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Protective Actions of Cilnidipine: Dual L/N-Type Calcium Channel Blocker Against Hypertensive Renal Injury in Rats

Gouher Banu Shaikh[1]*, Surekha Hippargi[1], Dewan S. A Majid[2] and Kusal K Das[1]

[1]Laboratory of Vascular Physiology and Medicine, Department of Physiology, Shri B.M. Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura-586103, Karnataka, India.
[2]Department of Physiology, Hypertension and Renal Center of Excellence, Tulane University School of Medicine, New Orleans, USA.
*Corresponding Author E-mail: gouher.banu@bldedu.ac.in

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Cilnidipine belongs to fourth generation dihydropyridine calcium channel blocker (CCB). It is a dual L & N-type CCB. L- type calcium channels are present on the vascular smooth muscle and N-type calcium channels are present on the presynaptic nerve terminals. Cilnidipine has a vasodilating effect, its action is slow and long lasting. Aim of present study was to demonstrate the beneficial effects of cilnidipine on the hypertensive renal injury rats. And our objectives is to assess renal injury parameters (Proteinuria, Creatinine clearance, Renal fibrosis/ glomerulosclerosis) in response to chronic NG-nitro-L-arginine methyl ester hydrochloride (L-NAME) treatment in the presence or absence of cilnidipine treatment. Male albino Wister rats were procured from institutional animal house, divided into 4 groups (n=6 in each group). Group1 treated with vehicle (control), group2 treated with cilnidipine, group3 treated with L-NAME, group4 treated with L-NAME & cilnidipine. 24 hour urinary protein and creatinine clearance were measured. Serum urea and creatinine levels are also measured. Urinary and serum Angiotensin II levels were measured. Histopathological examination of kidneys was performed. Our results demonstrate that treatment with cilnidipine (group4) there is reduction in 24hr urinary protein, improvement in creatinine clearance. We observed there was renal glomerulosclerosis and tubular degeneration of kidney tubules in group3 rats and reduction of renal injury in group4 rats. We also found reduced urinary and serum Angiotensin II level in cilnidipine treated (group 4) rats. Conclusion: These findings indicated that cilnidipine act as renoprotective agent and reduces glomerular damage in L-NAME induced hypertensive rats.

Keywords: Creatinine Clearance; Hypertensive Arts; 24Hour Protein; L and N-type Calcium Channel Blocker; Renal Injury.

Persistent hypertension causes loss of glomeruli and several morphological and quantitative alterations in the kidney and progressively leads to chronic renal failure. The angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are the prime drugs used for the treatment of chronic nephropathy.[1] The nitric oxide deficient rat hypertension model has

been shown to be a useful tool for studying both the development and treatment of renal lesions resembling those found in human hypertension. [2]

As complications of chronic hypertension mainly occurs in the kidneys, hence purpose of the therapy should be to reduce hypertensive renal injury. Sympathetic over activity plays a major role in progressive renal injury. Treatment which modulate sympathetic nerve activity may be of benefit to chronic renal disease.

Cilnidipine as a double L&N type calcium channel blocker may have greater beneficial effect compare to only L-type CCB in hypertensive renal injury rats. [3, 4, 5]

There are very less studies to elucidate the effects of N-type calcium channel blocker against nitric oxide inhibited hypertensive rats.

Hence we investigated the renoprotective effects of cilnidipine on L-NAME induced hypertensive rats.

METHODS

Experimental Animals

Procured 24 male Albino Wister rats (Weighing 160-200gms) from institutional animal house. The rats were acclimated to handling for one week before intervention. Animals were housed in standard conditions, two rats in a cage, with 12-hour light and dark cycle and given rodent food and water. All experiments are conducted according to the guide lines of (CPCSEA) Committee for the Purpose and Control and Supervision of Experiments on Animals, Government of India.

Animal Intervention

After one week of acclimatisation rats were divided into 4 groups, group1 (Control) treated with vehicle (0.5% sodium carboxy methyl cellulose (Na CMC). Group2, treated with cilnidipine (2mg/kg/day) in 0.5% (Na CMC). Cilnidipine purchased from Laksh Fine chem. Pvt. Limited, Gujarat, India. Group 3, treated with L-NAME (40mg/kg/day in distilled water). Purchased L-NAME from Pro Lab Marketing Pvt. Limited, New Delhi, India. Group 4, treated with both L-NAME and cilnidipine. All drugs are given by oral gavage at morning hours for 28days.

