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Anti-inflammatory Effects of Telmisartan and Valsartan in Animal Model of Airways Inflammation

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ASTRACT

Background: The Renin-Angiotensin System (RAS) was potentially implicated in the pathogenesis of pulmonary disorders through its involvement in inducing pro-inflammatory mediators in the lung tissues. The present study evaluates the effects of the angiotensin receptor blockers (ARBs), telmisartan and valsartan on the inflammatory changes in sensitized rats. **Materials and Methods:** Twenty-four Wister female rats were randomly allocated into four groups: 1st, negative control; 2nd, positive control; 3rd, valsartan-treated group and 4th, telmisartan-treated group. The rats in the groups 2-4 were sensitized and challenged with ovalbumin (OVA). Negative control rats were sensitized and challenged with normal saline. Rats in 3rd and 4th groups were treated with either valsartan or telmisartan (5 mg^{-1} kg^{-1} day^{-1}), respectively. The effects of administered ARBs on Bronchoalveolar Lavage (BAL) concentrations of TNF-α, IL-4 and inflammatory cells were evaluated, in addition to serum level of IgE. **Results:** Treatment with telmisartan significantly decreases the BAL levels of TNF-α, IL-4 and inflammatory cells compared with both positive control and valsartan treated group after OVA-challenge. Serum IgE shows similar pattern of changes. **Conclusion:** Telmisartan demonstrates greater anti-inflammatory activity in sensitized compared with valsartan in animal model of lung inflammation.

Key words: Telmisartan, valsartan, pulmonary inflammation, cytokines, BAL

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INTRODUCTION

Inflammatory lung disease, like Chronic Obstructive Pulmonary Disease (COPD), is a general term that covers many lung disorders including frequent exacerbations and emphysematous conditions (Hogg, 2004; Han *et al*., 2010; De Oca *et al*., 2012). The pathophysiology of inflammatory lung diseases is mostly related to disorders in both the lung parenchyma and airways with evidence for the involvement of multisystem disorders like skeletal muscle dysfunction, cardiac disorders and systemic inflammation (Barnes and Celli, 2009; Bolton *et al*., 2004; Sin *et al*., 2006). Recently, many researchers focus on the involvement of the Renin Angiotensin System (RAS) in the pathogenesis of pulmonary manifestations of COPD. Angiotensin II (Ag II) receptors are expressed in lung tissues (Kakar *et al*., 1992) while the Angiotensin-Converting Enzyme (ACE) is well defined in the lung capillary blood vessels (Carter *et al*., 2005). Moreover, both types of Ang II receptors (AT1 and AT2) are expressed in human lungs (Malendowicz *et al*., 2000). Chronic inflammation of the airways is recognized as a central feature of COPD whichis associated with parenchymal cells destruction, lung remodeling and development of emphysema (Stockley, 2009). Meanwhile, the role of RAS activation in the pathogenesis of inflammatory lung disorders is well established, where Ang II proved to stimulate the release of IL-6, TNF-α and monocyte chemotactic protein-1 (MCP-1) (Hanif *et al*., 2010). It also shows immunomodulatory effects on T-cell responses that mediate pulmonary tissue damage associated with COPD (Kaparianos and Argyropoulou, 2011). The RAS also mediates generation of Reactive Oxygen Species (ROS) through the activation of AT1 receptors whichconsequently initiating mitochondrial dysfunction (Benigni *et al*., 2010) and oxidative damage at the site of generation within the pulmonary tissues (Rahman and Adcock, 2006). Increasing evidence has suggested that RAS blockade exerts an anti-inflammatory action in many systems and experimental models (Fliser *et al*., 2004; Al-Hejjaj *et al*., 2011). Raupach *et al*. investigated the effects of the AgII Receptor Blocker irbesartan (ARB) in emphysema mouse model and reported improvement in the histological findings and exercise capacity following

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treatment (Raupach *et al*., 2011). Such activity is correlated with the decrease in the secretion of inflammatory cytokines reported after treatment with losartan in a pilot study of COPD (Morrell *et al*., 2005). The present study was designed to determine the anti-inflammatory activity of ARBs telmisartan and valsartan in a rat model of inflammatory asthma.

