Differential Effects of Antihypertensive Drugs on Renal and Glomerular Hemodynamics and Injury in the Chronic Nitric-Oxide-Suppressed Rat

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Key Words

Hemodynamics · Glomerulosclerosis · Rat model · Nitric oxide synthase inhibition

Abstract

Background/Aims: Prolonged nitric oxide synthase (NOS) inhibition with N^ω-nitro-*L*-arginine methylester in normotensive and hypertensive rats has been demonstrated to produce severe systemic and glomerular hypertension with glomerular sclerosis, and these changes have become a useful experimental model of hypertensive nephrosclerosis. This review summarizes data from our serial studies as well as work of others who are also investigating the effects of the commonly used antihypertensive drugs (including calcium antagonist, angiotensin-converting enzyme inhibitor, angiotensin II type 1 receptor blocker, aldosterone antagonist and thiazide diuretic) on renal and glomerular hemodynamics, renal function and glomerular histopathology using this model. **Methods:** A Medline search was performed to identify the relevant literature describing renal effects of antihypertensive drugs in models of hypertension and nephrosclerosis produced or exacerbated by NOS inhibition. **Results:** Existing data have indicated that most of

these drug classes have produced dramatic renoprotective effects, structurally or functionally, on nephrosclerosis induced by prolonged NOS inhibition. **Conclusion:** This review of experimental studies has provided strong evidence supporting the clinical benefits of antihypertensive drugs for hypertensive patients with renal impairment particularly those with endothelial dysfunction associated with NOS deficiency.

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Introduction

Nitric oxide is generated by the enzyme nitric oxide synthase (NOS) in the cardiovascular, renal, central nervous, gastrointestinal and immune systems [1–4]. It plays a critical role in regulating endothelial function [4, 5], systemic, renal and coronary hemodynamics [4–6], platelet adhesion and aggregation [7], cardiomyocyte hypertrophy [8] and vascular smooth muscle cell proliferation and fibrosis [9]. Chronic NOS inhibition with N^ω-nitro-*L*-arginine methylester (*L*-NAME) has been shown to activate both the systemic and local renal renin-angiotensin systems [10–13] and to increase sympathetic nerve activity [11, 14], thereby promoting persistent hypertension

through cardiovascular and renal damage. In normotensive rats, prolonged NOS inhibition results in sustained systemic and glomerular hypertension with proteinuria and focal glomerular sclerosis [15], marked renal vasoconstriction, arteriolar hypertrophy and focal arteriolar obliteration, and renal interstitial inflammatory responses [16]. In hypertensive rats, studies from our laboratory [10, 17, 18] and others [19, 20] have shown that prolonged NOS inhibition impaired renal and glomerular hemodynamics by significant glomerular arteriolar constriction and produced severe hypertensive nephrosclerosis in association with reduced glomerular tuft area, glomerular cell loss and apoptosis, tubular atrophy and interstitial fibrosis. Moreover, chronic NOS inhibition exacerbated preexistent hypertension to develop malignant hypertension and severe renal injury, even a high mortality [19]. These findings suggest that suppressed NO generation has emerged as a sufficient risk factor in the severe hypertensive nephrosclerosis process. Furthermore, prolonged NOS inhibition with *L*-NAME in younger (20-week-old) adult spontaneously hypertensive rats (SHR) produced pathophysiological alterations that were similar to naturally occurring nephrosclerosis in the aged SHR [17, 18, 21].

A number of experimental models have been employed for investigating progression of chronic renal disease in the past decades [22–28]. These models have usually involved surgical renal ablation of one or both kidneys with or without additional salt loading, steroidal administration or renal infarction; none of them have involved the natural development of renal dysfunction in animals with genetic hypertension [18]. Of particular note, a commonly used experimental model of 5/6 renal ablation (right uninephrectomy and infarction of two thirds of the left kidney), over time, develop proteinuria and progressive glomerulosclerosis of the initial normal remnant nephrons, which is hemodynamically characterized by systemic hypertension, glomerular arteriolar dilation, glomerular hypertension and consequently hyperfiltration [24, 25]. Obviously, this remnant kidney model is significantly distinct from the *L*-NAME/SHR model described above. Moreover, that remnant kidney model involves extreme reduction in renal mass, an initial deficit in renal function far beyond that in essential hypertensive patients who may slowly and progressively develop renal dysfunction.