Recording of Mean Arterial Pressure

Mean arterial pressure (MAP) of conscious rats was measured weekly during intervention. Animals were trained in the restainer everyday for one week before recording blood pressure. Blood pressure was recorded noninvasively by tail cuff (NIBP). Three readings were taken for each rat using Bio Pac Instrument (Bio Pac MP100:PC windows based animal electrophysiology). All the parameters will be analyzed by Bio Pac Student Lab 4.1 software.). Mean value of three recordings was considered.[6]

Assessment of Biochemical Parameters

For assessment of serum urea and creatinine, blood was collected from supra orbital plexus. Serum was separated and stored at -20 °C, serum urea and creatinine levels were assessed by fully automated dry chemistry analyser (VITROS 5.1/FS chemistry system).

Collection of Urine

Rats were kept individually in metabolic cages, 24 hour urine was collected from 10:00 a.m. to next day 10am to determine the 24hr urinary protein and creatinine excretion. Sediments were removed by centrifuging all urine samples.[7] The 24 hour protein concentration was measured with auto analyser (VITROS 5.1/FS chemistry system). Creatinine Clearance of all the rats was measured by using formula (urine creatinine in mg/ml ×urine volume / day in mL ÷ 1440 min ÷ serum creatinine in mg/ml ÷ both kidney weight in gm .[8]

Estimation of Angiotensin II Levels in Urine and serum

Urinary and serum Angiotensin II level were estimated in pg /ml by ELISA Kit method (Cat No-k11-0656).

Histological examination of Kidney

Kidneys were fixed by using 10% formalin at pH 7.4, paraffin embedded sections were made. Thin slices of 4ìm were prepared. Histological examinations was done under 10x and 40x.

Ethical considerations

Taken permission from Institutional Animal Ethics Committee (IAEC) before commencement of experiment. Ethical clearance certificate (Ref: BLDE/BPC/644/2018-2019 dated 15.12.2018).

Statistical Analysis

Statistical analysis was done by using SPSS Soft ware (version 16 software). Values were expressed as the mean± SD. Statistical comparisons were made by one-way ANOVA.

p-value of less than or equal to 0.05 were considered to be statistically significant.

RESULTS

Effect of Cilnidipine on Mean Arterial Blood Pressure (MAP)

Basal mean arterial blood pressure among all the groups was not significant. There is no statistical significant difference was observed in mean arterial pressure of control group through out the intervention period. Mean arterial pressure was progressively increased in L-NAME administered rats. We found significant increase in MAP from first week onwards in group3 rats. There was significant decrease in MAP on third week and fourth week in group4 rats compared to group 3 rats. (Fig1)

Urinary protein and creatinine excretion

We found that there is significant increase in excretion of 24hour protein and decrease in creatinine clearance in group 3 rats, with simultaneous treatment of L-NAME & cilnidipine (group4 rats) there is significant reduction in excretion of 24 hour protein and increase in creatinine clearance compare to group3 rats. (Table 1)

Urinary Angiotensin II levels

We observed there is significant increase in Angiotensin II level in group 3 rats. There is significant decrease in Angiotensin II levels in urine with L-NAME & cilnidipine treated (Group 4) rats. (Table 1)

