COMPARISON BETWEEN RENOPROTECTIVE EFFECTS OF CARVEDILOL AND LISINOPRIL IN L-NAME-TREATED RATS

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ABSTRACT

Aim The present study compared the renoprotective effects of curative and prophylactic doses of carvedilol and lisinopril in L-NAME treated rats.

Material and methods Rats were divided into seven groups: the first and second (normal control and hypertensive control) groups received distilled water and L-NAME (L-nitro argentine methyl ester, 50 mg/kg per day), respectively, for eight weeks. The third and fourth groups received L-NAME (50mg/kg per day) in combination with a prophylactic dose of carvedilol (15mg/kg per day) or lisinopril (20mg/kg per day), respectively, for eight weeks. The fifth, sixth and seventh groups received L-NAME (50mg/kg per day) for eight weeks, followed by curative doses of carvedilol (30mg/kg per day), lisinopril (40mg/kg per day) or carvedilol (15mg/kg per day) in combination with lisinopril (20mg/kg per day), respectively, for five weeks. The drugs were administered by gastric gavage.

Results During prophylactic therapy, carvedilol and lisinopril decreased L-NAME induced rise in systolic blood pressure (SBP) and serum creatinine levels. Furthermore, both drugs improved microalbuminuria and renal histopathological changes and increased serum nitric oxide (sNO). During curative therapy, carvedilol improved microalbuminuria and renal histopathological changes, lisinopril or the combination of carvedilol and lisinopril improved all L-NAME induced changes. The combination improved renal histopathological changes more significantly than each individual drug.

Conclusion carvedilol and lisinopril have prophylactic and curative renoprotective effects in L-NAME treated rats. Carvedilol has similar effectiveness as the converting enzyme inhibitor, lisinopril, in reducing the hypertension, proteinuria and glomerulosclerosis associated with L-NAME induced chronic hypertensive renal failure in rats making chronic renal disease as a therapeutic target for this drug. Moreover, a combination of both drugs at half the therapeutic doses significantly ameliorated hypertension-induced renal damage and improved renal functions, indicating the role of this low-dose combination as an effective therapeutic option if a multi-anti-hypertensive regimen is desirable.

Key words: carvedilol, lisinopril, L-NAME, nitric oxide

INTRODUCTION:

The renin-angiotensin system plays a major role in blood pressure regulation and hypertension. Angiotensin converting enzyme (ACE) inhibitors reduce production of angiotensin II (Ang II) and have proven to be very useful antihypertensive agents. They have pleiotropic effects and studies have reported that both captopril and ramipril are cardioprotective in an animal model of the metabolic syndrome and cardiovascular disease (Russell, et al., 1998). ARBs have been viewed as useful adjuncts to the treatment of cardiovascular disease because of their antihypertensive effects. However, there are recent indications that the effects of ARBs may be broader and include direct beneficial effects on diabetic vasculopathy (Karalliedde, et al., 2008; Negro, 2008).
The blood pressure-independent role of the renin-angiotensin system (RAS) in progressive deterioration of renal function has been firmly established in animal experiments (Rennke, Klein, 1989; Remuzzi, Bertani, 1998). In addition, in renal patients, angiotensin-converting enzyme (ACE) inhibitors as well as angiotensin receptor blockers have been shown to ameliorate progression (Lewis, et al., 2001; Brenner, et al., 2001). It is possible that better control of glomerular hypertension and/or inhibition of the proliferative effects of angiotensin II by ACE inhibitors may be important factors in the possible advantage of this class of drugs over other antihypertensive agents.

Rump et al. (1999) documented activation of the sympathetic nervous system (SNS) in models of renal damage. In addition, blockade of sympathetic activity either by a central sympathetic inhibitor (Amann, et al., 2000) or a β-blocker (Amann, et al., 2001) attenuated progression independently of blood pressure in the remnant kidney model. One of the main effects of the SNS in the kidney is activation of the juxtaglomerular apparatus (Ye, et al., 2002). Carvedilol is known to be an adrenoreceptor blocker and free radical scavenger, used in hypertension and cardiac failure. However, its therapeutic actions cannot be fully explained by these mechanisms.