For these reasons, the prolonged NOS-inhibited rat has become a useful experimental model of hypertensive nephrosclerosis that mimics alteration of a naturally occurring physiological process. Unfortunately, only a small

number of studies have investigated the effects of various pharmacological interventions on nephrosclerosis, structurally and functionally, in this experimental model. This report reviews data from our serial studies and others involving effects of several classes of antihypertensive agents on the prevention, development, progression and even reversal of nephrosclerosis induced by prolonged NOS inhibition.

Calcium Antagonists

The effects of calcium antagonists on the kidney have been widely investigated in vitro in the isolated perfused rat kidney [29–31] and blood-perfused juxtamedullary nephrons [32–34], and in vivo in experimental models including the SHR [35, 36], deoxycorticosterone salt-hypertensive [37, 38] and remnant-kidney rats [39–41]. However, some of these findings are contradictory and might be explained by differences in the structure, pharmacology, dosing profiles and duration of administration of the drugs as well as the experimental models themselves.

Several studies have been carried out in the chronic NOS-inhibited rat model to investigate the effects of various calcium antagonists on renal function and histopathology (table 1). Ribeiro et al. [42] found that Munich-Wistar rats, treated with *L*-NAME, exhibited extremely variable plasma renin activity, glomerular tuft collapse and renal interstitial fibrosis. Simultaneous nifedipine treatment normalized the dispersion of plasma renin levels while preventing renal morphological abnormalities. Navarro-Cid et al. [43] demonstrated that diltiazem normalized proteinuria induced by *L*-NAME in Sprague-Dawley rats, and Akuzawa et al. [44] reported that another calcium antagonist, amlodipine, ameliorated urinary albumin excretion and nephrosclerosis induced by *L*-NAME in Wistar rats. Another study, by Mandarim-de-Lacerda and Pereira [45], demonstrated that verapamil efficiently prevented *L*-NAME-induced glomerular sclerosis/hypertrophy and tubular remodeling in Wistar rats. The above-mentioned studies were performed in the *L*-NAME-treated normotensive rat, and all these calcium antagonists provided beneficial effects on renal function and histopathology. Studies performed in the *L*-NAME-treated hypertensive rat model also showed favorable effects of calcium antagonists on renal injury. For example, Qiu et al. [46] documented that mibefradil prevented the development of proteinuria and decreased creatinine clearance while preventing renal structural dam-

Table 1. Renal outcomes of various calcium antagonists in the chronic nitric-oxide-suppressed rat

Calcium antagonist	Experimental model	Renal outcomes	Investigators
Nifedipine	<i>L</i> -NAME/Wistar rats	Prevented glomerular tuft collapse and renal interstitial fibrosis	Ribeiro et al. [42]
Diltiazem	<i>L</i> -NAME/Sprague-Dawley rats	Normalized proteinuria	Navarro-Cid et al. [43]
Amlodipine	<i>L</i> -NAME/Wistar rats	Ameliorated urinary albumin excretion and nephro-sclerosis	Akuzawa et al. [44]
	<i>L</i> -NAME/SHR	Improved glomerular dynamics and reduced glomerular and arteriolar injury scores	Nakamura et al. [50]
Verapamil	<i>L</i> -NAME/Wistar rats	Prevented glomerular sclerosis/hypertrophy and tubular remodeling	Mandarim-de-Lacerda and Pereira [45]
Mibefradil	<i>L</i> -NAME/SHR	Prevented proteinuria, decreased creatinine clearance and protected renal structure	Qiu et al. [46]
	<i>L</i> -NAME/SHR	Improved glomerular dynamics and reduced glomerular and arteriolar injury scores	Nakamura et al. [50]
Efonidipine	<i>L</i> -NAME/SHR	Prevented severe proteinuria and nephrosclerosis, inhibited glomerular cell apoptosis and proliferation	Watanabe et al. [47]
Felodipine	<i>L</i> -NAME/SHR	Improved glomerular dynamics and reduced glomerular and arteriolar injury scores	Francischetti et al. [49]
Cilnidipine	<i>L</i> -NAME/SHR	Improved renal pathophysiology and inhibited apoptosis and glomerular cellular proliferation	Zhou et al. [51]

