ANTI-INFLAMMATORY ACTIVITY OF LOSARTAN IN EXPERIMENTALLY-INDUCED RESPIRATORY DISEASE IN RAT MODEL

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ABSTRACT

This study was carried out to evaluate the anti-inflammatory effect of losartan in 18 female rats which were divided into 3 groups. Respiratory disease were experimentally induced by the intraperitoneal injection and spray inhalation of ovalbumin (OVA) in first and second group while third group left as negative control group. First group were treated with losartan orally at dose rate of 5 mg/kg body weight, on the other hand second group considered as positive control group. ELISA test were used to estimate the concentration of TNF-α, IL-4 in BALF and total IgE in serum samples. Total WBC, neutrophil, eosinophil and lymphocyte were counted in bronchoaleveolar lavage (BAL). First and second rat groups show signs of pulmonary disease. Losartan treated group showed significant ($p<0.05$) decrease in concentration of TNF-α (152.483 pg/ml), IL-4 (39.733 pg/ml) in BALF and total serum IgE (56.006 pg/ml) in comparison with positive control group**.** A significant decrease ($p<0.05$) were also detected for total WBC (101.33 $x10^3$), neutrophil (8.83 $x10^3$), eosinophil (15.50 $x10^3$) and lymphocytes (15 $x10^3$) in BALF of losartan treated rat group in compare to positive control group.

INTRODUCTION

Angiotensin II (ANG II), is a potent vasoactive hormone that plays an important role in regulation of vasomotor tone and sodium and water homeostasis(1). High ANG II concentrations have been demonstrated in normal rat lung(2). ANG II modulates certain inflammatory responses, it has a pro-inflammatory effect, increasing local production of interferon-c and TNF-α (3). Some white blood cells (WBC) like monocytes produce ANG II (4), it also express AT1 and AT2 receptors for ANG II (5). Moreover, ANG II triggers many responses in monocytes and macrophages, such as chemotaxis, adhesion of endothelial cells and enhancement of phagocytosis (6). ANG II, and AT1 receptor antagonists, such as losartan, regulate the differentiation of dendritic cells (DCs) (7). Some such drugs suppress TNF-α and IL-1 synthesis by human peripheral blood mononuclear cells (PBMCs); thus, they have anti-inflammatory effects (8). The effectiveness of anti-inflammatory effect of losartan was found to be correlated to prevent monocyte development (9). Alveolar macrophages (AM) arise from circulating blood monocytes, which colonize the tissues under inflammatory and non-inflammatory states. AM-derived monocytes chemo-attractant protein-1 has a significant role in the recruitment of monocytes to the inflamed tissue (10,11). The low-affinity IgE receptor, FcεRII (CD23), is a Ca-dependent lectin that is expressed on B cells, as well as T cells, Langerhan cells, macrophage, monocytes, eosinophils, and platelets. The receptor consists of a large extracellular domain with the lectin head that binds IgE, a single transmembrane domain, and a short cytoplasmic tail. Like the FcεRI receptor, expression of CD23 is upregulated by IgE and IL-4 (12). Current study was designed to investigate the anti-inflammatory effect of losartan during the course of respiratory disease induced experimentally in rat model.

MATERIALS AND METHODS

Experimental animals

Eighteen Wister albino female rats weighing 180-250 gm were obtained from animal house of the College of Pharmacy/University of Baghdad. Animals were maintained on normal conditions of temperature and humidity. They were fed standard rodent pellet diet and they have free access to water. They were divided into 3 groups each group include 6 animals. First and second groups were sensitized by intraperitoneal injections of 1mg OVA (CHADWLL Heath ESSEX, England) and 100mg $Al(OH)_3$ dissolved in 1ml of phosphate buffer saline (PBS) at the first day. Second I/P injection of 100mg OVA , 100mg Al(OH)3 in 1ml of PBS was administered at fourth day. At eighth day, the rats were challenged by inhalation with 1% OVA (1gm OVA in 100ml PBS) for 30 minutes. On the other hand third group was challenged with inhaled PBS alone and considered as negative control group.

Inhalation were continued daily for 7 days for all groups. Sixty minutes prior to the challenge, the rats in the treated first group were gavage 0.5 ml suspension of 5mg/kg B.W. of Losartan (Actavis, New Zealand); while second and third groups were administered orally a comparable volume of distilled water.

Blood sample

After 30 days of the first treatment, rats were anesthetized by intraperitoneal injection of 70 mg/kg B.W. of sodium pentobarbital and blood were collected directly from the heart. Blood were left for 20 minutes to coagulate and centrifuged at 10000 rpm for 10 minutes to separate the serum which kept at -70 C° for measurement of total serum IgE using readymade ELISA kit according to the manufacturer's protocols (13).

