The Effect of Olmesartan on Inflammation and Oxidative Stress in Animal Model of Hypertension

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الخلاصة:
ارتفاع ضغط الدم هو أحد أهم عوامل الخطورة في أمراض القلب و الأوعية الدموية، وهو يعد الآن عملية التهاب مزمنة حيث إن كلا من الالتهاب و زيادة الإجهاد ألتأكسدي وما يسببه من تأكسد الدهون تساهم وبشكل أساسي في ارتفاع ضغط الدم. الاولميسارتان يعتبرمن الأدوية المضادة للالتهاب عن طريق تثبيط عوامل الالتهاب الأساسية وكذلك له خصائص مضادة للأكسدة. وعليه إن هذه الدراسة اجريت لتقييم تأثير دواء الاولميسارتان على ارتفاع ضغط الدم المحدث في الحيوانات من خلال تثبيط الالتهاب والإجهاد التاكسدي

الطريقة:
اربعة وعشرون جرذ أنثوي استخدموا في الدراسة وتم توزيعهم على ثلاثة مجاميع: المجموعة الأولى هي مجموعة السيطرة الطبيعية و أعطيت غذاء جرذان قياسي طبيعي فقط و عددها ثمانية جرذان. المجموعة الثانية هي مجموعة السيطرة ذات ارتفاع الضغط المحدث أعطيت حقن الكورتيكوتروفين الصناعي بجرعة (٠.٥ مغ/ كغم/ يوم) لمدة خمسة عشر يوم و عددها ثمانية. المجموعة الثالثة أعطيت حقن الكورتيكوتروفين الصناعي بجرعة(٠.٥مغ/ كغم/ يوم) لمدة خمسة عشر يوم ثم أعطيت دواء الاولميسارتان بجرعة (٨ مغ/ كغم/ يوم) عن طريق الفم لمدة ثمانية أسابيع و عددها ثمانية جرذان. تم اخذ نماذج من الدم بعد إحداث ارتفاع ضغط الدم قبل إعطاء العلاج وبعد ثمانية أسابيع من العلاج وتم قياس مستوى عوامل الالتهاب الأساسية (CRP,IL6,TNF-α) وقياس مستوى عامل التأكسد المالون ثنائي الالديهايد وكذالك مستوى الكلوتوثايون المختزل في الدم .وفي نهاية الدراسة بعد ثمانية أسابيع تم اخذ القلب وفحص التغيرات النسيجية فيه.

النتائج:
مقارنة مع مجموعة السيطرة الطبيعية مستوى كل من (hsERP, IL6, TNF-α ,MDA وضغط الدم) ارتفعت ارتفاعا معنويا ($P<0.05$) في الحيوانات ذات الضغط المرتفع المحدث بينما انخفض مستوى الكلوتاثايون المختزل .ولم تظهر تغيرات نسيجية على المستوى النسيجي.
ان العلاج بالاولميسارتان اظهر تاثيرا معنويا ($P < 0.05$) على كل من عوامل الالتهاب وعلى الاجهاد التاكسدي
الاستنتاج: نتائج دراستنا اظهرت الاولميسارتان خفض ارتفاع الضغط في الحيوانات ذات الضغط المرتفع المحدث من خلال تثبيط الالتهاب والاجهاد التاكسدي.

Abstract

Hypertension is one of the most common cardiovascular disease ; it is now widely accepted as a chronic inflammatory process. Low-grade inflammation, enhanced oxidant stress and lipid peroxidation play important roles in hypertension. Olmesartan exert a potent anti inflammatory activity by inhibition of inflammatory cytokines also it have anti oxidant effects..

The objective of present study was to assess the effect of olmesartan on hypertension via interfering with inflammatory and oxidative pathways

Twenty Four white laboratory female Wistar rats were assigned to three groups (Eight rats) in each group. Two groups of these three groups were induced with hypertension by giving intraperetonial injections of synthetic ACTH (0.5mg/kg/day) for Fifteen days. One of these tow induced groups were Olmesartan treated group: The other one was control untreated. Blood samples were collected at the end of induction of hypertension (2ed week) and after 8 weeks of treatment for measurement of serum (hsCRP, IL6, TNF-α ,malondialdehyde (MDA), and reduced glutathione (GSH) At the end of the eight weeks the hearts were removed for assessment the histopathological changes.

