L-NAME Induced Hypertension and Cardiac Remodeling as Modified by Angiotensin Receptor Blockade (ARB) in Experimental Animals (Rats)

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ABSTRACT

Chronic nitric oxide blockade constitutes a new model of severe arterial hypertension. L-NAME or N-nitro-L-arginine methyl ester (NO synthase inhibitor) produces inhibition of nitric oxide biosynthesis and promotes arterial hypertension & cardiac hypertrophy. As hypertension is a multifactorial syndrome, other factors beside the sympathetic nervous system overactivity, include the renin angiotensin aldosterone system & tonically active endothelium derived autacoids, nitric oxide (NO) and endothelin (ET1). The present study was carried out to assess & evaluate the contribution of the renin angiotensin system in the production of L-NAME hypertension syndrome and how angiotensin receptor blockade by ARB (losartan) can ameliorate the severe hypertension & cardiac hypertrophy & remodeling in this syndrome. In the present study, the systemic effects of 4 weeks oral administration of daily dose 40 mg/kg nitric oxide inhibitor L-NAME in male albino rats was evaluated on blood pressure & cardiac hypertrophy in this animal model. Age-matched untreated rats were used as control. In an additional group, nitric oxide blockade was carried out in conjunction with oral administration of angiotensin II receptor blocker losartan in a dose 30 mg/kg daily. The last group was given angiotensin II receptor blocker losartan alone. Measurement of the systolic blood pressure by indirect tail cuff method (Harvard apparatus), revealed progressive significant rise of blood pressure in L-NAME treated rats reaching 166.2 ± 7.13 mmHg after 4 weeks, compared with 105.3 ± 6.97 mmHg in control group. The magnitude of rise was 57.83% (P<0.001). However, in rats treated concomitantly with ARB losartan, blood pressure reached only 128.6±7.02 mmHg. This value although markedly reduced, yet was still significantly higher when compared with those encountered in control group. In rats treated with ARB losartan alone, their blood pressure reached 100.7±5.98 mmHg with no significant difference from control group -4.37%† (P>0.05). This experiment showed that, although treatment with angiotensin II receptor blockade losartan largely attenuated the L-NAME induced hypertension, yet arterial blood pressure still remained elevated than in losartan treated & control groups. The other part of the study was carried out on cardiac hypertrophy that accompanied L-NAME administration for 4 weeks. Cardiac histological examination of myocardium of L-NAME treated rats revealed marked myocardial hypertrophy, enlarged myocytes & fibrosis with fibroblast infiltration (remodeling).Left ventricular hypertrophy was attenuated by ARB losartan, verifying the presence of intracardiac renin angiotensin system. It is concluded that angiotensin II receptor blockade can ameliorate the rise in the systolic blood pressure & cardiac hypertrophy in this model of L-NAME severe arterial hypertension, revealing the role of renin angiotensin system in this respect.*

INTRODUCTION

In search for a model of hypertension instead of renal clipping, L-NAME was introduced. Other models include spontaneous hypertensive rats (SHR), spontaneous hypertensive obese rats (SHROB), fructose induced hypertensive rats, in addition to DOCA salt hypertension. L-NAME is N-nitro-L-arginine methyl ester (NO synthase inhibitor) produces inhibition of nitric oxide biosynthesis and promotes arterial hypertension & cardiac hypertrophy, following deprivation of nitric oxide in rats[1,2].

It is now established that nitric oxide (NO) plays a pivotal role in the regulation of arterial pressure & hemodynamics[3,4,5,6,7,8]. Nitric oxide (NO) released from endothelial cells has been found to account for the biological properties of endothelium derived relaxing factor (EDRF)[4,9]. Accordingly, the continuous release of endogenous NO maintains a dilator tone in vascular tissues[3, 10].

Nitric oxide formed from L-arginine, through the action of NO synthase (NO), activates guanylate cyclase and leads to an increase in cyclic guanosine monophosphate (cGMP) which in turn causes relaxation in vascular smooth muscle[9,10]. Chronic blockade of nitric oxide biosynthesis from L-arginine by L-NAME, through inhibition of NO synthase, causes vasoconstriction, producing increase in the peripheral resistance and sustained hypertension[11, 12, 13, 14].