age as assessed by scoring glomerular, tubulointerstitial and vascular lesions in the *L*-NAME/SHR model. Watanabe et al. [47] reported that another calcium antagonist, efonidipine, also prevented severe proteinuria and nephrosclerosis and significantly inhibited the increase in glomerular cell apoptosis index and the proliferative cell nuclear antigen index in the *L*-NAME/SHR model.

The actions of calcium antagonists on afferent and efferent arteriolar resistance had been an intriguing topic. Most traditional calcium antagonists have been shown to dilate afferent arterioles preferentially and to promote glomerular hypertension; however, newly developed calcium antagonists dilate both afferent and efferent arterioles and ameliorate glomerular hypertension [48]. In the past several years, we have carefully studied the effects of three different types of calcium antagonists on renal hemodynamics and glomerular dynamics in the *L*-NAME/SHR model using classical renal micropuncture techniques, which include felodipine and amlodipine (L type calcium antagonists), mibefradil (an L and T type calcium antagonist) and cilnidipine (an L and N type calcium antagonist) [49–51]. Each of these three types of calcium antagonists dilated not only the afferent but also the ef-

ferent arterioles, improved single-nephron plasma flow and increased single-nephron glomerular filtration rate in SHR co-treated with *L*-NAME (representing the preventive effects of calcium antagonists, fig. 1a–c) or post-treated after *L*-NAME (signifying the reversing effects of calcium antagonists, fig. 2a–c). They also prevented and reversed the glomerular and arteriolar injury in this *L*-NAME/SHR model (fig. 1d, 2d). In summary, although there were some minor differences in the responses among these calcium antagonists, they did not differ substantially with respect to their overall renoprotective effects.

Thus, in general, calcium antagonists, regardless of their subclasses, produced dramatic protective effects on renal pathophysiology in this particular experimental model of chronic NOS inhibition in normotensive or hypertensive rats. Their precise mechanisms remain unclear although inhibition of angiotensin II or norepinephrine may very well participate.

Angiotensin-Converting Enzyme Inhibitor and Angiotensin II Type 1 Receptor Blocker

Renin-angiotensin system inhibition by an angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin II type 1 receptor blocker (ARB) has distinct renoprotective effects in animals and humans [52–54]. Their favorable actions are believed to arise from inhibiting angiotensin II generation or directly inhibiting angiotensin II type 1 receptor stimulation [55–57]. However, ACE inhibition also reduces degradation of bradykinin [58–60], a potent vasodilator [61], which promotes selective efferent arteriolar dilatation during ACE inhibition [62]. On the other hand, the angiotensin II type 1 receptor antagonism may promote type 2 receptor upregulation [60, 63] and thereby modulates the opposing effect of angiotensin II type 1 receptor on arterial pressure [64, 65]. Furthermore, the angiotensin II type 2 receptor may also exert an anti-proliferative effect, counteracting the growth action of angiotensin II type 1 receptor [66–71]. Therefore, these potential effects might constitute differential outcomes for these two classes of renin-angiotensin system inhibitors on renal diseases. However, which type of angiotensin II inhibitor confers greater renoprotection remains unresolved.

