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Role of selective blocking of bradykinin receptor subtypes in attenuating allergic airway inflammation in guinea pigs.

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Abstract

The present study was designed to evaluate the potential role of bradykinin antagonists (R-715; bradykinin B₁ receptor antagonist and icatibant; bradykinin B₂ receptor antagonist) in treatment of allergic airway inflammation in comparison to dexamethasone and montelukast. R-715 as dexamethasone significantly decreased peribronchial leukocyte infiltration, bronchoalveolar lavage fluid (BALF) albumin & interleukin 1 β as well as serum OVA-specific IgE level. Also, R-715 like montelukast significantly decreased BALF cell count (total and eosinophils). Icatibant showed negative results. The current findings suggest that selective bradykinin B₁ receptor antagonists may have the therapeutic potential for the treatment of allergic airway inflammation.

Keywords: Bradykinin; R-715; Icatibant; Airway hyperresponsiveness; Bronchoalveolar lavage; Asthma

1- Introduction

Bronchial asthma is a complex disease or a syndrome characterized clinically by reversible bronchial obstruction (Haahtela, 2009). Asthma affects more than 334 million people worldwide (GAR, 2014).

Bradykinin and its metabolite des-Arg⁹-bradykinin mediate their effects through cell membrane attached receptors called bradykinin receptors that belong to the G protein-coupled family of receptors. Two bradykinin receptors are well characterized;

bradykinin B₁ and B₂ receptors. The former is up-regulated in many inflammatory conditions, while the latter is constitutively expressed (Marceau and Regoli, 2004).

Bradykinin first isolated by Rocha e Silva, Beraldo, and Rosenfeld, is a "slow" smooth-muscle-contracting substance, derived from plasma globulins by the proteolytic action of trypsin (Ambache and Silva, 1951).

R-715 is very highly selective antagonist for bradykinin B₁ receptors (Marceau et al., 1998). R-715, described initially in 1996 by Gobeil et al, is a synthetic peptide (Ac-Lys-[D-betaNal7,lle8]desArg9-bradykinin) that is partially resistant to degradation by angiotensin converting enzyme (Gobeil et al., 1996). R-715 reversed zymosan-induced hyperalgesia in a rat model of localized inflammatory hyperalgesia (Bélichard et al., 2000). Twice daily administration of R-715 for 7 days, significantly attenuated the hyperalgesic effect developed in streptozotocin-diabetic mice (Gabra and Sirois, 2003). Also, R-715 decreased cellular migration at the inflammatory site, in carrageenan-induced mouse pleurisy (Costa et al., 2006), reduced diabetes-induced Evans blue extravasation in diabetic rat retina (Abdoun et al., 2008) and significantly inhibited the capsaicin-induced ear oedema in mice when administered 15 min before the application of capsaicin (Pietrovski et al., 2009).

Icatibant is a potent synthetic selective bradykinin B₂ receptor antagonist. Icatibant, distantly related to the first peptide antagonists of bradykinin B₂ receptors described by Vavrek and Stewart (1985), incorporates several non-natural residues that confer rigidity to its backbone and resistance to peptidases (Hock et al., 1991; Wirth et

al., 1991). It is stable and not degraded by bradykinin-cleaving enzymes such as carboxypeptidase N (or kininase I) and angiotensin-converting enzyme (or kininase II) (Bas et al., 2010). Icatibant is a 10–amino acid peptide prepared by direct biosynthesis (Frank and Jiang, 2008). Icatibant is administered by subcutaneous injection at a dose of 30 mg and was licensed in the United States in August 2011 for physician- or patient-administered treatment of acute attacks in adults 18 years and older with Hereditary Angioedema (Riedl, 2012). Also, administration of icatibant was followed by rapid relief of symptoms in patients with angiotensin-converting enzyme inhibitor induced angioedema (Bas et al., 2010). The plasma half-life of icatibant is two to 4 hours. It is degraded by peptidases, and the degradation products are excreted via the kidneys (Bork et al., 2007). Icatibant has been associated with minor cutaneous reactions at the site of injection. Little is known about the long-term effect of bradykinin inhibition (Frank and Jiang, 2008).

Taken together, the present study was designed to evaluate the potential role of bradykinin antagonists (R-715 and icatibant) in treatment of allergic airway inflammation.

2- Materials and Methods

2.1 Animals

Experiments in this study were carried out on male English short hair tricolor guinea pigs (weight 300 – 350 g) that were housed and placed under a 12h light/dark cycle. Animals had free access to food and water throughout the experiments. All experiments and procedures were in accordance with the local regulations of Cairo University as well as the eighth edition of the Guide for the Care and Use of Laboratory Animals.