Bronchoalveolar Lavage (BAL)

A cannula was inserted into the trachea in situ, lungs were lavaged three times with 5ml PBS solution, and BAL fluid was collected (14). Bronchoalveolar lavage fluid (BALF) were centrifuged at 10000 rpm for 10 minutes at 4 C°. The supernatant were stored at -70 C° for measurements of TNF-α and IL-4 using readymade ELISA kit according to the manufacture's protocols (15). Pellets that results after centrifuge were used to estimate total and deferential leukocytes count using automated hematology analyzer designed for in Vitro diagnostic use in clinical laboratories (CELL-DYN RUBY® system, USA).

Statistical analysis

Data were expressed as means ± standard error (SE) of means. The statistical significance of the differences between various groups was determined by PostHoc test (LSD alpha 0.05) and one-way analysis of variance (ANOVA) using SPSS version 18.0 software. Differences were considered statistically significant for $p<0.05$.

RESULTS

Tumor necrosis factor alpha (TNF-α) and IL-4 in bronchoaleveolar lavage fluid (BALF) as well as serum IgE were estimated using ELISA test, and the results are shown in table 1.

Table (1) The ELISA results of BALF TNF-α and IL-4 and serum IgE.

		TNF-α	IL-4	IgE
Groups	N	Mean ± SE	Mean ± SE	Mean ± SE
Group treated with Losartan	6	152.4833 ± 3.16369 *	39.7333 ± 2.18901*	56.0067 ± 1.69130*
Positive control group	6	246.1167 ± 2.82199	54.4083 ± 0.71401	102.5200 ± 2.59891
Negative control group	6	37.9667 ± 1.29735	10.0217 ± 0.42134	4.1617 ± 0.22715

* = p<0.05.

Figure 1, 2 and 3 revealed significant differences (p<0.05) between treated and negative control group according to TNF-α, IL-4 and total serum IgE concentrations respectively.

Rat group treated with losartan show significant decrease (p<0.05) in concentration of TNF-α (152.483 pg/ml) and IL-4 (39.733 pg/ml) in BALF, and total serum IgE (56.006 pg/ml) comparing to positive control group (246.116 pg/ml, 54.408 pg/ml and 102.52 pg/ml respectively)

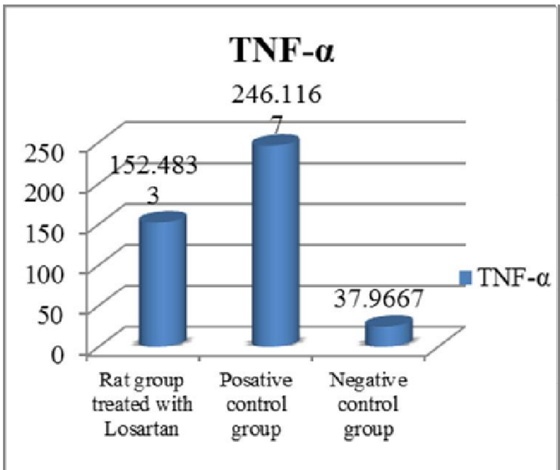

Figure (1) ELISA results of BALF TNF-α expressed as pg/ml

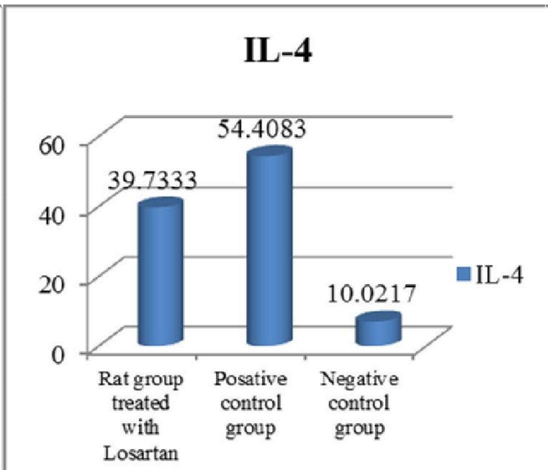

Figure (2) ELISA results of BALF IL-4 expressed as pg/ml

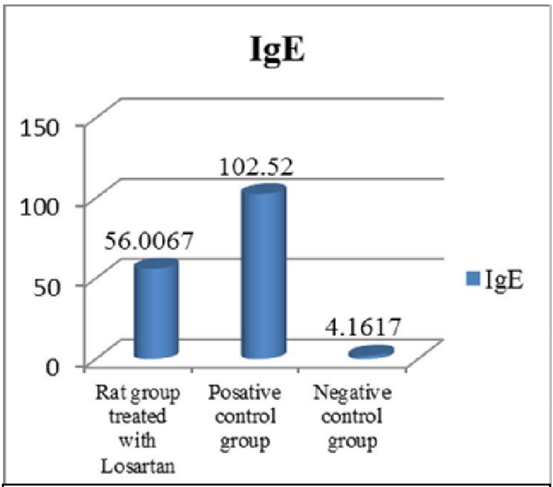

Figure (3) ELISA results of serum IgE expressed as pg/ml

Results of total WBC, neutrophils, eosinophils and lymphocytes count in BALF are shown in Table 2.