In experimental models of chronic renal disease, most studies have reported that ACE-I and ARB afforded similar beneficial effects including amelioration of proteinuria and glomerulosclerosis, suggesting that their key role is angiotensin II inhibition [72]. A few reports have suggested greater renoprotection with the ARB candesartan than with ACE-I enalapril [73, 74]. Data from recent clinical trials suggest that, in nondiabetic renal disease and in type 2 diabetes with microalbuminuria, ACE-I and ARB share a similar renoprotective effect [75–77]. Nevertheless, combined treatment with the ACE-I and ARB showed enhanced renoprotective benefit in patients with nondiabetic renal disease [78, 79] or both type 1 and type 2 diabetes [76, 80, 81]. However, taken together, comparative data on renal protective effects of ACE-I and ARB remain insufficient to permit the arrival at a conclusion as to which of these two types of agents may be better.

We have exploited the *L*-NAME/SHR model of severe hypertensive nephrosclerosis to investigate the effects of ACE-I and ARB on the prevention, development, progression and even reversal of nephrosclerosis. Our earlier results demonstrated that the ACE inhibitors quinapril, enalapril or lisinopril dilated both afferent and efferent arterioles, reduced glomerular capillary hydrostatic pres-

sure and thereby prevented and reversed the development of proteinuria and glomerular sclerosis [49, 82–84] (fig. 3a–d). We have also designed a study to determine potentially differential effects between candesartan and enalapril on systemic, renal and glomerular hemodynamics and pathological changes in SHR with *L*-NAME-exacerbated nephrosclerosis [84]. Furthermore, we also investigated the contribution of bradykinin to the renal effects of enalapril in that study. Our data clearly showed that angiotensin II type 1 receptor antagonism and ACE inhibition had very similar renopreventive effects and that the ACE inhibition of bradykinin degradation provided little evidence in support of a bradykinin renoprotective action in this model. We therefore concluded that ARB and ACE-I were equally effective in renoprotection in the *L*-NAME/SHR model as in other experimental models [72]. Notwithstanding, it seems reasonable to conclude that angiotensin II plays a crucial role in influencing glomerular pathophysiological changes, whereas bradykinin only participated partially in reducing arterial pressure. Moreover, bradykinin had little effect on renal hemodynamics, glomerular dynamics, renal function and histopathology.

In addition, the effects of ACE-I and ARB have been explored in the *L*-NAME-treated normotensive rat model. In general, the ACE inhibitors such as ramipril [85], quinapril [86], and imidapril [87], and the ARB (e.g. losartan) [88–90] significantly prevented hypertension and renal functional as well as morphological damage induced by prolonged NOS inhibition. Furthermore, one comparative study demonstrated that severe histological and biochemical injuries induced by *L*-NAME were equally ameliorated by either ACE-I or ARB [91].

Data from our laboratory and others (table 2) using the prolonged NOS inhibition model further confirmed the renoprotective effects of ACE-I and ARB. The comparative results of ACE-I and ARB in *L*-NAME-treated hypertensive and normotensive rats provided strong evidence for the equivalent effectiveness of these two classes of agents with respect to renoprotection. Unfortunately, detailed dose-response effects have not been explored, and combination of these two agents dealing with their renal benefits has not been examined in this model. It is possible, however, that dual blockade of the renin-angiotensin system with an ACE-I and an ARB could offer further benefit beyond the value of either agent when used alone, which must be awaited to be explored.

Fig. 1. Co-treatment with *L*-NAME and 3 types of calcium antagonists for 3 weeks. * $p < 0.05$ at least. **a** Effects on renal hemodynamics. MAP = Mean arterial pressure; RVR = renal vascular resistance; RPF = renal plasma flow; GFR = glomerular filtration rate. **b, c** Effects on glomerular dynamics. R_A and R_E = Afferent and efferent glomerular arteriolar resistances, respectively; SFP = stop-flow pressure; P_G = glomerular capillary pressure; SNPF = single-nephron plasma flow; SNGFR = single-nephron glomerular filtration rate; SNFF = single-nephron filtration fraction; K_f = ultrafiltration coefficient. **d** Effects on renal function and pathology. GIS = Glomerular injury score; AIS = arteriolar injury score.