In establishing a model of hypertension in rats by using a high dose of L-NAME, **Ribeiro et al.**[15] succeeded in developing a severe and progressive form of hypertension together with glomerulosclerosis. **Morton et al.**[16] recorded persistent hypertension, even after NO inhibition was discontinued after 4 weeks of treatment, suggesting that once NO has been inhibited, the hypertension no more depends exclusively on inactivation of the arginine /NO pathway, but may involve structural alteration of the myocardium & vascular walls[17]. **Zatz and Baylis**[18] emphasized persistent hypertension after L-NAME.

The underlying mechanisms & factors responsible for structural changes include, lack of tissue vasodilatation produced by NO, which control blood pressure and role of nitric oxide in central sympathetic outflow[19]. Involvement of the renin angiotensin system was also first proposed by **Ribeiro et al.**[15], through studying the effect of administration of angiotensin receptor blocker (ARB) losartan, and then by **Pollock et al.**[20] **& Qiu et al.**[21], indicating the major role of the renin angiotensin system in this aspect. Moreover, superoxide production in endothelial cells has also been implicated & superoxide radicals may underlie the activation of RAS (renin angiotensin system) in vascular tissues[22].

The structural changes produced by L-NAME through chronic inhibition of nitric oxide synthase, involve organs and tissues other than vascular blood vessels. They are recorded in the kidney & the heart, producing in the latter left ventricular hypertrophy & remodeling[2]. There is difference between simple hypertrophy and remodeling[23,24,25].

The pathological alterations in cardiovascular structure may involve hypertrophy (an increase in tissue mass) or remodeling (redistribution mass within a structure with matrix changes). This is due to direct trophic effect on cells as well as increased extracellular matrix. The cells involved include vascular smooth muscle cells, cardiac myocytes and / or fibroblasts.

Concerning the myocardium, the interstitial space of the myocardium is composed of non-myocyte network which serves to maintain the architecture & mechanical behavior of the myocardium, which constitutes extracellular matrix. It is the myocardial collagen matrix that determines myocardial stiffness in the normal and structurally remodeled myocardium. The hypertrophic growth of cardiac myocytes leads to an increment of myocardial mass, while non-myocyte growth is reflected on structural remodeling of the cardiac interstitium. Disproportionate growth between myocytic & non-myocytic cells accounts for heterogenecity in myocardial structure and sets the stage for abnormal myocardial function[23].

There is now a conclusive evidence for the existence of a local intracardiac renin angiotensin system, which is capable of synthesis of all components of the system. Losartan, the angiotensin receptor blocker (ARB) blocks AT1 receptors **[26].** It is a new class of antihypertensive drugs that superceded angiotensin converting enzyme inhibitors (ACEi).

In the present study, angiotensin receptor blocker (ARB) losartan was used to elucidate the involvement of other underlying mechanisms in L-NAME animal model, rather than nitric oxide inhibition, mainly renin angiotensin system in the production of hypertension and cardiac remodeling.

MATERIAL & METHODS

I- Experimental Animals:

40 white male albino rats of the local strain weighing 150-200 grams were used. The rats were fed a standard chow diet add libitum and housed in a room during the 4-weeks of experimental period under the room temperature. They were divided into several groups, including normal control; L-NAME induced hypertension rats either alone or in conjunction with angiotensin receptor blocker losartan; and those treated with angiotensin receptor blocker losartan alone.

II-Drugs:

1. **L-NAME (NO synthase inhibitor) (Sigma Chemicals, USA**): It is N-nitro-L-arginine methyl ester. It is supplied as white powder soluble in water, given in a daily dose of 40mg/kg by gavage according to **Gerova et al.[27].**
2. **Losartan (Cozaar) (Merk, Sharp & Dohme, USA):** It is supplied as white losartan potassium powder that is soluble in distilled water. The dose of losartan to rats: 30mg/kg/ day given by gavage according to **Ribeiro et al.[15].**

III- Apparatus:

1- **Harvard apparatus (52-3050),** for measuring of systolic blood pressure by indirect tail cuff method.