MATERIALS AND METHODS

Chemicals and reagents: Valsartan was a gift from Novartis Pharma (Basle, Switzerland). Telmisartan was obtained from Boehringer Ingelheim (Germany). Ovalbumin (OVA) was purchased from Chadwll Heath (Essex, UK). A ready-made kits for ELISA were obtained from Wuhan Huamei Biotech Co (Shanghai, China).

Animals and treatment protocol: Twenty-four pathogen-free female Wistar rats (weighing 150-200 g, 4 weeks old) were obtained from the College of Pharmacy, University of Baghdad and housed in the animal house, College of Pharmacy, University of Basra; they were maintained on normal conditions of temperature, humidity and light/dark cycle and received food and water *ad libitum*. The local Research Ethics Committee in College of Pharmacy, University of Baghdad, approved the research protocol. The animals randomly allocated into four groups (each of 6 rats) according to the type of treatment; negative control, received only distilled water; positive control group, treated with distilled water (0.5 mL day^{-1}); valsartan-treated group, treated with valsartan (5 mg^{-1} kg^{-1} day^{-1}); and telmisartan-treated group, treated with telmisartan (5 mg^{-1} kg^{-1} day^{-1}). Both drugs and the vehicle (distilled water) administered orally as single daily doses using gavage tube.

Sensitization of animals and outcome measurements: According to the methods of Xue *et al*. (1998), the rats in the positive control group and ARBs-treated groups were sensitized by intraperitoneal injections on days 0 and 7 with 100 mg ovalbumin (OVA) and 100 mg $Al(OH)_3$ in 1 mL saline. On day 15, the rats were challenged with inhaled nebulized 1% OVA for 30 minutes, every other day for 30 days. 60 min prior to OVA exposure, the rats in positive control and ARBs-treated groups were given orally valsartan, telmisartan or distilled water, respectively. The rats in the negative control group were sensitized and challenged with 0.9% saline, every other day for 30 days. Challenges took place in a special glass chamber (20 cm×30 cm×40 cm) with free-breathing animals. After challenging the rats, they were anesthetized by intraperitoneal injection with 70 mg kg^{-1} sodium pentobarbital in saline within 24 h after the last treatment. Blood samples were collected directly from the heart.

The collected blood samples were left to coagulate for 20 min, centrifuged at 10000 rpm for 10 min to separate the serum whichis stored at -70°C for measurement of IgE using an ELISA readymade kit. The right main-stem bronchus was occluded with a Satinsky clamp and left lungs were lavaged via the tracheal cannula with 5 mL of sterile saline at 37°C three times. The Bronchoalveolar Lavage Fluid (BALF) was recovered manually by gentle aspiration with a disposable syringe after each infusion, the recovery rate of BALF was >80%. The lavage fluid was centrifuged (4°C, 10000 10 min^{-1}). Supernatant was stored at -70°C for TNF-α and IL-4 assays using ELISA readymade kit. The pellet of cells that remains after centrifugation of BALF samples was re-suspended in an equal volume of fresh PBS (1.0 mL). Total and deferential white blood cells count were measured using automated hematology analyzer (CELL-DYN RUBY®, USA), designed for *in vitro* diagnostic purposes in clinical laboratories. The instrument utilizes MAPSS (Multi-Angle Polarized Scatter Separation) technology, Laser flow cytometry, coupled with state of the art software to provide the latest in automation available from Abbott Hematology.

Statistical analysis: Results were expressed as Mean±S.D. Data were analyzed using GraphPad Prism software for Windows version 5.0 (GraphPad Software Inc, San Diego, CA, USA). Multiple comparisons among groups were made by unpaired student's t-test and one-way analysis of variance (ANOVA), supported by Bonferroni's *post hoc* analysis. Values with $p<0.05$ were considered significant.