Compared with the control normal group, levels of hscRP, IL6, TNF-α ,MDA, and Blood pressure were increased and reduced GSH was decreased in hypertensive

animals (P<0.05). Histologically, all induced- untreated rats showed no significant changing (P>0.05). Olmesartan decreased the levels in hsCRP, IL6, TNF-α, MDA significantly compared with hypertensive untreated group (P<0.05), It also caused significant decrease in blood pressure and significant increase in GSH (P<0.05), it didn't show significant histological changing compared with induced untreated group p>0.05. Olmesartan possess antihypertensive effects in experimentally induced hypertensive rats via interfering with inflammatory and oxidative pathways.

Key Words: Olmesartan, , inflammatory markers, Oxidative stress

Introduction

Hypertension acts as a major determinant of endothelial dysfunction and vascular damage, promoting inflammatory activation of endothelial cells, recruitment of inflammatory cells in the arterial wall and activation of vascular resident elements systemic activation [1,2]. Mechanisms leading to this inflammatory response are not clarified and can include both mechanical stresses of the arterial wall and proinflammatory effects of humoural factors, such as Angiotensin II (AngII). [2]

Accumulating evidence from basic science researches and clinical studies showed that AngII, besides regulating the vascular tone, may exert some inflammatory effects on the arterial wall [2,3,4]. AngII, in fact, induces (NF-kB) activation triggering the production of inflammatory cytokine**s**, promotes the activation of NADPH oxidase followed by the release of reactive oxygen species (such as superoxide anion) and impairs endothelium-dependent vasodilatation by reducing nitric oxide (NO) generation [3] .The treatment of animal models of hypertension with Angiotensin receptor blockers reverses most of the detrimental effects of AngII on endothelial function and reduces the level of inflammatory activation in the vessels [4].

There is an increasing evidence base to support a relationship between elevation in vascular inflammatory markers and hypertension [5,6,7].

The selective AT_1R antagonism improved remodeling of resistance arteries beyond BP control, which could result in improved cardiovascular outcomes.[8]. Therefor, the aim of the present study was to assess the effect of olmesartan on hypertension via interfering with inflammatory and oxidative pathways**.**

Materials and Methods

In this study we have used twenty four white laboratory female Wistar rats that are of 200-250 gm body weight. Their ages range from(three to four) months, these rats were divided in to tree groups (eight in each group). The period of this study was continued from 7th Des . 2009 to 22nd Feb. 2010 in the animal house of the Collage of Medicine /University of Kufa and exposed to controlled temperature around 25 º C and humidity was kept at 60-65% .The rats were given water and standard chow diet. The amount of standard chow diet taken by each rat was 20gm daily. The rats were bred in standard cages. All rats groups except control normal group were induced with hypertension by giving intraperetonially injections of synthetic ACTH (0.5mg/kg/day)for fifteen days [9].

Olmesartan Medoxomil

Olmesartan medoxomil was given in dose of 8 mg/kg once a day(mid therapeutic dose) before morning feed [10]. by suspending each single rat a daily dose in water and then given orally by gavage for eight weeks.

Preparation of Samples

Blood Sampling

From each rat, about 3 ml of blood was collected by intracardiac aspiration at the subxiphisternal approach at the beginning of treatment and at the end by large gage needle . Sera were removed, and analyzed for determination of (hsCRP, TNF-a, IL-6., reduced Glutathione, malondialdehyde (MDA)).

Tissue Sampling for Histopathology

At the end of the protocol (10 weeks on their respective diets), rats were anesthetized with high concentration of chloroform and then killed. Rats were dissected through the chest wall to make the heart accessible for resection. The heart was exteriorized, cleaned of adherent connective tissue excised. All specimens were immediately fixed in 10% formaldehyde solution. After fixation, they were processed in a usual manner. The sections were examined by microscope under magnification power of (4×10) then the histological changes in the ventricle wall thickness were examined[11].

Methods

Blood Pressure Measurement

A physograph MK III tonometer transducer was used to measure indirect blood pressure (Bp) of rat by inserting the tonometer by a holder to the medial aspect of the rat thigh in the left femoral artery. All the rats blood pressure were measured at the same time throughout the induction and treatment course every 7 days.