2-**Microtome & Light microscopy for histological examination** of cardiac myocytes & measuring LVH and degree of cardiac remodeling using sections of paraffin-embedded LV tissue stained with hematoxylin & eosin.

METHODS

The animals were divided into 2 main groups:

(1) Group I: (Control group) 10 normal rats with free access to tap water. Each rat received 1 ml distilled water by gastric gavage equal to the volume used as vehicle for drugs for 4 weeks.

(2) Group II: 30 rats were subdivided into 3 subgroups each of 10 rats. They received tested agents for 4 weeks.

-Group IIA: Rats treated with L-NAME in a daily dose 40mg/kg according to **Gerova et al.**[27] given by gavage for 4 weeks.

-Group IIB: Rats treated with L-NAME in a daily dose 40mg/kg together with angiotensin receptor blocker losartan in a daily dose 30mg/kg according to **Ribeiro et al.**[15] given by gavage for 4 weeks.

-Group IIC: Rats treated with angiotensin receptor blocker losartan alone in a daily dose 30mg/kg given by gavage for 4 weeks.

The animals were tested by measuring systolic blood pressure by indirect tail cuff method (Harvard apparatus) every week for 4 weeks. After 4weeks, the heart was dissected for measuring LVH and degree of cardiac remodeling. Chest was opened and the heart was removed, whereas all adventitious tissues were cut away. The heart was squeezed properly to evacuate its blood content & washed with distilled water. The heart was then dissected to separate the left ventricle and fixed in buffer formalin routinely prepared. Then sections of paraffin-embedded LV tissue stained with hematoxylin and eosin were examined by light microscopy for histopathological study.

Statistical analysis:

The results were expressed as means ± S.D. Statistical analysis was performed using computer program MICROSTAT Rel.4.1.13 version statistical software. For all parameters comparison between two groups were done using Student t-test (unpaired-t-test). Differences were considered statistically significant at P values less than 0.05[28].

RESULTS

The results were tabulated, statistically analyzed and graphically illustrated. They were presented as tables (1-2) & figures (1-2).

Table (1) demonstrated the effect of oral administration of L-NAME either alone or in concomitant with ARB losartan and losartan alone, by gavage for 4 weeks on systolic blood pressure as determined by indirect tail cuff method (tail cuff pressure or TCP).

As seen in **table (1)**, L-NAME (40 mg/kg/day) oral administration for 4 weeks produced time dependent increase in systolic blood pressure. The mean values as evaluated at first, second, third & fourth week were 121.2±6.13, 130.2±6.42, 147.3±6.56 and 166.2±7.02 mmHg respectively compared with 105.3±6.97 mmHg in

age matched untreated control rats. At the end of the fourth week, the difference was highly significant as demonstrated in **table (2)** and **figure (1, 2).**

Table (1) also revealed the effect of administration of losartan, the angiotensin AT1 receptor blocker (ARB) 30mg/kg/day on systolic blood pressure of L-NAME hypertensive rats. The tail cuff pressure was reduced in rats concomitantly receiving losartan and L-NAME and the reduction at the end of the fourth week proved to be highly significant as demonstrated in **table (2)** and **figure (1, 2).**

Depicted also in **table (1)**, the effect of administration of losartan alone 30mg/kg/day on systolic blood pressure in rats for 4 weeks and the difference in tail cuff pressure before and after treatment proved to be non significant as shown in **table (2)** and **figure (1, 2).**

Table (2) and **figure (1)** demonstrated the percentage change and significance of change of systolic blood pressure in the normal control group compared with the corresponding values in L-NAME treated rats, and in rats receiving L-NAME together with losartan after 4 weeks and before sacrifice of the animals. It is evident that the changes were highly significant. The mean values of systolic blood pressure were 166.2±7.13 mmHg in L-NAME hypertensive rats compared with 105.3±6.97 mmHg in age matched untreated control rats with percentage change of +57.83%* (P<0.001). There was also significant reduction in tail cuff pressure in rats concomitantly receiving losartan and L-NAME , with percentage reduction of -22.62%*(P<0.001) reaching 128.6±7.02 mmHg in comparison with 166.2±7.13 mmHg in L-NAME hypertensive rats without losartan. However, tail cuff pressure reaching 128.6±7.02 mmHg in the latter group was still elevated in comparison with untreated control rats with percentage change of + 22.13%* (P<0.001). So, losartan can significantly reduce systolic blood pressure in L-NAME treated rats, although systolic blood pressure remains partly elevated compared to control.