2.2 Drugs and chemicals

The following drugs were used: R-715 (Tocris, UK), icatibant (Tocris, UK), dexamethasone as Fortecortin® ampoules (Sigma-Tec Pharmaceutical Industries S.A.E.), montelukast as Singulair® tablets (Global Napi Pharmaceuticals-Egypt), acetylcholine (MP Biomedicals, USA), mepyramine maleate (MP Biomedicals, USA), urethane (Sigma-Aldrich, Germany) and succinylcholine as Succinylcholine Chloride® ampoules (Misr Co. for Pharm. Ind. S.A.E.). The following chemicals were used: ovalbumin (Bio Basic®, Canada) and aluminum hydroxide containing adjuvant (Imject® Alum, Pierce Biotechnology, USA).

2.3 Exposure box

A transparent box assembled from Perspex sheets, that measure 50 cm × 30 cm × 30 cm as length, width and depth (or height) respectively, was made to in order to expose animals to ovalbumin (OVA) aerosol during sensitization and challenge protocols. The box has been equally divided into 3 separate cells by perpendicular holed Perspex sheets to accommodate just two animals in each. As a means to adequately ventilate the box, inflow and outflow air ports were installed and fresh air was delivered by air compressor

(Norditalia Elettromedicali, Italy, Model: Air Family). Two inlets in one sidewall were used to deliver OVA aerosol generated by the ultrasonic nebulizer (Shanghai MedEco Industry Co., Ltd. China, Model W002).

2.4 Animal sensitization and allergen challenge

Guinea pigs were sensitized as previously described with minor modifications. Initially, 100 µg OVA (200 µl of 0.05% solution) adsorbed to 100 µl of Imject[®] Alum was intraperitoneally injected. One week later, the animals were exposed to an aerosol of 0.5% OVA solution for 5 min in the aforementioned exposure box. The ultrasonic nebulizer was set to produce a continuous aerosol (particle size of 1-5 µm) at a rate of 2 ml/min. Bias flow in the chamber was 11 L/min, driven by an air compressor. This was repeated a total of three times at one-week intervals as a booster (Fig. 1). One week after the final booster (on day 35), the animals were challenged with aerosolized 0.5% OVA solution for 60 min. To avoid anaphylactic shock, animals were pretreated with mepyramine (10 mg/kg, i.p.) 30min before each OVA exposure (Bergren, 2001; Mukaiyama et al., 2004).

2.5 Experimental groups

Animals were randomly allocated to the following groups (6 animals each). Negative control groups were represented by nonsensitized nontreated group, OVA-sensitized saline-challenged (60 min) nontreated (O/S-NT) and OVA-sensitized OVA-challenged nontreated (O/O-NT). Two sensitized groups were treated with R-715 and icatibant. R-715 was dissolved in distilled water and given at a dose of 1.14 mg/kg body weight (extrapolated from rats and modified). It was injected intraperitoneally 30 min before

OVA-challenge, 3 h after challenge and on the following day two h before testing airway reactivity (Abdouh et al., 2008). Icatibant was dissolved in distilled water and given at a dose of 130 µg/kg. It was injected intraperitoneally 30 min before challenge, 3 h after challenge and on the following day two h before testing airway reactivity (Ricciardolo et al., 1994). Two positive control groups were treated by dexamethasone and montelukast. Dexamethasone was given at a dose of 5 mg/kg. Dexamethasone was injected intraperitoneally a day before challenge, one h before challenge and on the following day two h before testing airway reactivity (Ozer et al., 2008). Montelukast was suspended in distilled water and was given at a dose of 5 mg/kg. Montelukast was administered by oral gavage (through a straight needle, 15 Gauge, 15 cm length with a blunt tip), a day before challenge, two h before challenge and on the following day two h before testing airway reactivity (Gobbato et al., 2012).

