated airway inflammation model. The total IgE in the BAL fluid collected from the animals 48 h after the last challenge were determined by ELISA. A notable increase in IgE (5,197.31767 pg/ml) production was observed in OVA treated mice. There was a significant decrease in the concentration of IgE among the groups treated with compound A (1,164 pg/ml, 1088 pg/ml, 1214.33 pg/ml). The standard H4R antagonist JNJ777120 at a concentration of 10 mg/kg (1239.33 mg/kg) also show suppressive effect in OVA treated mice (Figure 2).

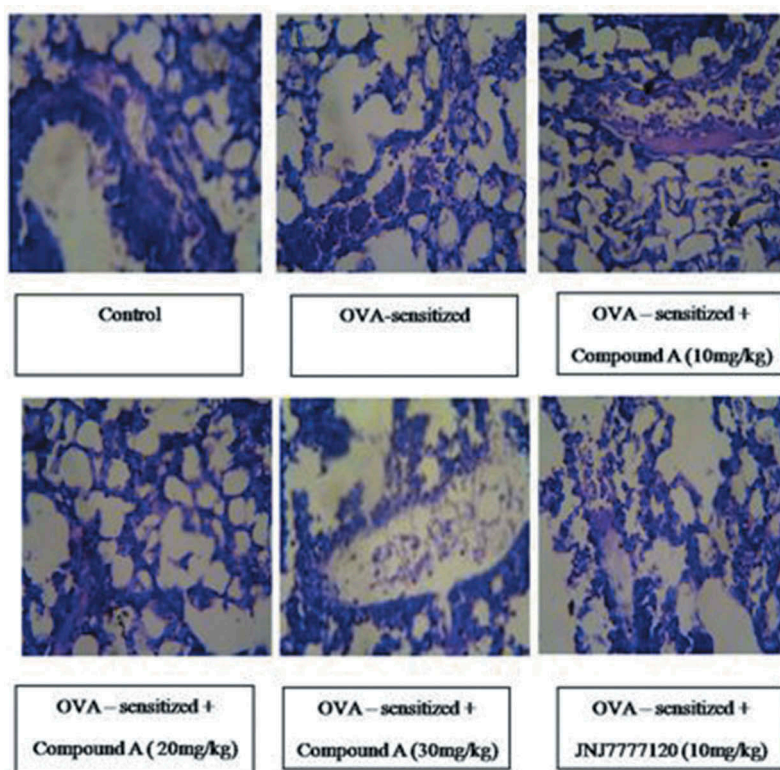


Figure 1. Effect of compound A in the lung tissue of allergen challenged lung tissue of Balb/c mice. Lungs from vehicle, OVA, compound A (10,20 and 30 mg/kg) and JNJ777120 (10 mg/kg) treated animals were sectioned and stained with H and E. 40 X magnification,6x645resolution.

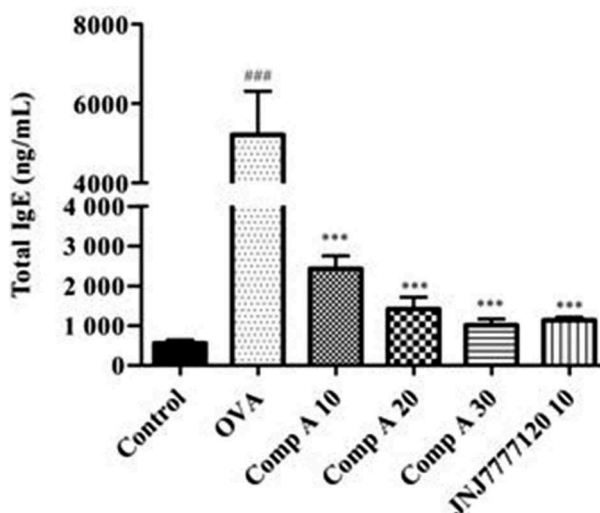


Figure 2. Effect of compound A on IgE production in BAL fluid in OVA-induced airway inflammation model. Control: mice were treated with PBS, OVA: OVA-sensitized mice, comp A 10, 20, 30: compound A(10 mg/kg, 20 mg/kg, 30 mg/kg) + OVA-sensitized mice. JNJ777120: JNJ777120 (10 mg/kg)+ OVA-sensitized mice. Values are expressed as mean $\pm$ S.E.M. (n = 4).