A digital sphygmometer is inflated up to 250 mmHg then gradually pressure declined with physiograph monitoring of pressure pulse oscillations. Immediately after reaching a maximal oscillation of tonometric pulsation a value of sphygmometer is recorded. That value corresponds to the MAP together with determining the maximal systolic and diastolic pressure for healthy group for calibrating the physiographic chart divisions [12]

Biochemical Measurement of (hsCRP, TNF-α, IL-6,MDA,and GSH)

ELISA Kits were used for quantitative determination of (hsCRP, TNF-α, IL-6,), (hs-CRP Elisa kit, Drg International Inc USA), (IL6 Elisa kit **,** ImmunoTech .France), (Tnf-α Elisa kit,ImmunoTech .France), respectively.

The level of MDA was determined by modified procedure [13]. Quantichrom™ Glutathione assay Kit is designed to accurately measure(GSH) **[14]**.

Statistical Analysis

Statistical analysis were done by using (SPSS, Mat Lab. 2010a) ,;For continuous data a paired t test was used whereas regression coefficient r was used to assess blood pressure responses .P value less than 0.05 regarding as significant

Results

Effect on Mean Arterial Pressure

There was a significant increase in MAP in induced control untreated group as compared with control normal group; $P < 0.05$. Olmesartan had potent antihypertensive effect. The drug caused a significant decrement of MAP after eight weeks of treatment; $P < 0.05$. as shown in table (1)

Effect on Serum Level of (hsCRP, TNF-α, IL6)

There was a significant increase in serum level of**(** hsCRP, TNF-α, IL6) noticed with induced untreated group as compared with control normal group after fifteen days of induction of hypertension; $p < 0.05$.

Olmesartan induced significant reductions in(hsCRP, TNF-α, IL6) levels as compared with control induced untreated group after 8 weeks of treatment course $p < 0.05$. as shown in table (2)

Effect on Serum Reduced Glutathione Level (GSH).

There was a significant reduction in serum reduced GSH level noticed with induced untreated group as compared with control normal group ; $P < 0.05$, Olmesartan caused a significant rise in reduced GSH in comparison with the untreated group, $P < 0.05$ After the eighth week of treatment. as shown in table (3)

Effect on Serum Level of Malondialdehyde MDA

There was a significant increase in serum MDA level noticed in induced untreated control group as compared with control normal group; $P < 0.05$,Olmesartan caused a significant decrease in serum MDA level in comparison with the untreated group, $P < 0.05$. After the eighth week of treatment. as shown in table (4)

Histological Effect of Induction of Hypertension and Effects of Olmesartan Treatment

induced untreated Control group (picture no. two) upper right showed mildly thickened wall and although Olmesartan (picture no. Three) showed a mild difference from the untreated group however this difference was not significant at $P <0.05$. This finding was expected since duration of induction of hypertension was not sufficient to revealed left ventricular wall thickness. as shown in figure (1)

Discussion

In the current study, a significant increase in mean arterial pressure was found in rats induced with 0.5 mg /kg /day for fifteen days as compared with the normal group. This increase of mean arterial pressure by ACTH can be explained by the mechanism that ACTH-induced secretion of the major rat glucocorticoid corticosterone, The increase in transcription and expression of glucocorticoid receptors might be one of the mechanisms involved Glucocorticoids also modulate vascular permeability and decrease production of NO as well as of other vasodilator factors [9,15]. .

There was a significant decrease in mean arterial pressure (MAP) in olmesartan treated rats (8mg/kg/day) as compared with the induced untreated group. This decrease of mean arterial pressure by olmesartan can be explained by the mechanism that olmesartan displace angiotensin II from the angiotensin I receptor and produce their blood pressure lowering effects by antagonizing angiotensin II–induced vasoconstriction [16].,