2.6 Determination of airway-hyperresponsiveness (AHR)

Airway resistance was measured according to the method of Konzett and Rossler (1940) with minor modifications. Briefly, Twenty-four hours after OVA last exposure (day 36), guinea pigs were anaesthetized with intraperitoneal administration of urethane (50% solution, 2 g/kg of body weight) (Plevkova et al., 2010). A tracheal cannula was connected to a small animal respirator (Searle BioScience, UK, Model: Miniature Ideal Pump). The animals were artificially ventilated with a constant volume respiration at a tidal air volume of 10 ml/kg and a frequency of 37 strokes/min (Muraki et al., 2009). The jugular vein was cannulated for intravenous injection of Ach (Takami and Tsukada, 1998). To eliminate spontaneous respiration, guinea pigs were

treated with succinylcholine chloride (2.5 mg/kg, s.c.). After a stabilization period of 15 min, changes in the peak inflation pressure (PIP; mmHg) at constant airflow were measured using a pressure transducer connected to a side arm of the tracheal cannula at one end and to the computerized data acquisition system (Powerlab[®], ADInstruments, Australia) at the other end (Eric et al., 2003). PIP was measured at baseline and following intravenous administration of increasing doses of acetylcholine from a wide range of concentrations (1/10,000- 1/1000 - 1/100). Doses were later corrected according to actual body weight of each animal (Xiong et al., 2012).

2.7 Determining Provocative Dose 200 (PD₂₀₀)

The provocative dose 200 (PD₂₀₀) is the dose at which airway peak inflation pressure (PIP) was 200% of its baseline value before administering acetylcholine. PD₂₀₀ was calculated using GraphPad Prism 6, San Diego, U.S.A.

2.8 Measurement of serum OVA-specific IgE antibody

After measurement of AHR, a 'Y'-shaped incision in the abdomen was made and the intestines were gently removed and the caudal vena cava (between the kidneys) was identified. Blood was collected in a plastic syringe connected to a 21G needle from the caudal vena cava (Parasuraman et al., 2010). Blood was allowed to clot at room temperature for one hour and then centrifuged at 550 g for 15 min. Aliquots of serum were stored at – 80 °C until analyzed for OVA-specific serum IgE by enzyme-linked

immunosorbent assay (ELISA) (Kim et al., 2007) using Guinea pig (OVA-specific IgE) ELISA kit (Sunred Biological Technology Co., Ltd, China).

2.9 Bronchoalveolar lavage fluid collection and analysis

Bronchoalveolar lavage fluid (BALF) was collected from each animal. A blunt stainless steel cannula with a female Luer hub (14G) was inserted into the trachea, and then warm saline solution (1ml/100 gram) was introduced into the lungs via a 10-ml syringe and then recovered 5 min later. After BALF collection, animals were euthanized by exsanguination via transection of the aorta and vena cava. The recovered lavaged fluid was centrifuged (Hettich, Germany, Model: Mikro 22R) at 500 g for 10 min at 4 °C; the resulting supernatant was collected and stored at – 80 °C (Smith and Broadley, 2010). The supernatant of BALF was employed to test total albumin turbidimetrically using a spectrophotometer (PG Instruments Limited, UK, Model: T80) (Schuh and Pahl, 2009) and interleukin 1 β (IL-1 β) using commercial ELISA kit (Sunred Biological Technology Co., Ltd, China) with an ELISA reader (Awareness Technology, USA, Model: Stat Fax 2100) while the cell pellets were resuspended in one milliliter of normal saline. By taking 50 μ l from the cellular suspension, the total number of cells was counted by a hemacytometer (Sysmex, Japan, Model: KX-21N). The remaining fluid was spread on the slide, fixed, and stained with Leishman's stain for differential cell counts. At least 200 cells were counted per slide. The different cell types were enumerated based on their morphology and staining profile (Tripathi et al., 2010; Vasquez-Pinto et al., 2010).

2.10 Histopathological examination

Lungs were removed after bronchoalveolar lavage fluid collection, immersed in 10% formalin for 24h until embedding in paraffin. Tissues were sliced with a microtome, and then sections were stained with hematoxylin-eosin. To determine the severity of inflammatory cell infiltration, peribronchial inflammatory cell number was blindly counted and the severity was evaluated by a 5 point scoring system. Briefly, the scoring system was: 0, no cells; 1, a few cells; 2, a ring of cells 1 cell layer deep; 3, a ring of cells 2–4 cells deep; 4, a ring of cells > 4 cells deep (Duan et al., 2004).

2.11 Statistical analysis

Data were analyzed using the SPSS version 19.0 statistical software (SPSS, Chicago, USA). All results were expressed as the mean $\pm$ standard error of the mean. Normality of data was assessed using a Shapiro–Wilk test and Levene's test was used to check for homogeneity of variance between groups. Binary comparisons were made by using the Student t test (two-sided) and Mann–Whitney U test. Comparisons between multiple groups were made by using the parametric One-way analysis of variance (ANOVA) test, followed by post hoc comparison with Games-Howell test or the nonparametric Kruskal–Wallis one-way analysis of variance, with Mann–Whitney U test as post Hoc analysis. A *P* value less than 0.05 was considered as statistically significant for all tests.