RESULTS

Table 1 showed that exposure of rats in positive control, valsartan and telmisartan groups to OVA-sensitization/challenge resulted in a significant elevation in the level of TNF-α in BALF compared to negative control group ($p<0.05$). Treatment with telmisartan and valsartan significantly decreased the level of TNF-α compared to that in positive control group (untreated sensitized group) ($p<0.05$); meanwhile, telmisartan shows significantly greater effect compared with that showed in valsartan-treated group ($p<0.05$). Table 1 also shows that challenging of rats in positive control and ARBs-treated groups with OVA results in a significant elevation in the levels of IL-4 in BALF compared to

Table 1: Effects of valsartan and telmisartan on BAL fluid concentrations of TNF-α and IL-4 in sensitized rats

Treatment group	TNF-α (pg mL^{-1})	IL-4 (pg mL^{-1})
Negative control	36.63 ± 1.3^{a}	$8.20.8^{a}$
Positive control	$261.8\pm2.7\star^{b}$	$64.4\pm0.7\star^{b}$
5 mg kg^{-1} valsartan	$230.1\pm1.6\star^{c}$	$56.1\pm0.8\star^{c}$
5 mg kg^{-1} telmisartan	$104.4\pm1.9\star^{d}$	$24.7\pm1.2\star^{d}$

Values expressed as Mean±SE. No. of rats in each group = 6, ⋆significant difference with negative control ($p<0.05$), values with non-identical superscripts (a, b, c, d) among different groups are significantly different ($p<0.05$)

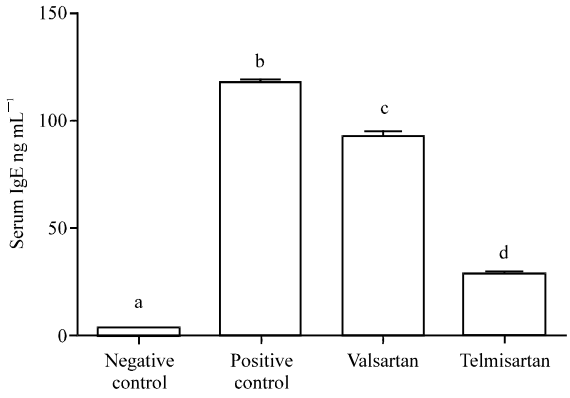

Fig. 1: Effects of Valsartan and Telmisartan on serum concentration of immunoglobulin E (IgE) in sensitized rats. Values with non-identical letters (a, b, c, d) are significantly different ($p<0.05$)

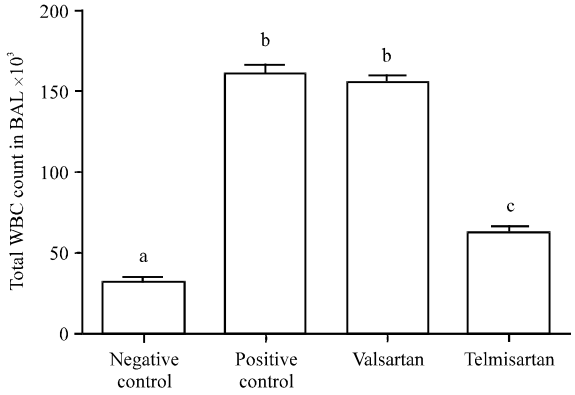

Fig. 2: Effects of Valsartan and Telmisartan on BAL white blood cells count in sensitized rats; BAL: Bronchial Alveolar Lavage; values with non-identical letters (a,b) are significantly different ($p<0.05$)

negative control group ($p<0.05$) and treatment with telmisartan and valsartan significantly decreases IL-4 levels compared to that in positive control group ($p<0.05$). However, telmisartan produces greater reduction in IL-4 concentrations in BALF whichis significantly different compared to that reported in valsartan-treated group ($p<0.05$). The results presented in Fig. 1 showed that exposure of rats in positive control, valsartan and telmisartan groups to OVA-sensitization/challenge results in significant elevation in serum IgE levels compared to negative control group (31, 24 and 7 folds, respectively; $p<0.05$). Meanwhile, treatment with telmisartan and valsartan suppresses the elevation of serum IgE levels significantly compared to untreated positive control group with significantly higher effect for telmisartan in this respect ($p<0.05$). Figure 2