In this study, we tested the hypothesis that carvedilol action is associated with the synthesis/release of nitric oxide (NO). In addition, we evaluated the hypothesis that low prophylactic doses, single curative doses or combined prophylactic doses of lisinopril and carvedilol might be protective against renal damage in hypertension. To prove this, we used L-nitro-arginine methyl ester (L-NAME)-treated rats because this model of hypertension is characterized by the severity of renal lesions (Xu, et al., 1995).

MATERIAL AND METHODS
1-Animals:

The present study was conducted in adult male albino rats, weighting (120-150gm). They were brought from Experimental Animal Breeding Farm, Helwan - Cairo. All animals were housed in controlled laboratory condition at 20 -25°C in a 12h light/dark cycle and had free access to standard laboratory chow (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and water. They were acclimatized for one week and were caged (10/cage) in fully ventilated room (at room temperature) in Pharmacology Department, Benha Faculty of Medicine from September 2011-February 2012. All experimental protocols were approved by the ethics committee of Benha University.

2-Experimental model: induction of hypertension in rats:

L-NAME (50mg/kg day) was administered to rats by gastric gavage for eight weeks. By the inhibition of nitric oxide synthase (NOS), chronic L-NAME treatment increases systolic load because nitric oxide and its donors increase cyclic GMP, causing vasorelaxation; a withdrawal of constitutive NO induces vasoconstriction; causing severe hypertension (Bartunek, et al., 2000) Systolic blood pressure (SBP) was measured by the direct tail-cuff method (Harverd apparatus, 52-0338) and registered by a chart recorder (Pfeffer, et l., 1971) SBP was recorded at the start of the experiment, weekly and after eight weeks. Rats were considered to be hypertensive if SBP was > 120 mmHg. In the curative therapy protocol, SBP was measured weekly and at the end of the fifth week of treatment.
3-Treatment protocols:

The rats were divided into seven groups (n=6): the first and second (normal control and hypertensive control) groups received distilled water and L-NAME (50 mg/kg day), respectively, for eight weeks. The third and fourth groups received L-NAME (50mg/kg/ day) plus prophylactic doses of lisinopril (5mg/kg/ day) or carvedilol (15mg/kg day), respectively, for eight weeks. The fifth, sixth and seventh groups received L-NAME (50mg/kg /day) for eight weeks, followed by curative doses of lisinopril (10mg/kg per day), carvedilol (30 mg/kg /day) or lisinopril plus carvedilol (5+15mg/kg/ day), respectively, for five weeks. SBP was recorded at the start of the experiment, weekly and after eight weeks then during treatment with drugs it was measured weekly and at the end of the fifth week. Biochemical data and histopathological changes were performed at the end of 8 weeks in the control and prophylactic groups and at the end of 5 weeks of treatment in the curative groups.

The drugs were administered by gastric gavage. The dosages of the drugs used were based on the literature reported: Zoja et al. (2003) used lisinopril (40mg/kg / day) and reported that it prevented the development of renal damage in an L-NAME rat model. Kozlovski et al. (2006) used carvedilol (3-30mg/kg/day) and reported that it prevented the development of cardiac damage in L-NAME hypertension.

Estimation of renal excretion of albumin:

All rats were housed individually in metabolic cages for 24 hours with free access to water and a normal chow. Albumin concentrations were measured in 24-hour urine (mg/24 hours) samples using a Minineph microalbumin kit (The Binding Site, Birmingham, UK) (Showell, et al., 2002).

Measurement of serum creatinine and nitric oxide:

Blood samples were collected from the retro-orbital plexus (Timm, 1979).