3- Results

3.1 Effect of R-715 and icatibant on airway hyperresponsiveness

Exposure of anaesthetized guinea pigs to successive intravenous doses of acetylcholine (20 –1280 µg/kg) induced a dose-related increase in PIP. An important parameter reflecting AHR is PD₂₀₀, which was determined by interpolation from acetylcholine Log dose response curves in all studied groups (Fig. 2). Repeated exposure of OVA-sensitized guinea pigs to OVA solution resulted in a significant increase in airway reactivity to acetylcholine and a decrease in the PD₂₀₀ value from 403.00 µg/kg in the nonsensitized group to 59.00 µg/kg in the sensitized OVA-challenged nontreated group.

PD₂₀₀ value was significantly increased from 59.00 µg/kg to 251.33 µg/kg in the group treated with R-715 (1.14 mg/kg, i.p.) in comparison to sensitized OVA-challenged nontreated group. However, result of icatibant at the dose of (130 µg/kg, i.p.) produced insignificant change. Dexamethasone and montelukast treated groups significantly increased PD₂₀₀ in comparison to R-715 treated group.

3.2 Effect of R-715 and icatibant on Serum OVA-Specific IgE Level

The mean serum level of OVA-Specific IgE immunoglobulin in OVA-sensitized OVA-challenged nontreated animals was significantly elevated (from 245.97 19 ng/ml to 381.19 ng/ml) compared to nonsensitized nontreated group. Serum IgE level was significantly decreased from 381.62 19 ng/ml to 334.52 ng/ml in the group treated with R-715 in comparison to sensitized OVA-challenged nontreated group (Fig. 3). However,

icatibant produced insignificant change. Further statistical analysis testing R-715 against dexamethasone and montelukast showed insignificant difference.

3.3 Effect of R-715 and icatibant on BALF inflammatory cells, albumin & IL-1 β

Hemocytometer was used to count cells in BALF collected from animals of all groups at 24 h after the last challenge. The differentiation between cells was done by light microscopy. Cells that were identified include macrophages/monocytes, lymphocytes, eosinophils, neutrophils and epithelial cells. OVA challenge in OVA-sensitized guinea pigs caused a marked infiltration of leukocytes into the lung, significantly increasing the total number of leukocytes (18.28×10^3 cells/ml) recovered in BALF by about 2.2 fold compared with that in the nonsensitized nontreated group (8.35×10^3 cells/ml) and 1.75 fold compared with that in OVA-sensitized saline-challenged group (10.45×10^3 cells/ml) (Table 1). The number of eosinophils recovered in BALF 24 hours after OVA challenge in sensitized guinea pigs was significantly increased in comparison to nonsensitized group, reaching up to mean of 9.94×10^3 cells/ml while was nearly undetectable in the nonsensitized group. Significant difference was observed in case of OVA-sensitized saline-challenged group with a mean eosinophil count of 0.50×10^3 cells/ml in comparison to OVA-sensitized OVA-challenged animals.

The mean total leukocyte count in BALF was significantly decreased from 18.28×10^3 cells/ml to 12.33×10^3 cells/ml in the group treated with R-715 (1.14 mg/kg, i.p.) in comparison to sensitized OVA-challenged nontreated group (Table 1). However, icatibant at the dose of (130 μ g/kg, i.p.) produced insignificant change. Further

statistical analysis testing R-715 against dexamethasone and montelukast revealed insignificant difference between R-715 and montelukast. Eosinophil count in BALF was significantly decreased from 9.94×10^3 to 3.36×10^3 cells/ml in the group treated with R-715 in comparison to sensitized OVA-challenged nontreated group (Table 1). However, icatibant induced insignificant change (from 9.94×10^3 to 9.75×10^3 cells/ml). Further statistical analysis testing showed insignificant difference between R-715 and montelukast. On the other hand, dexamethasone induced significant decrease in eosinophil count in comparison to R-715.