Effect on Inflammatory Parameter hsCRP, TNF _α, and IL6

There was a significant increase in,(hsCRP,IL6,TNF-α), level in rats induced with 0.5 mg/kg/day (ACTH) for fifteen days as compared with the normal group. ($p<0.05$). This increase of (hsCRP, IL6,TNF –α) level by ACTH can be explained by the mechanism that ACTH-induced hypertension in rat and as hypertension acts as a major determinant of endothelial dysfunction and vascular damage lead to promoting inflammatory activation, these effects characterized by the expression of inflammatory markers hsCRP,IL6,TNF -α. The mechanisms leading to this inflammatory response include both mechanical stress of the arterial wall and proinflammatory effects of humoural factors, such as Angiotensin II (AngII). AngII, besides regulating the vascular tone, may exert some pro-inflammatory effects on the arterial wall. AngII, in fact, induces (NF-kB) activation triggering the production of inflammatory cytokines [3].

Olmesartan showed highly significant decrease in (hsCRP,IL6,TNF –α) in comparison with untreated group. This effect of olmesartan could be explained by the relation between angiotensin suppresses ATI receptor that have a role in mediation of cytokines release [17].

Effect on Oxidative stress

In our study hypertension was associated with increases in the serum levels of the lipid peroxidation product MDA and decrease in the level of serum GSH . This effect of ACTH could be explained by that Administration of adrenocorticotropic hormone (ACTH) to rats provides means of inducing hypertension by stimulating the adrenal production of corticosterone and this lead to disruption of the nitric oxide synthase (NOS) pathway at various points, affecting NO bioavailability, oxidative stress and function of the endothelium [18].

Olmesartan had significant effect on serum MDA and GSH levels hence it inhibited the(increase of serum MDA caused by ACTH induced hypertension in rats .This suggesting decrease in ROS and subsequent lipid peroxidation. Also olmesartan caused high increase in rats serum GSH level,. This effects of olmesartan might be attributed to that Angiotensin II activates the AT1 receptor resulting in superoxide anion generation, oxidative stress, and endothelial dysfunction [18], ARBs diminish production of intracellular superoxide anions by reducing activity of angiotensin II-dependent oxidizes in the endothelium and vascular smooth muscle. This protects endothelium-derived NO from oxidant degradation to inert or toxic molecules([19].

Table (1): Changes of MAP in (mmHg) in different groups form zero time to the 10th week Data are presented as mean ± SEM at P < 0.05.
NS = not significant

Groups	Zero time 2nd	3rd	4rth	5fth	6th	7th	8th	9th	10th	Correlation test P< 0.05
Healthy control	123 ± 5	121± 2	120 ± 3	121± 3	122± 5	123 ± 6	120 ± 7	121 ± 7	122 ± 5	NS
Induced untreated	184 ± 7	189± 5	186 ± 6	184± 2	183± 5	182 ± 4	186 ± 3	185 ± 4	188 ± 4	NS
olmesartan	188 ± 6	122± 3	96± 2	94± 2	85 ± 3	86 ±3	87± 2	88 ± 3	85 ± 2	P < 0.05

Table (2): Sequential Changes of Serum hsCRP Level mg/L,(TNF-α, IL6) level ng/L of the Three Experimental Groups at the end of the induction period and at the end of the treatment. The Data Expressed as Mean ± SEM (N=8 in each group) p<0.05.

Groups	Zero time 2 weeks			10 weeks			P-values
	hscrp	TNF- α	IL6	hscrp	TNFα	IL6	
Normal control	1.55 ± 0.40	1.52 ± 1.2	1.8 ± 0.55	1.58 ± 0.22	1.52 ± 1.37	1.875 ± 1.56	p > 0.05
Induced control (untreated)	4.78 ± 1.23	3.04 ± 1.03	2.68 ± 1.49	4.86 ± 1.17	3.06 ± 1.003	2.84 ±1.61	p < 0.05
Olmesartan group	4.64 ± 1.03	3.02 ± 1.04	2.52 ± 1.33	2.95 ± 0.19	1.625 ± 0.35	1.175 ± 0.47	p < 0.05

Table (3): Sequential Changes of Serum GSH level µmol/L of the Three Experimental Groups at the end of the induction period and at the end of the treatment. The Data Expressed as Mean ± SEM (N=8 in Each group). P < 0.05.