Creatinine:

The kit used for measuring creatinine levels (Randox Laboratories, Crumlin, Country Antrim, UK) is based on the Jaffe reaction. In an alkaline solution, creatinine combines with picric acid to form an orange-red complex (the creatinine-picric acid complex). The increase in absorbance using a spectrophotometer at 510 nm is proportional to creatinine concentration (in μmol/L) (Jaffe,1886).

Nitric oxide:

The concentration of serum nitrate (a stable end product of NO, in μmol/l) was measured by a one-step enzymatic assay using nitrate reductase (Roche diagnostic group, Basel, Switzerland). The concomitant reduction of nitrate o nitrite by NADPH was reflected by the oxidation of the coenzyme and the decrease in absorbance using a spectrophotometer at 340 nm (Bories , Bories, 1995).

Histopathological kidney examination:

The rats in all groups were sacrificed, and their kidneys were harvested; each kidney was divided into two halves. The specimens were preserved in 10% formalin, dehydrated in ascending grades of ethyl alcohol (50%, 70%, 90% and 100%) and cleared; the two halves were embedded in soft and hard paraffin, respectively. Paraffin blocks were generated, and sections (3-μm thick) were cut on a microtome and subjected to haematoxylin and eosin staining. For statistical analysis, the number of affected renal tubules per 100 tubules was counted in each section, as evidence by nuclear changes (e.g. pyknosis, karyorrhexis and
karyolysis), cytoplasmic changes as (vacuolation) or sloughing of epithelial debris into the lumen of the tubules (Ono, et al., 1995).

**Statistical analysis:**

Data are presented as mean± SEM. Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by Turkeys' test as a post-hoc test. The 0.05 level of probability was used as the criterion for significance.

**RESULTS:**

**1-L-NAME –induced changes in rats:**

L-NAME administration (50mg/kg/ day for eight weeks) significantly reduced serum NO levels and increased SBP, renal albumin excretion and serum creatinine levels. L-NAME produced significant widening of Bowman's space, congestion of glomerular capillaries and areas of haemorrhage between the renal tubules.

**2- Effects of carvedilol and lisinopril administration on L-Name-Induced changes:**

Prophylactic doses of carvedilol and lisinopril significantly reduced the effect of L-NAME on SBP, but no significant difference was noted on comparison with the normal control and between the two treatment groups (Figure 1A). In addition, the curative dose of lisinopril, carvedilol and the combination produced a significant reduction in SBP with no such difference between the latter two groups (table 1, 2)

**Table 1:** Effects of L-NAME administration (50mg/kg/ day for eight weeks) on systolic blood pressure (mmHg) in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Start</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
<th>7th week</th>
<th>8th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>78.5±4.89</td>
<td>79.17±2.70</td>
<td>82±5.62</td>
<td>100.5±1.95</td>
<td>98±1.93</td>
<td>99.5±1.61</td>
<td>100.2±2.87</td>
<td>105.3±2.39</td>
<td>102.6±8.32</td>
</tr>
<tr>
<td>HC</td>
<td>81.45±2.39</td>
<td>99.33±1.15</td>
<td>114.17±3.01</td>
<td>117.5±3.82</td>
<td>124.17±2.5</td>
<td>137.8±2.5</td>
<td>158.7±2.26</td>
<td>162.7±0.7</td>
<td>173.3±11.4*</td>
</tr>
</tbody>
</table>

**Table 2:** Effects of lisinopril and carvedilol given prophylactically (L-N+L, L-N+C: L-NAME (50mg/kg/ day) + (20mg /kg/ day) lisinopril or carvedilol (15mg/kg/ day) for eight weeks) on systolic blood pressure (mmHg) in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Start</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
<th>7th week</th>
<th>8th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NAME+C</td>
<td>79.17±2.7</td>
<td>92.3±2.77</td>
<td>98.5±1.73</td>
<td>97.8±2.11</td>
<td>99.67±0.67</td>
<td>104±1.71</td>
<td>117.5±3.82</td>
<td>121.3±3.2</td>
<td>128.6±6.21*</td>
</tr>
<tr>
<td>L-NAME+L</td>
<td>82±5.6</td>
<td>96±1.48</td>
<td>99.33±1.15</td>
<td>98.33±1.76</td>
<td>100.27±0.87</td>
<td>92.27±2.76</td>
<td>111.33±6.13</td>
<td>121.8±2.7</td>
<td>123.6±11.21*</td>
</tr>
</tbody>
</table>