Serum urea, creatinine and Angiotensin II levels

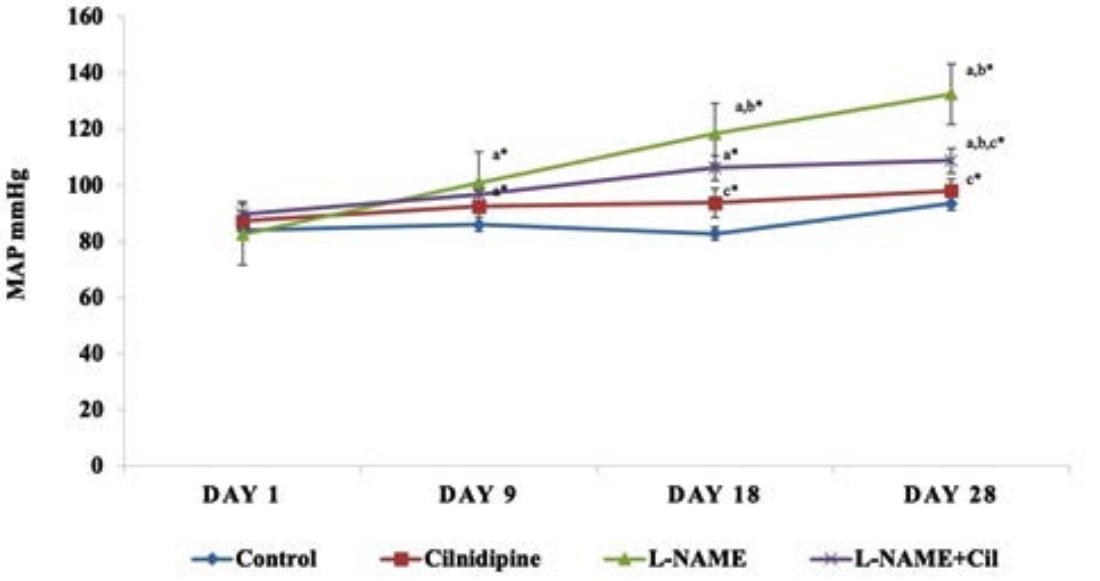
We found there is no significant change in serum urea levels in all four groups.

We observed there is significant increase in serum Angiotensin II levels in group 3 hypertensive rats, simultaneous treatment with L-NAME and cilnidipine there is significant decrease in Angiotensin II levels. (Table2)

Histological study of Kidney

Microscopic structure of kidney in each rat of all groups are stained with haematoxylin & eosin (10 x & 40x) depicted in two rows. Control group we observed normal renal histology. Cilnidipine group is also showing normal renal histological features. L-NAME group we observed (arrow A) glomeruli are hyper cellular with increased mesangial proliferation. Focal tubular epithelial hydropic degeneration (arrow B). L-NAME plus Cilnidipine treated group we found there is normal renal histology.

Histopathology of Kidney



Graph showing the effect of cilnidipine on mean arterial pressure in L-NAME induced hypertensive rats. Superscript a, b, c, indicate significant difference between group1, group2 and group3 respectively . *p<0.05 is statistically significant

Fig. 1. Effect of cilnidipine on mean arterial blood pressure

Table 1. Proteinuria, creatinine clearance & Urinary Angiotensin II level (n=6 in each group)

Parameters	Group1	Group2	Group3	Group4	ANOVA	
					F value	P value
24 hr Urinary protein in mg/ml	1.56±0.043	1.72±0.045^{c,d}	4.58±0.28^{a,b,d}	3.24±0.23^{a,b,c}	346	0.0001*
Creatinine Clearance in ml/min/gm of kidney tissue	0.073±0.0026	0.075±0.0032^{c}	0.063±0.0028^{a,b}	0.077±0.0026^{c}	28.499	0.0001*
Urinary Ang II in pg/ml	329.74±7.13	403.99±4.58	450.84±10.30^{a,b}	410.49±6.8^{a,c}	272.143	0.0001*

Values are expressed in (Mean ± SD). Groups are compared by One-way ANOVA followed by Post Hoc multiple test. Superscript a, b, c, indicate significant difference between groups. 'a, b, c denotes comparison with group1, group2 and group3 respectively. *p<0.05. Ang II-Angiotensin II

Table 2. Serum urea and creatinine and Angiotensin II levels among groups. (n=6 in each group)

Parameters	Group1	Group2	Group3	Group4	ANOVA	
					F value	P Value
Serum urea mg/ml	41.37±2.72	43.13±3.26	44.01±2.92	41±3.34	1.2093	0.304
Serum Creatinine in mg/ml	0.40±0.03	0.42±0.04	0.611±0.03^{a,b}	0.40±0.03^{b,c}	41.672	0.0001*
Serum Ang II in pg/ml	152.53±25.22	209.12±42.59^{a,c}	321.61±25.86^{a,b,}	245.45±14.62^{a,c}	36.12	0.0001*

Values are expressed in (Mean ± SD). One-way ANOVA done for comparison between groups. Superscript a, b, c, indicate significant difference between groups. *p<0.05. Ang II-Angiotensin II