Demonstrated also in **Table (2)** and **figure (1),** tail cuff pressure in rats receiving ARB losartan alone reaching 100.7±5.98mmHg. There was no significant change compared to age matched untreated control rats recording105.3±6.97 mmHg (P>0.05).

Figure (2) is a compiled bar chart illustrating changes in systolic blood pressure (SBP) in the normal control group compared with the corresponding values in L-NAME treated rats, L-NAME + losartan treated rats and after treatment with losartan alone (30mg/Kg/day) every week for 4 weeks.

Figure (3) showed cardiac hypertrophy & remodeling at the end of the 4th week of L-NAME administration, revealing left ventricular hypertrophy, enlarged myocytes & fibrosis with fibroblast infiltration. Losartan produced regression of myocardial fibrosis and decrease of size of hypertrophied cardiac myocytes in L-NAME treated rats. There was no difference noted between control group and losartan alone treated group.

Table (1): Systolic Blood Pressure (SBP) in the normal control group compared with the corresponding values in L-NAME treated rats, L-NAME + losartan treated rats and after treatment with losartan alone (30mg/Kg/day)every week for4weeks.

Group of rats (n=10)	Normal control group	L-NAME treated group	L-NAME + losartan treated group	Losartan treated group
First week SBP (mmHg) (Mean ±SD)	105.3 ±6.97	121.2 ±6.13	118.6 ±5.47	103.7 ±5.61
Second week SBP (mmHg) (Mean ±SD)	105.3 ±6.97	130.2 ±6.42	121.6 ±6.24	102.6 ±6.02
Third week SBP (mmHg) (Mean ±SD)	105.3 ±6.97	147.3 ±6.57	125.8 ±6.81	100.9 ±7.02
Fourth week SBP (mmHg) (Mean ±SD)	105.3 ±6.97	166.2 ±7.13	128.6 ±7.02	100.7 ±5.98

Table (2): Systolic Blood Pressure (SBP) in the normal control group compared with the corresponding values in L-NAME treated rats, L-NAME + losartan treated rats and after treatment with losartan alone (30mg/Kg/day)after 4 weeks.

Group of rats (n=10)	Normal control group	L-NAME treated group	L-NAME + losartan treated group	losartan treated group
SBP (mmHg) (Mean ±SD)	105.3 ±6.97	166.2 ±7.13	128.6 ±7.02	100.7 ±5.98
%Change & Significance		+57.83%* (P<0.001) (From control group)	-22.62%* (P<0.001) (From L-NAME treated group)	-4.37%† (P>0.05) (From control group)
			+22.13%* (P<0.001) (From control group)	

* = *significant (P<0.001)*
† = *Insignificant (P>0.05)*

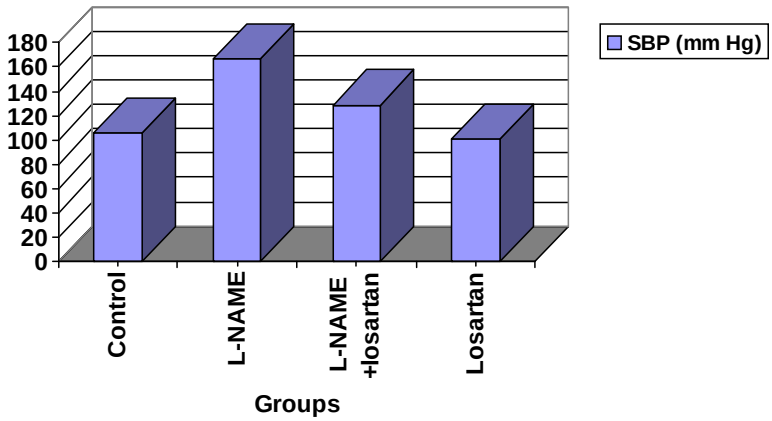

Fig. (1): Systolic Blood Pressure (SBP) in the normal control group compared with the corresponding values in L-NAME treated rats, L-NAME + losartan treated rats and after treatment with losartan alone (30mg/Kg/day)after 4 weeks.