Fig. 2. Posttreatment with 3 types of calcium antagonists after *L*-NAME (i.e. *L*-NAME for initial 3 weeks followed by a subsequent 3 weeks of calcium antagonist treatment). * $p < 0.05$ at least. For explanation of abbreviations, see figure 1. **a** Effects on renal hemodynamics. **b, c** Effects on glomerular dynamics. **d** Effects on renal function and pathology.

Fig. 3. Co-treatment with *L*-NAME and 3 ACE inhibitors for 3 weeks. * $p < 0.05$ at least. For explanation of abbreviations, see figure 1. **a** Effects on renal hemodynamics. **b, c** Effects on glomerular dynamics. **d** Effects on renal function and pathology.

Table 2. Renal outcomes of different ACE-I or ARB in the chronic nitric-oxide-suppressed rat

ACE-I or ARB	Experimental model	Renal outcomes	Investigators
Quinapril	<i>L</i> -NAME/SHR	Improved glomerular dynamics and reduced glomerular and arteriolar injury scores	Ono et al. [82]
	<i>L</i> -NAME/Wistar rats	Improved survival	Michel et al. [86]
Enalapril	<i>L</i> -NAME/SHR	Improved glomerular dynamics and reduced glomerular and arteriolar injury scores	Francischetti et al. [49] and Nakamura et al. [84]
	<i>L</i> -NAME/Wistar rats	Prevented glomerular sclerosis/hypertrophy and tubular remodeling	Mandarim-de-Lacerda and Pereira [45]
Lisinopril	<i>L</i> -NAME/SHR	Improved glomerular dynamics and ameliorated urinary albumin excretion and nephrosclerosis	Zhou et al. [83]
Ramipril	<i>L</i> -NAME/Wistar rats	Improved renal hemodynamics and renal function	Hropot et al. [85]
Imidapril	<i>L</i> -NAME/Wistar rats	Prevented nephrosclerosis	Nakamura et al. [87]
	<i>L</i> -NAME/Wistar rats	Ameliorated histological injuries and the increased expression of transforming growth factor β	Kashiwagi et al. [91]
Candesartan	<i>L</i> -NAME/SHR	Improved glomerular dynamics and ameliorated urinary albumin excretion and nephrosclerosis	Nakamura et al. [84]
	<i>L</i> -NAME/Wistar rats	Ameliorated histological injuries and the increased expression of transforming growth factor β	Kashiwagi et al. [91]
Losartan	<i>L</i> -NAME/Wistar rats	Normalized glomerular filtration rate	Ribeiro et al. [88]
	<i>L</i> -NAME/Sprague-Dawley rats	Prevented severe renal injury and hypertension	Verhagen et al. [90]

Aldosterone Antagonists

Recently, a number of experimental and clinical reports have demonstrated that aldosterone participates in hypertension and renal injury [92–98] and may be supported further by the antihypertensive and renoprotective effects of aldosterone antagonism [92–94, 96, 97, 99, 100]. In addition, the phenomenon of ‘aldosterone escape’ has been suggested to participate during ACE-I therapy [101–104]. Accordingly, aldosterone antagonism has been perceived as an important approach in preventing the progression of renal dysfunction in hypertension [105, 106] since it may also enhance the effectiveness of angiotensin II inhibition. Indeed, recent clinical trials including the Randomized Aldactone Evaluation Study [107] and the Eplerenone Post-AMI Heart Failure Efficacy and Survey Study [108] have demonstrated a greater benefit of the co-administration of an aldosterone antagonist with an ACE-I or an ARB in patients with cardiac dysfunction. Whether or not the combination of aldosterone antagonism with angiotensin II inhibition produces superior

renal benefits to angiotensin II inhibition therapy alone is, at present, a matter of speculation.

The experimental studies described above were explored in the aldosterone/salt-hypertensive rat [92], remnant-kidney rat [93], stroke-prone SHR [94, 95], *L*-NAME/angiotensin II/NaCl-treated rat [96] and radiation-induced nephrosclerotic rat [97]. One study by Usui et al. [109] reported that prolonged NOS inhibition also increased plasma aldosterone concentration and its mRNA level; however, the effect of aldosterone antagonism on the prolonged NOS-inhibition-induced hypertension and renal damage has been barely investigated.