**3- Serum NO levels:**

Prophylactic doses of carvedilol and lisinopril significantly mitigated the effect of L-NAME on sNO with no difference between therapies (figure 1; B). Furthermore, a curative dose of lisinopril and carvedilol significantly increase serum NO levels with no difference between therapies.

**4- Renal excretion of albumin:**

Prophylactic doses of lisinopril and carvedilol significantly reduced the effects of L-NAME on renal albumin excretion, but no significant differences were noted when compared with the normal control and between the two treatment groups (figure 1; c).
Moreover, curative doses of lisinopril, carvedilol and their combination significantly reduced renal albumin excretion, but no significant differences were noted when compared with normal control or between the three treatment groups.

5- Serum creatinine levels:

Prophylactic doses of lisinopril and carvedilol significantly inhibited the effects of L-NAME on serum creatinine, but no significant difference was noted when compared with the normal control or between the two treatment groups (figure 1; D). In addition, the curative dose of lisinopril and carvedilol significantly reduce serum creatinine levels with no difference between the three treatment groups.

6- Renal histopathological structure:

Prophylactic doses of lisinopril and carvedilol significantly reduced the effects of L-NAME on renal structure. Moreover, curative doses of lisinopril, carvedilol and their combination significantly decreased the percentage of affected renal tubules, this was more pronounced in the combination group. The combination produced a more significant decrease than each individual drug did (figure 1;E) and (figures 2; E,F and G).

Table (3): Effects of carvedilol (C, 30mg/kg/ day), lisinopril (L, 40 mg/kg/ day) or carvedilol + lisinopril (C+L: 15+20 mg/kg/day) given curatively for five weeks on systolic blood pressure (mmHg) in L-NAME-induced hypertensive rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Start</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>5th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>181.2±1.32</td>
<td>178.5±1.23</td>
<td>174.33±1.31</td>
<td>167.5±2.67</td>
<td>164±0.52</td>
<td>151.33±8.82</td>
</tr>
<tr>
<td>L</td>
<td>184±1.93</td>
<td>176.2±1.17</td>
<td>160.33±3.05</td>
<td>135.7±2.32</td>
<td>125.7±7.76</td>
<td>118.33±6.96*</td>
</tr>
<tr>
<td>C+L</td>
<td>178.5±1.23</td>
<td>169.7±3.06</td>
<td>158.7±2.26</td>
<td>138±2.75</td>
<td>121.3±2.17</td>
<td>120±7.68*</td>
</tr>
</tbody>
</table>

*Significant difference at p<0.05 vs. hypertensive control (HC).
Figure 1. Effects of carvedilol and lisinopril given either prophylactically (L-N+C, L-N+L: L-NAME (50mg/KG per day) + carvedilol (15mg/kg per day) or lisinopril (lisinopril, 20mg/kg per day) for eight weeks then carvedilol (C, 15mg/kg per day), lisinopril (L, 20mg/kg per day) for five weeks in L-NAME–treated rats on:
**DISCUSSION:**