The microvascular leakage of albumin in lung tissues after OVA sensitization and challenge was reflected on albumin concentration in bronchoalveolar lavage fluid. The albumin level in BALF of OVA-sensitized OVA-challenged NT animals was significantly elevated (from 115.98 to 195.28 mg/dl) compared to nonsensitized nontreated group. In OVA-sensitized saline-challenged group the albumin level in BALF was 144.95 mg/dl, which is statistically different in comparison to sensitized OVA-challenged animals. The mean albumin level in BALF was significantly decreased from 195.28 to 161.64 mg/dl in the group treated with R-715 (1.14 mg/kg, i.p.) in comparison to sensitized OVA-challenged nontreated group (Table 1). Further statistical analysis revealed insignificant difference between R-715, dexamethasone and montelukast. However, icatibant at the dose of (130 μ g/kg, i.p.) produced insignificant change.

The mean IL-1 β level in BAL fluid of OVA-sensitized OVA-challenged NT animals was significantly elevated (from 14.33 to 21.63 pg/ml) compared to non-sensitized nontreated group. In OVA-sensitized saline-challenged group the IL-1 β level in BALF was 17.50 pg/ml which is statistically different in contrast to OVA- sensitized OVA-challenged animals. The mean IL-1 β level in BALF was significantly decreased from 21.63 to 15.86 pg/ml in the group treated with R-715 (1.14 mg/kg, i.p.) in comparison to sensitized OVA-challenged nontreated group (Table 1). Further statistical analysis testing R-715 against dexamethasone and montelukast revealed insignificant difference.

Table 1. Effect of Effect of R-715 & icatibant on inflammatory cells and albumin level in BALF

Group	Total cells ($\times 10^3$ cells/ml)	Eosinophils ($\times 10^3$ cells/ml)	Albumin (mg/dl)	IL-1 β (pg/ml)
NS	8.35 $\pm$ 1.12	0.00 $\pm$ 0.00	115.98 $\pm$ 6.42	14.33 $\pm$ 0.88
O/O-NT	18.28 $\pm$ 1.31 ^b	9.94 $\pm$ 0.97 ^b	195.29 $\pm$ 5.90 ^b	21.63 $\pm$ 1.44 ^b
O/S-NT	10.45 $\pm$ 1.42 ^a	0.50 $\pm$ 0.06 ^a	144.95 $\pm$ 7.60 ^a	17.50 $\pm$ 0.58 ^a
O/O-Dexamethasone	6.37 $\pm$ 0.77 ^a	1.11 $\pm$ 0.18 ^a	129.10 $\pm$ 7.60 ^a	15.31 $\pm$ 0.73 ^a
O/O-Montelukast	11.07 $\pm$ 0.53 ^a	2.77 $\pm$ 0.27 ^a	141.11 $\pm$ 4.06 ^a	16.04 $\pm$ 0.69 ^a
O/O-R-715	12.33 $\pm$ 0.65 ^{ac}	3.36 $\pm$ 0.08 ^{ac}	161.64 $\pm$ 9.64 ^a	15.86 $\pm$ 0.95 ^a

O/O-Icatibant	17.77±0.75	9.75±0.64	184.51±4.98	20.30±1.05
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Values shown are the mean $\pm$ S.E.M. of 6 animals per group. NS; nonsensitized, O/O; OVA-sensitized OVA-challenged, NT; nontreated, O/S; OVA-sensitized saline-challenged. R-715 treated group was statistically compared to dexamethasone and montelukast treated groups and only significant results have been shown.

^a $P < 0.05$, significantly different from sensitized OVA-challenged non-treated group.

^b $P < 0.05$, significantly different from non-sensitized group.

^c $P < 0.05$, significantly different from dexamethasone treated group.

3.4 Effect of R-715 and icatibant on lung inflammatory cellular infiltration

Histopathological analysis revealed the pathological features of asthma in OVA-sensitized and OVA-challenged guinea pigs. Lung sections showed a marked infiltration of inflammatory cells, including eosinophils, mononuclear cells and lymphocytes, into the peribronchial and perivascular spaces. Fig. 4 illustrates inflammatory scores from zero to four (0, 1, 2, 3 & 4) applied to sections from lung specimens obtained from nonsensitized & sensitized treated and nontreated animals.

The scores of peribronchial lung infiltration (Table 2) were increased significantly at 24 h after OVA challenge in OVA-sensitized nontreated animals with a mean score of 3.83 ± 0.17 compared with the scores of nonsensitized naïve animals (mean score = 0.17 ± 0.17). It was also significantly elevated compared with the scores of OVA-sensitized saline-challenged animals (mean score = 1.00 ± 0.26).