#p < .05, control compared with OVA-sensitized mice., *p < .05, OVA compared with compound at different concentrations.

The inhibitory effect of compound A on Th2 cytokines (IL-4, IL-5, IL-13) and IL-17 level in BAL fluid

To determine the effect of compound A on Th2 cytokines (IL-4, IL-5, IL-13) and IL-17 released in BAL fluid, levels were measured using ELISA. A notable increase in cytokines IL-4 (31.33 pg/ml), IL-5 (66.9 pg/ml), IL-13 (65 pg/ml) and IL-17 (44.8 pg/ml) production was observed in OVA treated mice. There was a significant decrease in the level of Th2 cytokines IL-4 (8.12, 8.065, 7.67 pg/ml), IL-5 (31.57, 271, 20.304 pg/ml), IL-13 (30.5, 40.2, 36.2 pg/ml) (Figure 3a–c) and Th17 cytokine IL-17 (20.156, 28.146, 18.993 pg/ml) (Figure 3d) in the groups pre-treated with the compound A at different concentrations. The standard H4R antagonist JNJ777120 (10 mg/kg) has also reduced the production of IL-4, IL-5, IL-13 and IL-17 levels in OVA treated group (Figure 3).

The inhibitory effect of compound A on signalling molecules in lung tissue

NF- κ B plays a major role in immune and inflammatory responses. The activation of ERK1/2 and Akt was considered to be an essential signal for the production of various Th2 cytokines in mast cells and other immune cells in allergy and asthma. SAPK/JNK is stress activated proteins which plays a pivotal role in the pathogenesis of allergic asthma. Hence, the effect of compound A on signalling molecules such as ERK1/2, Akt, SAP/JNK and NF- κ B p65 in the lung tissue of animals induced with allergic asthma was investigated (Figure 4a). Interestingly we found that lung tissue of animals treated with OVA treated group was able to stimulate the phosphorylation of ERK1/2 (23%), Akt (28%), SAPK/JNK