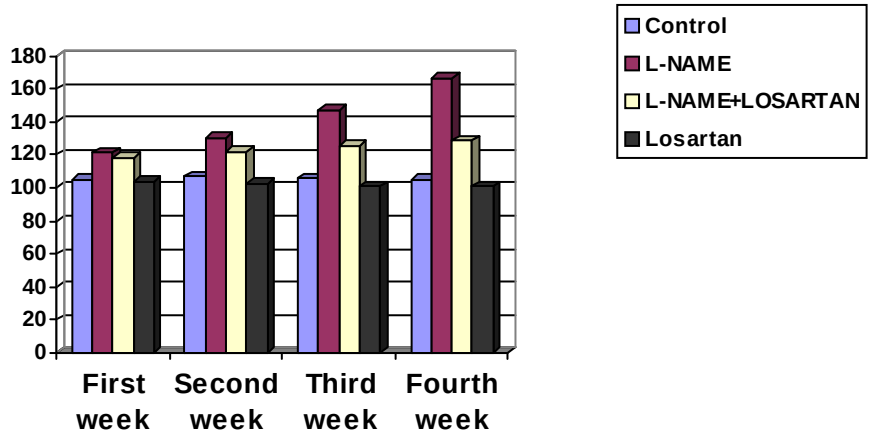

Fig. (2): Systolic Blood Pressure (SBP) in the normal control group compared with the corresponding values in L-NAME treated rats, L-NAME + losartan treated rats and after treatment with losartan alone (30mg/Kg/day)every week for4weeks.

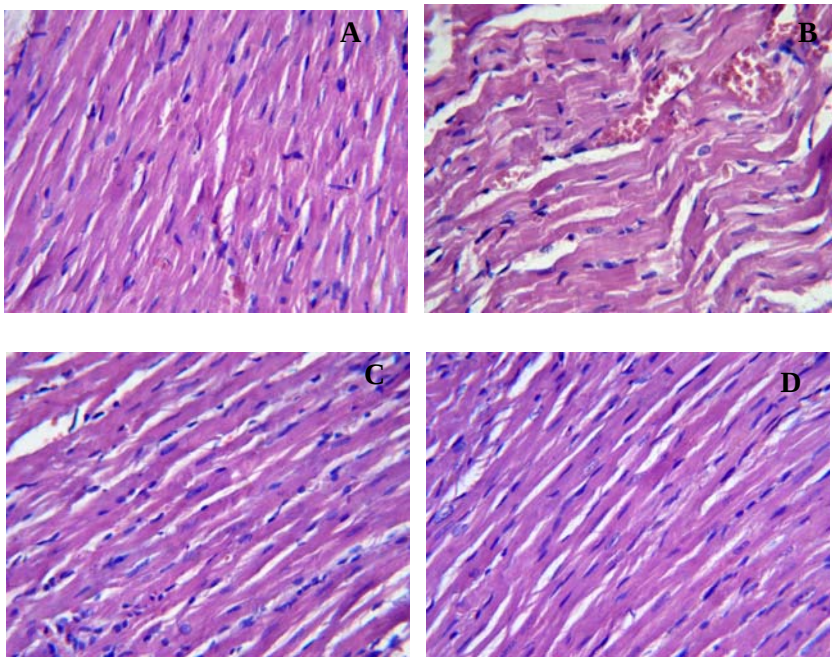

Fig. (3): Left ventricular muscle in the normal control group (A) compared with the corresponding in L-NAME treated rat (B), L-NAME + losartan treated rats (C) and after treatment with losartan alone (D) after 4 weeks.