With this background, we initiated a study designed to test the effects of the aldosterone antagonist eplerenone in *L*-NAME-exacerbated SHR nephrosclerosis and the outcomes of combined therapy of eplerenone with the ACE-I lisinopril to determine whether aldosterone antagonism enhances the effectiveness of ACE inhibition [83]. Our findings demonstrated that an aldosterone antagonist had no effect on systemic, renal hemodynamics and glomerular dynamics; however, it did significantly

ameliorate the proteinuria and markedly improve glomerular and arteriolar injuries and tubulointerstitial lesions in the *L*-NAME/SHR model. To this end, we suggested that the beneficial effects of eplerenone were most likely achieved through nonhemodynamic effects such as its anti-inflammatory actions [92]. We further found that combined therapy with an aldosterone antagonist and an ACE-I produced no further benefit than with the ACE-I alone on the renal pathophysiological alterations in *L*-NAME/SHR nephrosclerosis. The explanation for the latter unexpected finding remains unresolved, and it is possible that aldosterone escape had not emerged during the 3-week period of our study.

Therefore, existing data suggest that aldosterone plays a deleterious role in the progression of *L*-NAME-induced SHR nephrosclerosis, although angiotensin II may be considered as an essential determinant of inducing the advanced hemodynamic consequences such as systemic and glomerular hypertension as well as renal damage during chronic NOS inhibition [10–13]. Eplerenone, the selective mineralocorticoid receptor antagonist, has afforded favorable renal actions in the experimental model of chronic NOS inhibition.

Thiazide Diuretics

Since their introduction in the late 1950s, thiazide diuretics have been considered as a major antihypertensive drug class [110]. After being widely used in the major antihypertensive drug trials and clinically for decades, the thiazide diuretics were highlighted in recent clinical trials, the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial [111] and the Second Australian National Blood Pressure Study [112]. Thus, they are currently advised for initiating therapy by the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure [113].

However, although the thiazide diuretics have been demonstrated to be efficacious for preventing stroke and hypertensive cardiovascular disease and decreasing total mortality in numerous clinical trials [111, 114], studies investigating the effects of thiazide diuretics on hypertensive renal damage are scarce in both experimental and clinical settings [115–117]. Of particular note, a prior study from our laboratory evaluated the effects of hydrochlorothiazide on *L*-NAME-induced severe hypertensive nephrosclerosis; the data demonstrated that hydrochlorothiazide, alone, further impaired renal function while also

producing greater glomerular injury [116]. Recently, we have repeated this study in another protocol, and our findings showed only slightly salutary renal effects of hydrochlorothiazide in this experimental model (abstract for AHA council, 2004). The explanation for these seemingly different findings is uncertain but may result from the differences in SHR generations and the consumed *L*-NAME doses, thereby underscoring the importance of having special control groups for all studies.

In summary, although thiazide diuretics are recommended for first-step antihypertensive therapy, their long-term renal effects remain unknown. More experimental and clinical studies are expected.

Conclusion

This report reviews the pathophysiological renal outcomes and the renal responses of the commonly used antihypertensive agents in chronic nitric-oxide-suppressed rats. Thus, calcium antagonists, ACE inhibitors and ARBs have been demonstrated to prevent and even reverse the adverse renal hemodynamic, glomerular dynamic and renal pathological alterations induced by chronic NOS inhibition. An aldosterone antagonist impressively improved the severe proteinuria and renal histopathological alterations in the absence of any of the renal hemodynamic and glomerular dynamic effects produced by the calcium antagonists or angiotensin II inhibitors in the *L*-NAME/SHR model. These actions, therefore, provide important clinical implications for patients with essential hypertension and other cardiovascular and renal complications or diseases which are commonly associated with NOS deficiency.

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