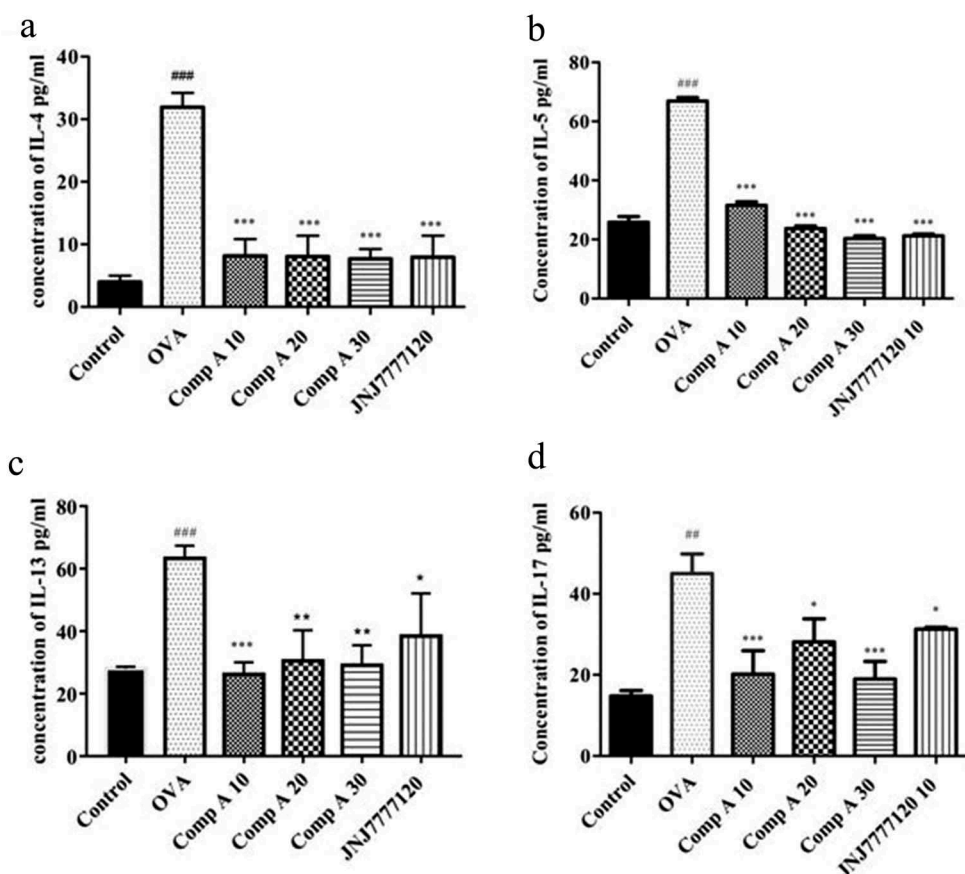


Figure 3. Effect of compound A on Th2 cytokines (IL-4, IL-5, IL-13) (a,b,c) and IL-17 (d) production in BAL fluid in OVA-induced airway inflammation model. Control: mice were treated with PBS, OVA: OVA-sensitized mice, Comp A 10, 20, 30: compound A (10 mg/kg, 20 mg/kg, 30 mg/kg) + OVA sensitized mice. JNJ777120: JNJ777120 (10 mg/kg) + OVA-sensitized mice. Values are expressed as mean $\pm$ S.E.M. (n = 4).

#p < .05, control compared with OVA-sensitized mice, *p < .05, OVA compared with compound at different concentrations.

(22.66%), and NF- κ B p65 (34%). Compound A (10 mg/kg, 20 mg/kg, 30 mg/kg body weight) was able to down regulate ERK1/2 (16%, 17%, 16%) (Figure 4b), Akt (12%, 18%, 15%) (Figure 4c), SAPK/JNK (16%, 17.33%, 16%) (Figure 4d) and NF- κ B p65 (10%, 15%, 10%) (Figure 4e) phosphorylation in the lung tissue. JNJ777120 the standard antagonist at a concentration of 10 mg/kg body weight was also able to down regulate the phosphorylation of ERK1/2, Akt, SAPK/JNK and NF- κ B p65 in the lung tissue.

Discussion

In the present study, we studied the effect of chemically synthesized H4R antagonist N-(2-aminoethyl)-5-chloro-1H-indol-2-carboxamide (compound A) (10 mg/kg, 20 mg/kg and 30 mg/kg bodyweight) that was generated using bioinformatics structure-based virtual screening strategies (Christopher et al. 2012) on allergic asthma mice model.