The selective bradykinin B₁ antagonist significantly decreased peribronchial lung inflammation in comparison to sensitized OVA-challenged nontreated group with a mean inflammatory score of 1.33 ± 0.21 (Table 2). Insignificant difference was observed between R-715 treated group and that of positive control groups (dexamethasone and montelukast). On the other hand icatibant showed insignificant effect compared to sensitized OVA-challenged nontreated group with a mean inflammatory score of 3.83 ± 0.17 (Table 2).

Table 2. Effect of Effect of R-715 & icatibant on lung histopathological scores

Group	H&E score
NS	0.17 ± 0.17
O/O-NT	3.83 ± 0.17^b
O/S-NT	1.00 ± 0.26^a
O/O-Dexamethasone	1.00 ± 0.37^a
O/O-Montelukast	2.00 ± 0.26^a
O/O-R-715	1.33 ± 0.21^a
O/O-Icatibant	3.83 ± 0.17

Values shown are the mean $\pm$ S.E.M. of 6 animals per group. NS; nonsensitized, O/O; OVA-sensitized OVA-challenged, NT; nontreated, O/S; OVA-sensitized saline-

challenged. R-715 treated group was statistically compared to dexamethasone and montelukast treated groups and no significant difference has been shown.

^a $P < 0.05$, significantly different from sensitized OVA-challenged non-treated group.

^b $P < 0.05$, significantly different from non-sensitized group.

4- Discussion

Asthma remains to be a major public health concern worldwide and is one of the greatest burdens to healthcare resources. The number of people with asthma in the world has increased from 235 to 334 million between 2011 and 2014 as stated in the latest Global Asthma Report 2014. Until now treatment of asthma remains to be unsatisfactory. The present study was designed to investigate the anti-asthmatic effect of blocking bradykinin receptors using R-715, a selective bradykinin B₁ receptor antagonist and icatibant; a well-known selective bradykinin-2 receptor antagonist in a guinea pig model sensitized by OVA.

In the present study, it was demonstrated that R-715 as dexamethasone significantly decreased peribronchial leukocyte infiltration, BALF albumin & IL-1 β as well as serum OVA-specific IgE level. Also, R-715 as montelukast significantly decreased BALF cell count (total and eosinophils). In fact, R-715 decreased IL-4 in BALF besides suppressing bronchial cyclooxygenase -2 & inducible nitric oxide synthase expression (unpublished results). On the other hand, icatibant failed to neither improve airway AHR nor modulate significantly any of the tested parameters.

The relative discrepancy of the results of R-715 in comparison to the two positive controls possibly reveals the role that can be played by selective bradykinin B₁ antagonists in asthma treatment plan. Glucocorticoids attenuate broader facets of the inflammatory cell profile including peribronchial mononuclear cell aggregation and the degree of the anti-inflammatory effect of other asthma controllers as leukotriene antagonists is generally mild relative to that attained with corticosteroids (Leick-maldonado et al., 2004; Ogawa and Calhoun, 2006).

As expected, AHR as well as other values of assessed parameters have considerably changed when sensitized animals were challenged with ovalbumin in comparison to saline challenged animals. However, the latter did show a milder degree of airway reactivity. The reasons behind this finding might be attributed to changes in the fluid lining of the respiratory tract upon saline challenge or that animals started to develop chronic allergic pulmonary inflammation (Smith and Anderson, 1989; Souza et al., 2013)

A putative role of bradykinin B₁ receptors in allergic inflammation is supported by the results of this research. Bradykinin B₁ receptor expression is induced by a number of different proinflammatory cytokines including IL-1 β , tumor necrosis factor-alpha (TNF- α), or interferon gamma (IFN γ). These stimuli turn on a common signaling pathway involving the transcription factor nuclear factor kappa (NF κ B), which is a pivotal manager for induction of bradykinin B₁ receptor. In addition, bradykinin B₁ receptor activation itself stimulates NF κ B activation, eventually leading to increase of

IL-1 β or TNF- α synthesis (Leeb-Lundberg et al., 2005; Schanstra et al., 1998). These findings suggest that bradykinin B₁ receptor expression may be autogenously boosted uninterrupted thus favoring chronicity.

DesArg9-bradykinin (potent bradykinin B₁ receptor agonist) is able to stimulate the production of inflammatory mediators such as prostaglandins E₂ and I₂, platelet activating factor by endothelial cells, IL-1 and TNF- α by macrophages (Eric et al., 2003; Toda et al., 1987).