DISCUSSION

In the present study, an experimental model of arterial hypertension & left ventricular hypertrophy was induced by L-NAME, through chronic inhibition of nitric oxide synthesis. Nitric oxide plays a pivotal role in the regulation of arterial pressure & hemodynamics[2].

Nitric oxide (NO) is released from endothelial cells in response to activation of a variety of receptors[1, 4, 9]. It diffuses from endothelium to vascular smooth muscles to produce vasodilatation. It increases intracellular cyclic guanosine monophosphate (cGMP) by activation of the enzyme guanylate cyclase leading to relaxation of the vascular smooth muscle[1, 3, 9,].

The formation of Nitric oxide from L-arginine, through the action of the enzyme NO synthase, is inhibited by L-NAME[5, 14]. L-NAME is N-nitro-L-arginine methyl ester (NO synthase inhibitor) produces inhibition of nitric oxide biosynthesis and promotes arterial hypertension & cardiac hypertrophy[1, 2].

Baylis et al.[5] were the first who reported that, chronic administration of nitric oxide synthase inhibitor (L-NAME) to rats resulted in systemic hypertension and glomerulosclerosis. Using a higher dose of L-NAME (40 mg/kg/day for 4 weeks in rats), **Ribeiro et al.**[15] succeeded in developing a severe progressive form

of hypertension, giving birth to a new model of hypertension. Six years later, chronic nitric oxide inhibition model by L-NAME became established[18]. Lately, **Hu et al.**[2] gave L-NAME to rats in different doses 10, 20, 40mg/kg/day for several weeks in rats and they found the most effective dose in producing ventricular hypertrophy & sustained hypertension was the higher dose 40 mg/kg/day.

In the present study, daily administration of L-NAME to rats in a daily dose of 40mg/kg by gavage for 4 weeks resulted in sustained systemic hypertension and cardiac hypertrophy, confirming the major role of NO in maintenance of normal vascular tone. There was increase of tail cuff pressure as measured by indirect tail cuff method (Harvard apparatus) at the end of 4 weeks daily administration of L-NAME by significant rise by +57.83%* compared to control & age matched rats ($P<0.001$).

During the whole experimental study, the blood pressure was measured every week (1st, 2nd, 3rd & 4th week) for 4 weeks until the maximum level of blood pressure was attained.

Cardiac hypertrophy & remodeling were verified at the end of the 4th week of L-NAME administration, revealing left ventricular hypertrophy, enlarged myocytes & fibrosis with fibroblast infiltration as shown in figure (3).

Losartan, the angiotensin receptor blocker (ARB) was also used to reveal other underlying mechanisms beside nitric oxide (NO) inhibition in L-NAME animal model to produce hypertension and cardiac remodeling. Losartan belongs to a new class of blockers of the renin angiotensin system that have recently been shown to be safe and effective antihypertensive agents in both animal and human studies. It blocks angiotensin II receptors type I (AT1 receptors)[29, 30].

Losartan in the present study was able to attenuate the rise of blood pressure induced by L-NAME in rats. It caused significant reduction in tail cuff pressure in L-NAME hypertensive rats, amounting to percentage change of -22.62%*($P<0.001$) reaching 128.6±7.02 mmHg in comparison with 166.2±7.13 mmHg in L-NAME hypertensive rats without losartan. However, although the systolic blood pressure was partly elevated compared to control recording 105.3±6.97 mmHg; losartan produced marked regression of left ventricular hypertrophy & remodeling with regression of the size of cardiac myocytes and fibrosis in this type of L-NAME hypertensive model. These findings point to direct involvement of the renin angiotensin system in the pathogenesis of L-NAME hypertension & cardiac hypertrophy. Further supporting evidence of direct trophic effect of angiotensin II arose in several experimental studies. There is now conclusive evidence of a local intracardiac renin angiotensin system which is capable of synthesis of all components of the system & of cleaving via the classic pathway, angiotensin peptides from the precursor angiotensinogen[25].