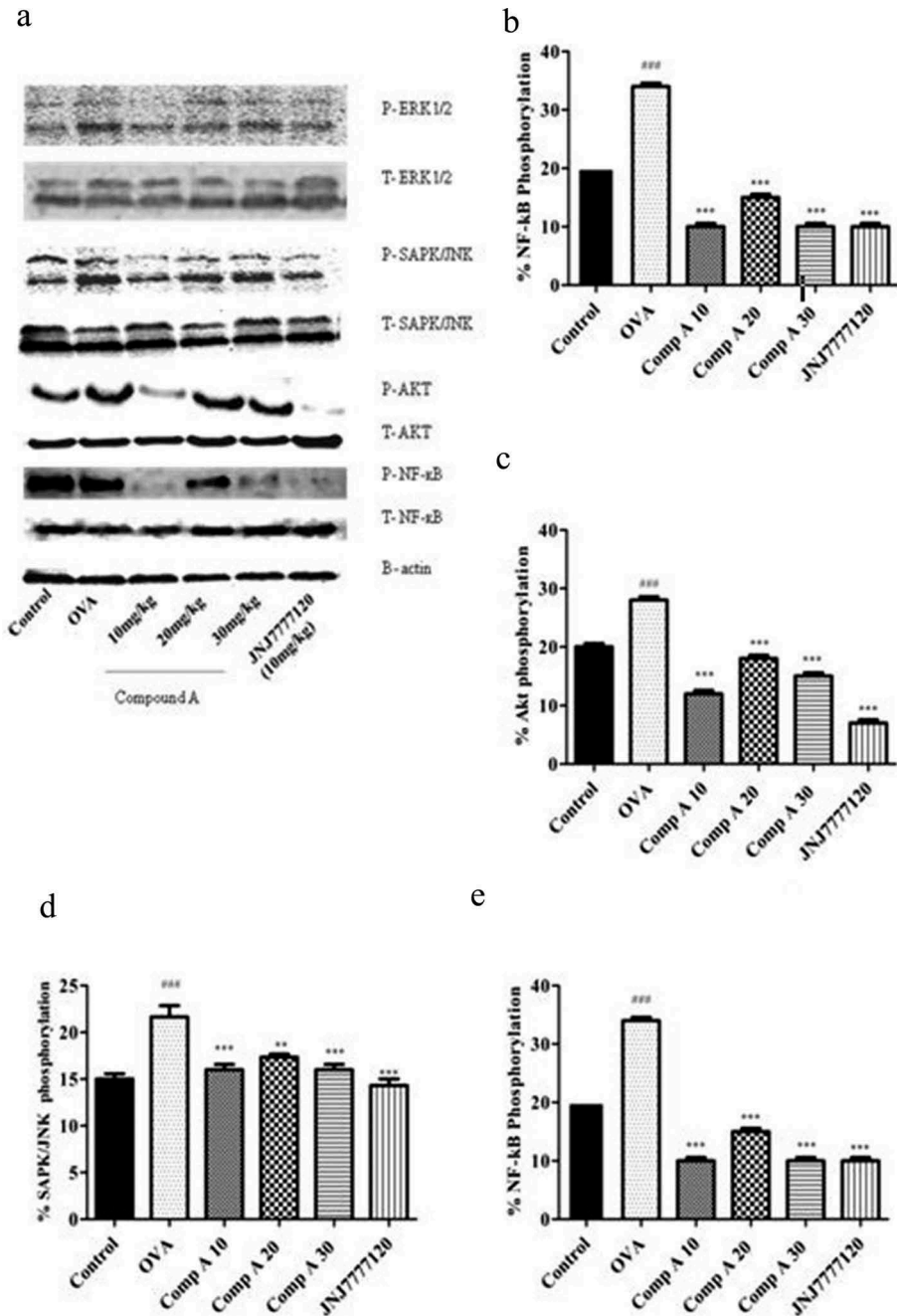


Figure 4. Female Balb/c mouse were sensitized with OVA and treated with compound A on day 28,29 and 30. The animals were sacrificed on day 32 and lung tissues were collected. The tissues were homogenised and cell lysates were separated on SDS-PAGE and blots were probed with anti-phospho-ERK1/2, anti-phospho-Akt, anti-phospho-SAPK/JNK and anti-phospho-NF-κBp65 antibody. Respective blots were stripped and reprobed with total or β-actin antibody followed by anti-mouse IgG-HRP antibodies. Immuno-reactive bands were visualized by SuperSignal West Pico chemi-luminescent substrate. Blots shown here are representative of three similar experiments. Bar graph represents summary of ERK1/2, Akt, SAPK/JNK and NF-κBp65 phosphorylation. Bands were quantified by densitometry and expressed as percentage. Values are analysed using one-way ANOVA with Dunnett's test. Data were expressed as mean $\pm$ SD of three experiments.

$p < .05$, was considered significantly different from control. * $p < .05$, was considered significantly different from OVA.

JNJ7777120 (10 mg/kg body weight) was used as the standard H4R antagonist. The IgE concentration, Th2 (IL-4, IL-5 and IL-13) and IL-17 cytokines estimation were done in BAL fluid whereas, the inhibitory effect of compound A on signalling molecules (ERK1/2, Akt, SAPK/JNK and NF- κ B) and inflammatory infiltrate cells were examination in lung tissue. Previously we have reported that JNJ7777120, compound A and compound L were able to inhibit the effect of histamine and 4-methylhistamine induced calcium mobilization, degranulation, Th2 cytokine release and activation of ERK1/2, Akt and NF- κ Bp65 when stimulated with histamine and 4-methylhistamine in HMC-1 cells (Nagarajan et al. 2016; Angel Jemima et al. 2014).

In the present study, we checked the effect of compound A on inflammatory cell infiltrates in lung tissue. Numerous studies have reported that the infiltration of eosinophils, T lymphocytes, and mast cells into the lung is the hallmark condition which contributes to the development of allergic airway inflammation (Elsner and Kapp 1999; Yuan et al. 2013; Hall and Agrawal 2014). Moreover, previous investigations also demonstrated that JNJ7777120 was able to therapeutically modify allergic inflammation and attenuate airway remodeling and hyperreactivity (Cowden et al. 2010). In our study we were able to show that pretreatment of compound A was able to inhibit infiltration of leukocytes into the lung tissue.