Groups	Zero time 2 weeks	10 weeks	P- values
Normal control	114.5± 4.123	115 ± 4.16	p > 0.05
Induced control (untreated)	36.25 ± 6.19	36.99 ±11.73	P < 0.05
Olmesartan group	38.7 ± 5.8	99 ± 12.8	P < 0.05

Table (4): Sequential Changes of Serum MDA Level mmol/L of the Three Experimental Groups at the end of the induction period and at the end of the treatment. The Data Expressed as Mean ± SEM (N=8 in each group). P < 0.05.

Groups	zero time 2 weeks	10 week	p values
Normal control	2.03 ±0.11	2.13 ± 0.17	p > 0.05
Induced control (untreated)	7.625 ± 0.35	7.625 ± 0,55	p<0.05
Olmesartan group	7.6 ± 0.29	3.225 ± 0.275	P < 0.05

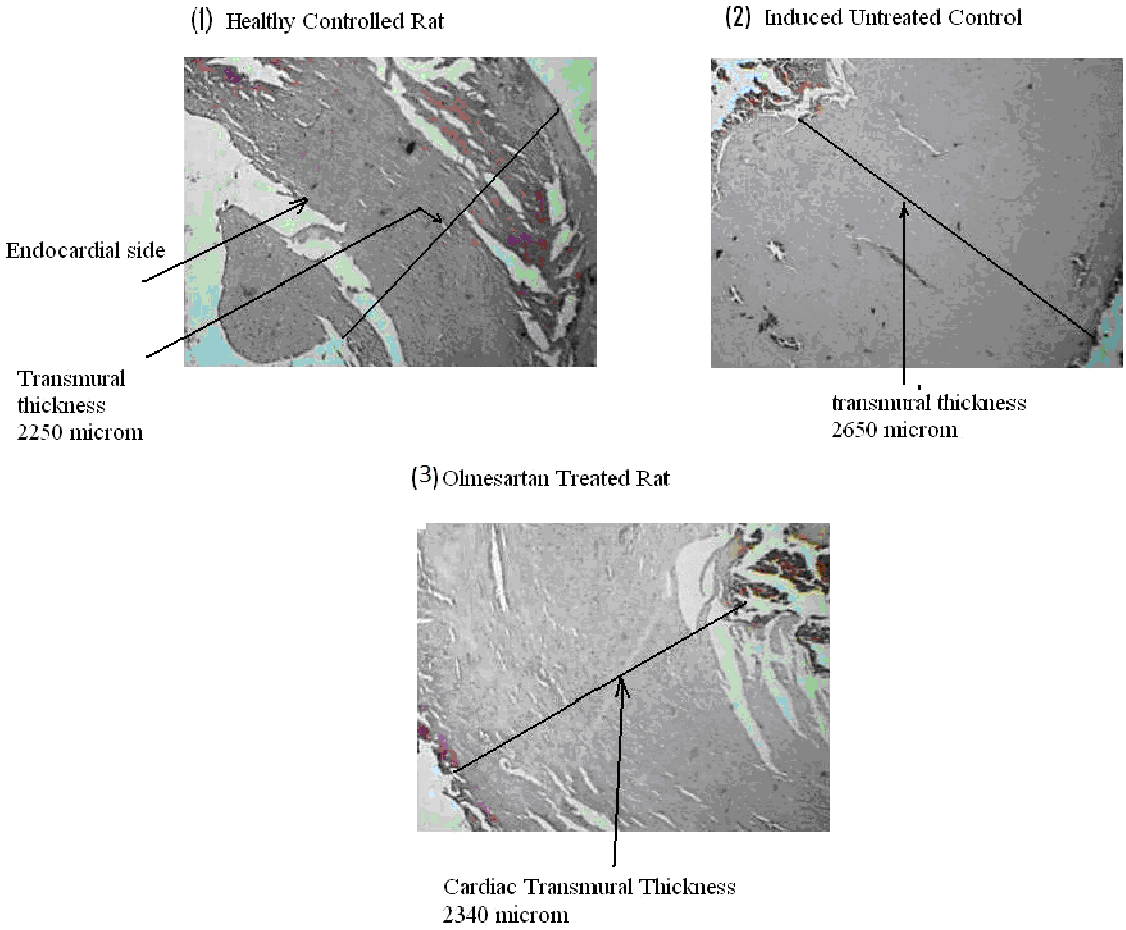

Figure (1): shows the Main Histopathological Finding of Transventricular Section for Rats Hearts(1-Healthy controlled, 2- Induced untreated control,3- Olmesartan treated) Revealed Minimal Chages in Transmural Cardiac Thickness Between Different Groups