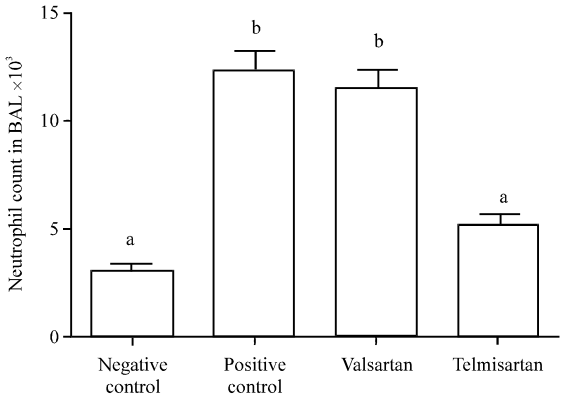

Fig. 3: Effects of Valsartan and Telmisartan on BAL neutrophils count in sensitized rats; BAL: Bronchial Alveolar Lavage; values with non-identical letters (a,b) are significantly different ($p<0.05$)

shows that total WBC number in BALF recovered from OVA-sensitized/challenged rats in positive control, valsartan and telmisartan were significantly higher than that from negative control group (4.1, 3.9 and 1.0 folds, respectively; $p<0.05$). However, telmisartan significantly decreases the total WBC count in BAL fluid compared with positive control group while valsartan did not show such effect. The results presented in Fig. 3 showed that the number of neutrophils in BALF recovered from OVA-sensitized/challenged rats from positive control and valsartan groups were significantly higher compared to negative control group (3.1 and 2.8 folds, respectively; $p<0.05$). Treatment with telmisartan significantly decreases neutrophils count compared to both the positive control and valsartan-treated groups ($p<0.05$) and comparable to that reported in group A ($p>0.05$). Similar pattern of changes were reported regarding the eosinophils and lymphocytes count in BALF, where telmisartan significantly attenuates the migration of eosinophils and lymphocytes to the alveoli after challenging the animals with OVA, compared with positive control and valsartan-treated groups (Fig. 4 and 5).

DISCUSSION

Chronic OVA-challenged rats exhibited marked upregulation of type 1 AngII receptor protein expressions, suggesting the association with airway inflammation and remodeling in a rodent model of asthma (Wang *et al*., 2008). Previous studies indicated that inhibition of ACE decreases the incidence of many disorders including vascular diseases, ischemic heart diseases, heart failure and pulmonary diseases (Dickstein and Kjekshus, 1999; Moller *et al*., 2004; Idell *et al*., 1987). Although, several studies strongly

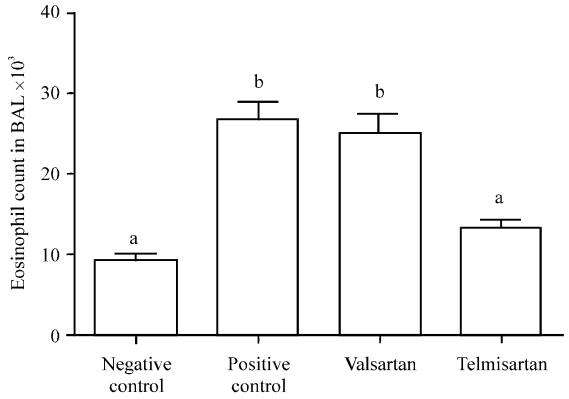

Fig. 4: Effects of Valsartan and Telmisartan on BAL eosinophils count in sensitized rats. BAL: Bronchial Alveolar Lavage; values with non-identical letters (a,b) are significantly different ($p<0.05$)