Many inflammatory cells show Bradykinin B₁ receptor expression including macrophages, fibroblasts, mast cells, epithelial cells, smooth muscle cells, and endothelial cells (Leeb-Lundberg et al., 2005). The main bradykinin B₁ receptor mediated effects include vasodilatation, plasma protein extravasation, pain mediation, activation of leukocyte–endothelial cell interactions and leukocyte recruitment (Ellis and Fozard, 2002).

Pesquero et al. (1996) showed that polymorphonuclear leukocytes was decreased by 65% in inflamed tissues from transgenic bradykinin B₁ knockout mice. Vianna and Calixto (1998) demonstrated that the intrathoracic administration of desArg9-bradykinin in a mouse model of pleurisy induced plasma leakage and neutrophil accumulation.

Another study demonstrated that the bradykinin B₁ receptors are also involved in the release of inflammatory cytokines (IL-6, IL-8, Granulocyte-colony stimulating factor, chemokine (C-C motif) ligand 2 (CCL2), and transforming growth factor beta by

human type II pneumocytes that are responsible for the modulation of lung inflammation (Koyama et al., 1998).

There is a strong evidence to support a role for endothelial bradykinin B₁ receptor expression and activation in inflammation. In this context, Duchene et al. (2007) demonstrated that bradykinin B₁ receptor induced neutrophil recruitment is, at least partly, due to endothelial chemokine C-X-C motif chemokine 5 (CXCL5) synthesis and it is possible that the C-fiber-dependent component of bradykinin B₁ receptor-induced cell recruitment is involved. In addition, various immune cells express bradykinin B₁ receptor such as neutrophils, macrophages and T-lymphocytes (Ehrenfeld et al., 2009; Eric et al., 2003).

Christiansen et al. (2002) demonstrated the presence of functional bradykinin B₁ receptor in the airways during allergic inflammation. This was confirmed by the marked increase in the expression of bradykinin B₁ receptor mRNA in individuals with allergic rhinitis, while no significant difference was found in bradykinin B₂ receptor expression.

Bradykinin antagonists were the focus of eyes of researchers to treat asthma. An early study was conducted by Akbary et al. (1996) to investigate the efficacy of oral inhalation of bradykinin B₂ receptor antagonist icatibant in adult patients with chronic asthma. After 4 weeks of inhaling nebulized icatibant three times daily, a statistically significant difference between icatibant and placebo of about 10% was achieved for all pulmonary function tests. This study differs from the current work in two important

aspects. First, icatibant was administered in that study by oral inhalation not systemic. Second, the long duration of icatibant administration that lasted for up to 4 weeks.

In a porcine model, Sylvin *et al.* (2001) examined the effect of blocking the bradykinin B₂ receptor in allergen-induced airway responses. They administered the bradykinin B₂ receptor antagonist NPC-567 to sensitized pigs before allergen (*Ascaris suum*) and during allergen challenge. NPC-567 was delivered as an aerosol. Respiratory functions were observed for 8 h immediately after challenge. NPC-567 inhibited mast cell degranulation as well as reduced the acute allergen-induced bronchoconstriction in sensitized pigs. Unlike the present study, the authors (Sylvin *et al.*, 2001) were verifying the early asthmatic reaction not the late phase, also, icatibant was given as an aerosol.

Involvement of bradykinin receptors in the allergic inflammation associated with AHR was evaluated in mice by means of the selective bradykinin B₁ receptor antagonists R-715 and R-954, or the selective bradykinin B₂ receptor antagonist icatibant by Eric *et al.* (2003). Cellular migration and AHR were examined 24 h after the second OVA challenge. R-715 and R-954 injected intravenously, 5 min prior to aerosol OVA challenges, decreased by approximately 50% the induced lung eosinophilia in OVA-sensitized mice but did not reduce AHR. Icatibant administered in the same manner, decreased mononuclear cell and eosinophil infiltration in BALF. Moreover, treatment of OVA-sensitized mice with icatibant completely suppressed the AHR to carbachol. The results of aforementioned study

are consistent to some extent with findings of the present work relevant to R-715 except that it was shown here that R-715 did improve AHR, yet there is conflict about icatibant. Worth noting, this study was done on mice not guinea pigs, plus the immunization protocol was just two weeks duration, comprised merely two OVA inhalation doses as well as the limitation of tested biochemical and histopathological parameters.

Closely matching results to the present research were concluded by Gama Landgraf et al. (2004) who studied the effect of bradykinin (B_1 or B_2) receptor antagonists in OVA-sensitized mice 24 h after induction of lung inflammation. The bradykinin B_1 receptor antagonist, R-954 and icatibant were given intraperitoneally, 30 min before induction. Pre-treatment with R-954 reduced eosinophil infiltration, mucus secretion and AHR to methacholine, while pretreatment with icatibant increased eosinophil infiltration and did not affect the other parameters. Again, this study was done on mice not guinea pigs, besides; the current work broadened the tested parameters.