Table (2) Results of total and deferential leukocyte count in BALF expressed as $1x10^3$

		Total WBC count	Neutrophil	Eosinophil	Lymphocyte
	N	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Group treated with Losartan	6	101.33 ± 2.246*	8.83 ± 0.792*	15.50 ± 0.806*	15.00 ± 1.095*
Positive control group	6	161.17 ± 4.875	13.33 ± 0.919	28.17 ± 2.120	22.50 ± 1.118
Negative control group	6	42.83 ± 3.877	3.83 ± 0.401	10.83 ± 0.601	8.00 ± 0.365

* = $p<0.05$.

A significant increase ($p<0.05$) in total WBC, neutrophil, eosinophil and lymphocytes were detected in BALF of rat group treated with OVA in compare with negative control group (figure 4, 5, 6 and 7 respectively). On the other hand, according to the mentioned figures, there were significant decrease ($p<0.05$) in rat group treated with losartan (101.33 $x10^3$, 8.83 $x10^3$, 15.50 $x10^3$ and 15 $x10^3$ respectively) comparing to positive group (161.17 $x10^3$, 13.33 $x10^3$, 28.17 $x10^3$ and 22.50 $x10^3$ respectively).

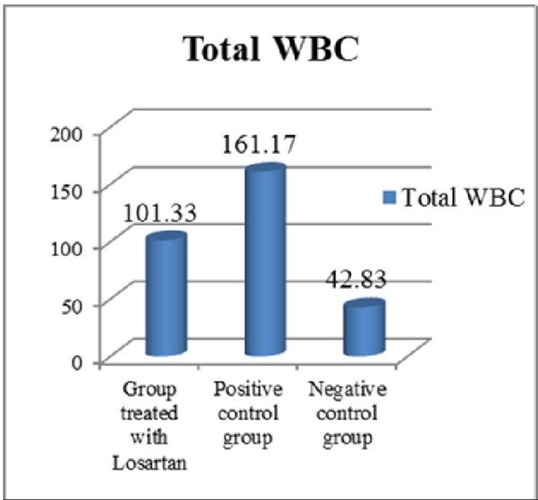

Figure (4) Total WBC count results in BALF expressed as $1x10^3$

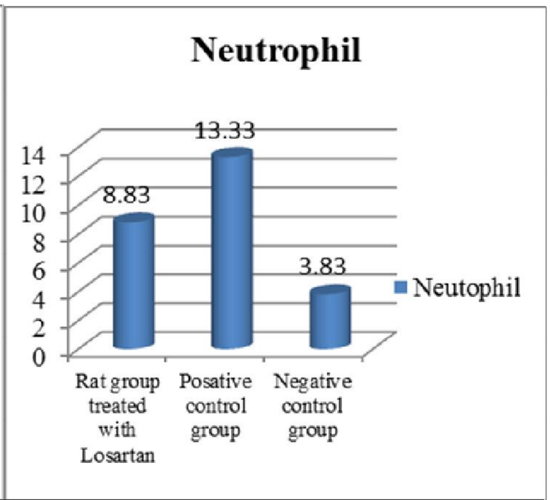

Figure (5) Neutrophil count results in BALF expressed $1x10^3$

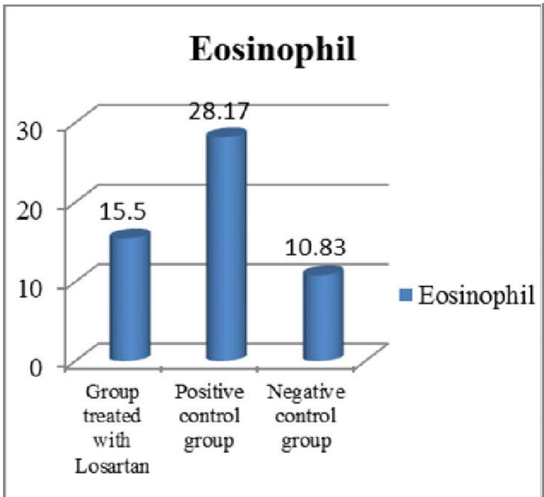

Figure (6) Eosinophil count results in BALF expressed $1x10^3$

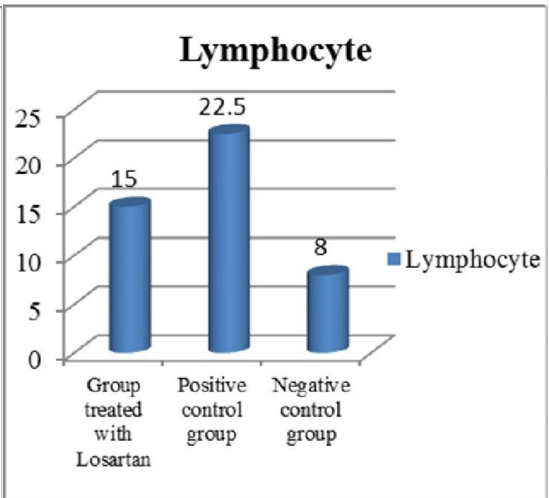

Figure (7) Lymphocyte count results in BALF expressed $1x10^3$

DISCUSSION

Pulmonary remodeling is occur as a result to combination of chronic repetitive injury to airway wall and the ensuing tissue process. Variety of cytokines, pro-inflammatory mediators, growth factors and enzymes are involved in the airway remodeling (16,17, 18, 19, 20). In the current study signs of respiratory disease was developed in groups sensitized by OVA. This results were in agreement with Palmans *et al*, (21), who found that prolong pulmonary sensitization with OVA result in remodeling the airway passage leading to respiratory disease (21). Although currently available ANG II receptor blockers biological actions are not identical (22), the findings in this study were in line of (23), who found that telmisartan and valsartan had an anti-inflammatory effect in rats with