The current study showed that prophylactic therapy with lisinopril significantly reduced the effects of L-NAME on SBP, sNO and serum creatinine levels and improved microalbuminuria and renal histological changes. Moreover, curative therapy with lisinopril yielded significant effects in decreasing SBP, increasing serum NO, reducing microalbuminuria, reduced renal structure alternation and reducing serum creatinine levels. These findings are
congruent with those of Buikema et al. (2000) who noted that ACE inhibitors has a potential advantage in improvement of endothelial dysfunction through increased activity of NO after release from the endothelium into the vessel wall in experimental heart failure rats. ACEIs can potentiate NO release and subsequent functional restoration of the vascular endothelium, perhaps by increasing tissue levels of bradykinin, up-regulating eNOS and scavenging basally released oxygen-derived free radicals (Fujiki, et al., 2005). Moreover, Oktem et al. (2011) reported that lisinopril significantly ameliorated proteinuria and improved glomerular, arteriolar and tubulointerstitial lesions in L-NAME hypertensive rats. The blood pressure independent tissue-protective mechanisms of lisinopril might be related to reduction of oxidative stress (Oktem et al., 2011).

In this study, prophylactic therapy with carvedilol significantly reduced the effects of L-NAME on SBP, sNO levels and serum creatinine levels and improved microalbuminuria and renal histopathological alternations. Furthermore, curative therapy with carvedilol significantly decreased and normalized SBP; increased sNO levels and reduced serum creatinine levels, microalbuminuria and renal alternations. The observed increase in sNO level by carvedilol corresponds with the finding of Afonso et al. (2006) who reported that simultaneous treatment of rats with L-NAME and carvedilol preserved sNOS activity without affecting iNOS levels. Moreover, carvedilol can potentiate NO release and subsequent functional restoration of the vascular endothelium, perhaps by up-regulating NOS and scavenging basally released oxygen-derived free radicals (Hayashi, et al., 2010). These findings correspond to those of Di Verniero et al. (2010) who reported that the simultaneous treatment of wistar rats with L-NAME and carvedilol prevented hypertension by lowering enhanced sympathetic tone. In addition, carvedilol increases NO bioavailability and improves impaired endothelial function by decreasing oxidative stress and generating anti-hypertensive and protective effects on renal injury (Yasar, et al., 2012).

In the present study, curative therapy with the combination of carvedilol and lisinopril significantly decreased and normalized SBP, increased sNO levels and reduced serum creatinine levels, microalbuminuria and renal alternations. This combination improved renal structure more significantly than either individual drug did. These findings are supported by those of previous studies, where the administration of carvedilol with ACEI significantly reduced albuminuria in renal transplant recipients (Tylicki, et al., 2006). In addition, the combination of carvedilol and ACEIs was observed to provide a better NO/O$^2$- balanced via markeded up-regulation of eNO by the ACEI and significant lowering of O2-formation by the carvedilol (Hamar, et al., 2007) Furthermore, pretreatment with carvedilol or lisinopril reduces protein excretion in urine and caused significant reduction of glomerular volume and podocyte hypertrophy, the effects being more profound when both drugs are combined (Hamar, et al., 2007).

Jawa et al. (2008) found that combination therapy of ACEI with carvedilol was more effective than ACEI monotherapy to reduce proteinuria in diabetic hypertensive patients with proteinuric renal diseases. In addition, Bakris et al. (2008) demonstrated that in patients whose blood pressure was not controlled with a liisopril, the addition of carvedilol over a 6-week period significantly lowered systolic BP. Carvedilol, therefore, may be useful add-on therapy in hypertensive patients inadequately controlled on ACE inhibitors alone.

In conclusion, carvedilol and lisinopril have prophylactic and curative renoprotective effects in L-NAME- treated rats. Moreover, a combination of both drugs at half the therapeutic doses significantly ameliorated hypertension-induced renal damage and improves renal functions, indicating the role of this low-dose combination as an effective therapeutic option if a multi-anti-hypertensive regimen is desirable.
REFERENCES:


Nebivovol and carvedilol induce NO-dependent coronary vasodilatation that is unlikely to be mediated by extracellular ATP in the isolated guinea pig heart. Pharmacol Rep.; 58 Suppl: 103-110.


Chronic renal failure – taming the sympathetic nervous system! New approach to delaying progression. MMW Fortschr Med;141:39–41