Additionally, the present data is in agreement with Vasquez-Pinto et al. (2010). They investigated bradykinin antagonists in a murine model of asthma using albino mice immunized with OVA/alum and challenged with aerosolized OVA (for three weeks). Icatibant and R-954 were administered intraperitoneally 30 min before each challenge. In sensitized mice, the antigen challenge induced eosinophil proliferation in the bone

marrow and their migration into the lungs. These events were reduced by treatment of the mice with the bradykinin B₁ receptor antagonist while the bradykinin B₂ antagonist increased the number of eosinophils in the airways.

None of the aforementioned studies included a positive control group for comparison unlike here where dexamethasone and montelukast were compared with groups treated with bradykinin antagonists (R-715 and icatibant).

In fact the first generations of bradykinin antagonists were peptide molecules susceptible to enzymatic degradation with short duration of action limiting their clinical use. However, non-peptide bradykinin antagonists represent a novel generation of molecules, which offer better oral bioavailability, and therefore may be superior as future therapeutic agents (Éles et al., 2012).

In conclusion, bradykinin B₁ receptor antagonist clearly modulated the late phase of allergic lung inflammation. This was seen from the comparable results of R-715 to dexamethasone in significantly decreasing peribronchial leukocyte infiltration, BALF albumin & IL-1 β as well as serum OVA-specific IgE level. Further, from the comparable results of R-715 to montelukast in significantly decreasing BALF cell count (total and eosinophils). However more studies are needed before its possible benefit in the treatment of asthma could be confirmed.

Conflict of interest statement

The authors state no conflict of interest

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Fig. 1.

Time line of asthma induction and allergen challenge.

Fig. 2.

Effect of R-715 and icatibant on airway hyperresponsiveness (AHR) of guinea pigs. Animals were sensitized intraperitoneal ovalbumin (OVA) injection then by aerosol inhalation. AHR was expressed as provocative doses of acetylcholine causing a 200% increase from control level of airway responsiveness. Bradykinin antagonists were given i.p. before and after the last aerosol challenge as follows: R-715 (1.14 mg/kg) and icatibant (130 µg/kg). Dexamethasone and montelukast were used as positive controls. O/O; OVA-sensitized OVA-challenged, NT; nontreated, O/S; OVA-sensitized saline-challenged.

Each column represents the mean ($\pm$ S.E.M.) for 6 guinea pigs. ^{*} $P < 0.05$, significantly different from sensitized OVA-challenged non-treated group, [#] $P < 0.05$, significantly different from non-sensitized group, [§] $P < 0.05$, significantly different from dexamethasone treated group and [‡] $P < 0.05$, significantly different from montelukast treated group.

Fig. 3.

The mean ($\pm$ S.E.M.) serum level of Ovalbumin-specific immunoglobulin E (IgE) levels of nonsensitized, sensitized treated and nontreated guinea pigs. Dexamethasone and montelukast were used as positive controls. O/O; OVA-sensitized OVA-challenged, NT; nontreated, O/S; OVA-sensitized saline-challenged.

Each column represents the mean ($\pm$ S.E.M.) for 6 guinea pigs. R-715 treated group was statistically compared to dexamethasone and montelukast treated groups and no

significant difference has been shown. $^*P < 0.05$, significantly different from sensitized OVA-challenged non-treated group and $^{\#}P < 0.05$, significantly different from non-sensitized group.

Fig. 4.

Representative micrographs of lung tissue stained with hematoxylin & eosin obtained from nonsensitized (A) (x200), sensitized OVA challenged nontreated (E) (x400), dexamethasone treated (F), R-715 treated (G) and icatibant treated groups (H) (x200). A score of 0 (A), 1 (B), 2 (C), 3 (D), & 4 (E) was applied to the tissue sections according to peribronchial cell infiltration. Black arrows point to leukocyte infiltration (B, C, D, E and H). Arrow heads indicate eosinophilic infiltration (E).

Highlights

- Role of bradykinin B_1 receptors in allergic inflammation is supported by the results of this research.
- Selective blocking of bradykinin B_1 receptors by R-715 suppressed allergic airway inflammation in guinea pigs.
- The importance of bradykinin B_2 receptors in sharing of allergic inflammation is degraded herein.