airway inflammation (23). TNF-α concentration in BALF was decreased significantly in group treated with losartan comparing to positive control group, these finding are in line of, (24), who stated that the treatment of patient with heart frailer by losartan can significantly decrease circulatory TNF-α (24). Moreover, (25), had demonstrated that DCs from AT1-deficient mice produce significantly lower levels of TNF-α (25). Furthermore, (26), indicated that losartan increase the production of IL-10 resulting in suppression of immune system (26); this findings explained the decrease in IL-4 and IgE as well as the decrease in total and deferential WBC in rat group treated with losartan.

التأثير المضاد للالتهاب لعقار اللوزارتان في الجرذان المصابة تجريبيا بالتهاب الجهاز التنفسي

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الخلاصة

اجريت هذه الدراسة لتقييم التأثير المضاد للالتهاب لعقار اللوزارتان في 18 انثى جرذ قسمت لثلاث مجموعات اذ استحدث التهاب الجهاز التنفسي مختبريا في المجموعتين الاولى والثانية بالحقن داخل الصفاق والاستنشاق البخاخي لمادة البومين البيض بينما تركت المجموعة الثالثة كمجموعة سيطرة سلبية. تم معالجة المجموعة الاولى بعقار اللوزارتان فمويا بجرعة مقدارها 5ملغ/كغم وزن الجسم، من ناحية اخرى اعتبرت المجموعة الثانية مجموعة سيطرة ايجابية. استخدم اختبار اليزا لتحديد تراكيز عامل النخر السرطاني الفا و انترلوكين-4 في السائل المستخلص من غسيل الرئة و IgE في مصل الدم. كما تم حساب العدد الكلي لكريات الدم البيض فضلا عن حساب كريات الدم العدلات والحمضات والخلايا اللمفاوية في السائل المستخلص من غسيل الرئة. اظهرت المجموعتين الاولى والثانية علامات التهاب الجهاز التنفسي بالمقارنة مع مجموعة السيطرة السلبية، فيما اظهرت المجموعة المعالجة بعقار اللوزارتان انخفاضا معنويا ($p<0.05$) في تراكيز عامل النخر السرطاني الفا (152.483 pg/ml) و انترلوكين-4 (39.733 pg/ml) في السائل المستخلص من غسيل الرئة وكذلك تركيز IgE الكلي في مصل الدم (56.006 pg/ml) بالمقارنة مع مجموعة السيطرة الايجابية. كما اظهرت المجموعة المعالجة بعقار اللوزارتان انخفاضا معياريا ($p<0.05$) في العدد الكلي لكريات الدم البيض (101.33×10^3) و العدلات (8.83×10^3) والحمضيات (15.5×10^3) والخلايا اللمفاوية (15×10^3) في السائل المستخلص من غسيل الرئة بالمقارنة مع مجموعة السيطرة الايجابية.

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