It is now evident that the role of renin angiotensin system (RAS) and the effect of angiotensin II are of

importance in regulating the myocardial collagen matrix. The major structural proteins of the interstitium are the fibrillar type I and type III collagen. Accumulation of fibrillar collagen occurs within the cardiac interstitium. The cardiac RAS is sufficiently activated in hypertrophic and failing heart. Angiotensin II has a direct trophic effect on mammalian myocytes and it also can stimulate fibroblast proliferation[25]. Moreover, it was observed that fibroblasts in culture increase their collagen synthesis in response to angiotensin II[23].

Diez et al.[31] reported a strong association between myocardial collagen content and LV chamber stiffness & hypertrophy in patients with essential hypertension. They used markers of collagen type I synthesis & degradation. Myocardial fibrosis is the result of an exaggerated accumulation of collagen type I & III. According to **Diez et al.**[31], angiotensin II plays a critical role in alteration of collagen type I metabolism & the development of myocardial fibrosis in arterial hypertension. Thus, the excess of myocardial collagen seen in LV hypertrophy is the result of both increased collagen synthesis and decreased collagen degradation. Losartan, the angiotensin II AT1 receptor blocker, produces its beneficial effect in hypertensive subjects by regression of myocardial fibrosis and also decrease of size of hypertrophic cardiac myocytes as a result of decrease of after load.

Kagami et al.[32] attributed the ability of angiotensin II to stimulate extracellular matrix protein synthesis, to the induction of transforming growth factor β expression in the rat, one of the factors that are responsible for fibrosis. **Spieker et al.**[1] also reported that the harmful effect of angiotensin II is mediated by reactive oxygen species mainly oxygen radicals and their interaction with endothelial NO.

The ability of ARB losartan in the present study to produce regression of LV hypertrophy & remodeling is in agreement with several studies. It was reported first with ACE inhibitors, which proved to be uniquely effective in inducing regression or preventing the occurrence of ventricular hypertrophy associated with systemic hypertension[25]. **Takemoto et al.**[33] reported that angiotensin converting enzyme inhibitors (ACEi) markedly reduced myocardial hypertrophy & remodeling induced by long term blockade of nitric oxide synthesis by L-NAME. However, although activation of bradykinin & inhibition of its metabolism contributes significantly to the hypotensive action of ACEi, yet it is apparently responsible for adverse effects as cough[26]. ACE inhibitors are now superceded by angiotensin receptor blockers (ARB) which are devoid of any action on bradykinin. Since then, supporting evidence pointed to the possible direct involvement of the renin angiotensin system in the pathogenesis of cardiac hypertrophy and the amelioration by the use of ARB losartan.

Recent clinical studies also documented that regression of left ventricular hypertrophy by ARB losartan, which represents independent risk factor for

cardiovascular morbidity & mortality, cause decreased incidence of new onset atrial fibrillation[34].

Lastly, this study has several implications. First; verification of the presence of local intracardiac renin angiotensin system and the beneficial effect of angiotensin receptor blockade. Second; interaction between NO, angiotensin II and oxygen free radicals. Thus the underlying mechanism of left ventricular hypertrophy and remodeling seems to be mulifactorial.

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دراسة ارتفاع ضغط الدم الشريانى المحدث بواسطة الـ L-NAME فى الفئران وتأثير غلق مستقبلات الانجيوتنسين ٢ على تضخم عضلة القلب بالبطين الأيسر وتغيير الخلايا العضلية وألياف الأنسجة بالقلب فى هذا النوع من حيوانات التجارب