REFERENCES

1. **Cheng ZJ, Vapaaealo H**, Antiinflammatory effect of angiotensin II subtype 1 receptor blockade in hypertensive patients with microinflammation. Circulation 2004; 110:1103-1107.
2- **Luvara G, Pueyo ME, Philippe M.** Chronic blockade of NO synthase activity induces a proinflammatory phenotype in the arterial wall: prevention by angiotensin II antagonism. Arterioscler Thromb Vasc Biol 1998; 18: 1408–1416
3- **Schieffer B, Bunte C, Witte J.** Comparative effects of AT1-antagonism and angiotensin-converting enzyme inhibition on markers inflammation and platelet aggregation in patients with coronary artery disease. J Am Coll Cardiol 2004; 44: 362-368.
4- **Calhoun DA, Bakir SE, Oparil S,** et al. Etiology and pathogenesis of essential hypertension. In: Crawford MH, DiMarco JP, eds. Cardiology.International.2003;3: 1-3.
5- **Blake GJ, Ridker PM.** C-reactive protein and other inflammatory risk markers in acute coronary syndromes. J Am Coll Cardiol 2003;41: 37-42.

6- **Tilg H, Dinarello CA, Mier JW.** IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. Immunol Today 1997; 18: 428-32

7- **Chia S, Qadan M, Newton R, Ludlam CA, Fox KA, Newby DE, et al.** Intra-arterial tumor necrosis factor-alpha impairs endothelium dependent vasodilatation and stimulates local tissue plasminogen activator release in humans. Arterioscler Thromb Vasc Biol 2003;23: 695-701

8- **Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, et al**. Effect of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med. 2001; 345: 861–869

9- **Vazir H, Whitehouse BJ, Vinson GP.** Effects of prolonged ACTH treatment on adrenal steriodogenesis and blood pressure in rats . Acta Endocrinol 1981; 97:533-1542.

10- **10 -Porteri E, Rodella L, Rizzoni D**.Effects of olmesartan and enalapril at low vascular interstitial matrix in spontaneously hypertensive rats. Blood Press 2005 ;14: 184-192.

11- **Wagner M, Mascareno E, Siddiqui MA.** Cardiac hypertrophy: signal transduction, transcriptional adaptation, and altered growth control. Ann N Y Acad Sci. 1999;874:1–10.

12- **John K-J Li**. Dynamics of the Vascular System. VOl. 1. (Department of Biomedical Engineering, Rutgers University, USA) 2004.P. 224

13- **Muslih RK, ALNimer MS, Yasser OM.** The level of malondialdehyde after activation with (HO and CUSO4) and inhibition by desferoxamine and molsidomine in the serum of patient with acute myocardial infarction.Nat.J chem..2005;1:139-148.

14- **Hu ML.** Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol.1994;233:381-385

15- **Freeman RH, Davis JO, Fullerton D.** Chronic ACTH administration and development of hypertension in rats. Proc Soc Exp Biol Med 1980 ;163:473-477.

16- **Püchler K, Laeis P, Gunther A.** Safety, tolerability and efficacy of the new oral angiotensin II (AT1)-receptor antagonist CS-866 in patients with mild to moderate hypertension JHum Hypertens 1999;13:4.

17- **Wu L, Iwai M, Nakagami H.** Roles of angiotensin II type 2 receptor stimulation associated with selective angiotensin II type 1 receptor blockade with valsartan in the improvement of inflammation-induced vascular injury. Circulation. 2001;104:2716-2721.

18- **Whitworth JA, Schyvens CG, Zhang Y,** The nitric oxide system in glucocorticoid-induced hypertension. J Hypertens 2002 **;** 20**:**1035-1043.

19- **Pueyo ME, Gonzalez W, Nicoletti A.** Angiotensin II stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kappaB activation induced by intracellular oxidative stress. Arterioscler Thromb Vasc Biol. 2000; 20:645–651.