Furthermore, IgE-mediated allergic responses are the most common inducer of AHR. Therefore, we estimated the level of IgE and Th2 cytokines (IL-4, IL-5 and IL-13) and IL-17 production in BAL fluid. Previous reports demonstrate that JNJ7777120 has shown to regulate the level of IgE in a murine asthma model (Cowden et al. 2010). Different compounds from plant extracts such as Ivermectin, astalbic acid has also shown to regulate the levels of IgE in serum and BALF in mouse model of allergic asthma (Yan et al. 2011; Yuk et al. 2011). In line with the previous reports, pre-treatment with compound A has shown to down regulate the levels of IgE compared to OVA group.

Increasing experimental evidence suggests that the Th2 cytokines play an important role in inducing inflammatory responses and the pathogenesis of allergic reactions. The Th17 cytokine IL-17 recruits the activated lymphocytes, monocytes, neutrophils and eosinophils to lungs. Earlier it was reported that JNJ7777120 has anti-puritic and anti-inflammatory function in a mouse model (Shin et al. 2012) and also has shown to suppress the Th2 associated cytokines IL-5 and IL-13 from BAL fluid and lung tissue in sub-chronic model of allergic airway inflammation (Cowden et al. 2010). The H4R antagonist JNJ7777120 and VUF6007 have shown to have anti-inflammatory effect in rat model (Coruzzi et al. 2007). In accordance with the previous reports, in the current study we have shown that the animals pre-treated with compound A at different concentrations were able to down regulate the Th2 cytokines and IL-17 levels.

Several investigators have demonstrated that NF- κ B is a key transcription factor which plays an important role in regulating many cytokines and mediators that are involved in the immune response to allergy, inflammation and infection (Flohe et al. 1997). Previously it was thought that H1R activates NF- κ B in cos-7 cells, later it was found that H4R was also involved in the activation of NF- κ B (Remko et al. 2001; Gutzmer et al. 2009). JNJ7777120 has also shown to inhibit the phosphorylation of NF- κ B in a rat model of inflamed knee tissue (Lorentz et al. 2003; Desai and Thurmond 2011; Ahmad et al. 2015). The activation of ERK1/2 was reported to be an essential signal for the production of IL-5, TNF- α , IL-3 and IL-13 in mast cell (Lorentz et al. 2003). Li et al., has

shown that TLR2- knock-out asthmatic mice had decreased Akt phosphorylation levels compared to wild type asthmatic mice (Li et al. 2014). SAPK/JNK plays a pivotal role in the pathogenesis of asthma and are expressed by both Th1 and Th2 cells (Pelaia et al. 2005). Another study by Gutzmer et al. has showed that histamine via H4 receptor activated JNK. H3/H4 antagonists, thioperamide, clobenpropit has also inhibited histamine mediated phosphorylation of SAPK/JNK (Gutzmer et al. 2005). Similarly, in line with the previous reports, we have found that compound A was able to down regulate the phosphorylation of ERK, Akt, SAPK/JNK and NF- κ B in the lung tissue of animals with allergic inflammation.

Taken together, our data demonstrate that compound A was shown to inhibit allergic inflammation in allergen induced Balb/c mice. We noticed histopathological changes in lung tissue treated with compound A. It was able to lower the production of IgE and Th2 cytokine IL-4, IL-5, IL-13 and Th17 cytokine IL-17 in BALF. It has also shown to down regulate signalling molecules such as ERK1/2, Akt, SAPK/JNK and NF- κ B p65 in lung tissue.

Conclusion

Therefore, the preliminary study suggests that compound A may inhibit H4R mediated allergic and inflammatory reactions in allergen induced inflammatory mice model. The study shows that H4R antagonists may be useful in treating allergic asthma and inflammatory disorders. However, more studies are required to explore its potential. Further, the effect of compound A on mast cell response and its signalling mechanism in mast cell mediated allergic asthma is under progress.

Acknowledgments

We thank DST-SERB, Government of India, Ref.No: EMR/2017/000933 for funding this project. We thank Dr.Vairamani Mariappanadar, Dean, School of Bioengineering, SRM Institute of Science and Technology for helping in the synthesis of Compound. We are grateful to G. Ariharasivakumar (Associate professor, KMCH College of pharmacy) for his help with animal studies. This work was supported by SRM Institute of Science and Technology, Chennai, Tamil Nadu, India.

Disclosure statement

The authors declare no conflict of interest.

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