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الغرض من البحث: هو دراسة ارتفاع ضغط الدم الشريانى المحدث بواسطة الـ L-NAME فى الفئران، والـ L-NAME هو مضاد لأوكسيد النيتريك يقلل من إنتاجه بتأثيره المثبط لعمل إنزيم أوكسيد النيتريك سينزاز الداخل فى تصنيعه، وأوكسيد النيتريك مضاد لتأثير الأدرينالين يعمل على توسعة الشرايين ولما كان ارتفاع الضغط الشريانى ناتجاً عن عدة أنشطة وعوامل أخرى هى الجهاز العصبى المركزى وكذلك مجموعة الرنين أنجيوتنسين وعوامل أخرى مثل المساعدة على الأكسدة. فالدراسة الحالية تهدف إلى التعرف على مدى أهمية مجموعة الرنين انجيوتنسين وهل لها دور فى هذا النموذج المقل لإنتاج أوكسيد النيتريك وهذا بإستعمال اللوسارتان الغالق لمستقبلات الانجيوتنسين٢ النوع الأول وكذلك مدى التأثير على تضخم عضلة القلب فى البطين الأيسر فى هذا النوع.

طرق البحث: فى هذه الدراسة تم استخدام أربعون فأراً تتراوح أوزانها ما بين ١٥٠–٢٠٠ جم ثم تقسيمهم أربعة مجموعة: المجموعة الأولى الضابطة والمجموعة الثانية تم اعطائها L-NAME ٤٠مجم/كجم يومياً عن طريق الفم لمدة أربعة أسابيع مع قياس لضغط الدم كل اسبوع من ذيل الفأر بواسطة جهاز هارفارد حتى يصل الضغط إلى الإرتفاع المطلوب حيث أن الضغط يرتفع تدريجياً كل اسبوع وهذا الارتفاع فى ضغط الدم مصاحب لتضخم عضلة القلب التى تحدث على مدى أربعة أسابيع أما المجموعة الثالثة فقد أعطيت الـ L-NAME مع اللوسارتان والأخير أعطى جرعة ٣٠مجم/كجم يومياً عن طريق الفم أما المجموعة الرابعة فقد أعطيت اللوسارتان بمفرده فقط بنفس الجرعة السابق ذكرها وعند انتهاء الأربعة الأسابيع بعد قياس الضغط تم تشريح عضلة القلب وفصل البطين الأيسر وفحصه هستولوجياً بواسطة الميكروسكوب بعد صبغه بالهيماتوكسلين وأيوسين.

نتائج البحث: عند إعطاء الـ L-NAME فى الفئران وصل ارتفاع ضغط الدم فى ذيل الفأر إلى مداه بعد أربعة أسابيع من المعالجة يومياً إلى المتوسط ١٦٦.٢±٧.١٣مم زئبق وهو ارتفاع ذو دلالة احصائية بنسبة ٥٧.٨% إلى المجموعة الضابطة، وكذلك عند اعطاء اللوسارتان مع L-NAME انخفض مستوى الضغط إلى المتوسط ١٢٨.٦±٧.٠٢ مم زئبق ووصلت النسبة المئوية للإنخفاض إلى مجموعة الـ L-NAME ٢٢.٦% وهو ذو دلالة احصائية ولكنه لم يصل إلى مستوى المجموعة الضابطة. أما المجموعة المعالجة باللوسارتان فقط فلم يحدث بها تغير ذو دلالة احصائية حيث وصل متوسط الضغط إلى ١٠٠.٧±٥.٩مم زئبق بالنسبة الى ١٠٥.٣±٦.٩مم زئبق للمجموعة الضابطة. وعند فصل البطين الأيسر وتشريحه وجد تضخم بالقلب والبطين الأيسر وصل إلى أقصى مداه فى المجموعة المعالجة بالـ L-NAME من حيث كثرة الألياف وتضخم خلايا عضلة القلب كما أن القلب رجع إلى قرب حالته الطبيعية فى المجموعة المعالجة باللوسارتان مع الـ L-NAME وكذلك المعالجة باللوسارتان فقط لما يدل على أن اللوسارتان الغالق لمستقبلات الانجيوتنسين ٢ له دور مهم وواضح فى تقليل تضخم عضلة القلب لو حدث تغييرات بها وأنه يقى من هذا التضخم الذى يحدث مع ارتفاع ضغط الدم مما يؤكد وجود مجموعة الرنين الانجيوتنسين داخل عضلة القلب وكذلك يؤكد البحث نتائج مماثلة فى الاستعمال الاكلينيكى لغالقات مستقبلات الانجيوتنسين ٢.