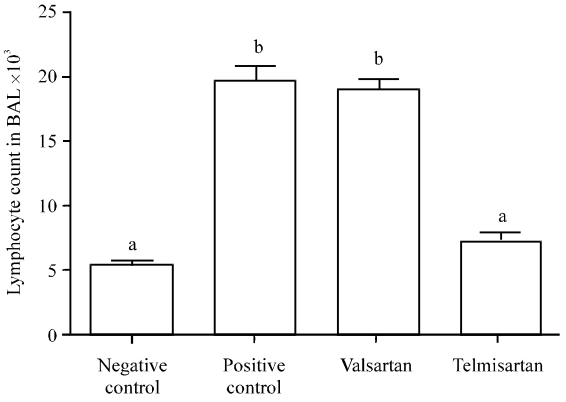

Fig. 5: Effects of Valsartan and Telmisartan on BAL lymphocytes count in sensitized rats; BAL: Bronchial Alveolar Lavage; values with non-identical letters (a,b) are significantly different ($p<0.05$)

suggest a potential role for RAS in lung disorders, particularly those of inflammatory origin, the role of different available approaches for interference with RAS components in the management if inflammatory lung diseases is poorly understood. The results of the present study suggest a role for AgII receptors in regulating the inflammatory reactions and recruitment of WBCs to the alveolar compartment after exposure to systemic inflammatory challenge with OVA. This finding is found in tune with that reported by others, where modulation of RAS decreases neutrophil influx to the lung after exposure to bacterial peptides (Raiden *et al*., 2000; Raiden *et al*., 2002). Moreover, blockade of ACE attenuates lipopolysaccharide-induced pulmonary neutrophils recruitment in mice model of inflammatory lung disorder (Arndt *et al*., 2006). Accordingly, the present study adds the observation that blockade of the RAS prevents the inflammation produced by sensitization and challenge with OVA; however, the significant differences between the effects of telmisartan and valsartan cannot be explained exclusively according to this idea. Recently, many research data suggest anti-inflammatory role for ARBs whichhave specific structural requirements, beyond AgII receptor blockade (Benson *et al*., 2004; Kramer *et al*., 2002), including losartan and telmisartan; however, conflicting data were obtained regarding valsartan (Algaem *et al*., 2013), where poor anti-inflammatory activity observed that attributed to poor activity as PPAR-γ agonist (Ushijima *et al*., 2013). However, others reported that valsartan inhibits inflammatory cell influx after chronic allergen exposure in sensitized Wistar rats (Wang *et al*., 2008). Similar conflicting results were observed in the clinical setting. In asthmatic patients, the use of candesartan (AT1 receptor blocker) slightly reduced bronchial hyperresponsiveness to methacholine (Tanaka *et al*., 2001). Meanwhile, the use of irbesartan in patients with COPD did not exert a significant effect on the primary end-point maximum inspiratory pressure (Andreas *et al*., 2006) while administration of losartan to asthmatic patients partially attenuates the hyperresponsiveness of airways to methacholine (Myou *et al*., 2000). The controversy regarding the influence of AgII receptor blockade in inflammatory lung disease highlights the involvement of other mechanisms, including PPAR pathway or others, behind such anti-inflammatory activity. Accordingly, more experimental and clinical data are required to support this idea. The existence of local RAS in the inflammatory cells, explains the ability of AngII to produce both autocrine and paracrine functions whichmostly associated with increased receptor protein expression and excessive production and release of many inflammatory mediators (Guo *et al*., 2011). This finding can explain the reported outcome of the present study, where blocking RAS was associated with reduction of inflammatory cells recruitment to the alveoli and decreased TNF-α and IL-4 release in BALF, in rats sensitized with OVA. Our results are in tune with an *in vivo* study that reported a significant increase in airways hyper-reactivity and accumulation of eosinophils in the alveoli after inhalation of an antigen and this effect was attenuated by candesartan (Myou *et al*., 2000). In conclusion, the ARBs telmisartan and valsartan attenuated the inflammatory cascade in the lungs of rats sensitized and challenged with OVA with predominant activity reported for the former.

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