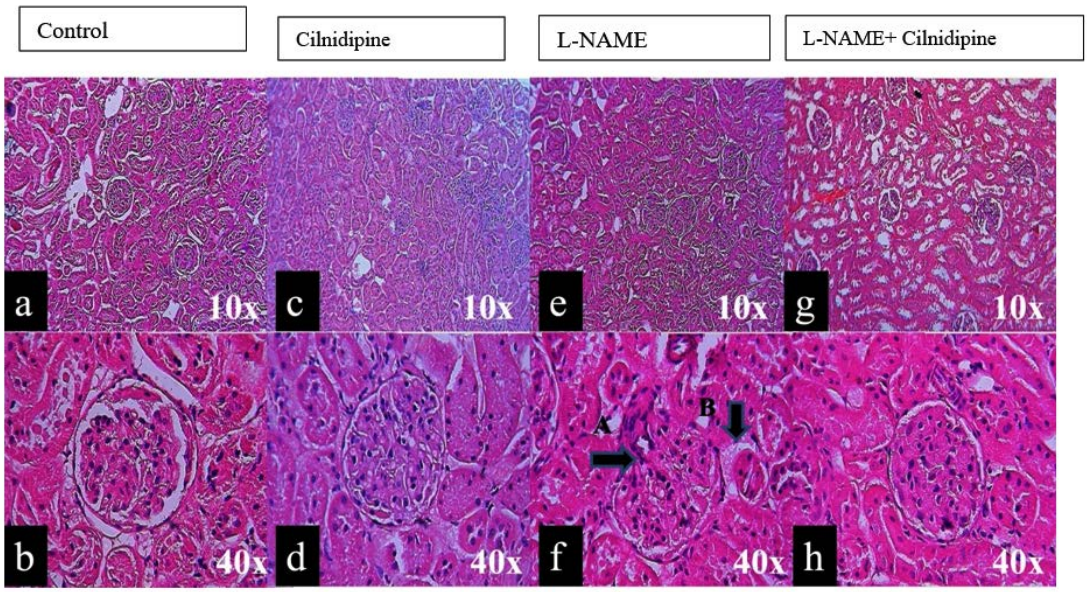


Fig. 2. Photomicrograph of the renal tissue stained with haematoxylin and eosin stain from (a) Group1(control) (x10); (b) group1 (control) (x40); (c) group2 (Cil) (x10); (d) group2 (Cil) (x40); (e) group3 (L-NAME) (x10); (f) group3(L-NAME) (x40); (g) group4 (L-NAME + Cil) (x10); (h) group4 (L-NAME + Cil) (x40). Arrow A- Glomeruli are hyper cellular with increased mesangial proliferation. Arrow B- Focal tubular epithelial hydropic degeneration. Cil-cilnidipine

DISCUSSION

We observed that administration of cilnidipine to the L-NAME induced renal injury hypertensive rat reduced proteinuria, improves creatinine clearance and reduces the glomerular damage.

Chronic nitric oxide synthesis inhibition induced by chronic L-NAME treatment, results in endothelial dysfunction, hypertrophy of vasculature, fibrosis of cardiac tissue, atherosclerosis and perivascular inflammation. Nitric oxide deficiency also contributes to renal failure and increased vascular responses to adrenergic stimuli. Several other factors, including renin angiotensin system (RAS), endothelial constrictor factors, sympathetic nervous system, arterial remodelling are also involved in these effects.[9]

Cilnidipine being both L and N-type calcium channel blocker, many of the actions are mediated by inhibiting specifically N-type calcium channels. Primary actions include the suppression of sympathetic nervous over activity,[10,11] cardiovascular and renal protective functions.[12,13] They also observed in spontaneously hypertensive rats, Plasma rennin activity and plasma angiotensin II levels doesn't increase with cilnidipine treatment. They also found that cilnidipine by inhibiting N-type calcium channels, directly suppresses secretion of aldosterone from adrenocortical cells.[14] These results suggest that N-type calcium channels plays a important role in regulation of renin–angiotensin–aldosterone (RAAS) activity. A clinical trial comparing CCBs cilnidipine and L-type CCB amlodipine, in combination with a RAAS inhibitor, showed that cilnidipine is much effective compared to other CCB in preventing the progression of proteinuria in hypertensive patients. They also proved that cilnidipine was more superior than L-type CCB amlodipine in preserving the glomerular slit membrane and preventing impaired kidney function.[15]

We found there is increase in Angiotensin II levels in plasma and urine in L-NAME treated rats and there is significant decrease with L-NAME and cilnidipine treatment.

Angiotensin-II, the prime bioactive peptide of the RAAS, has a important role in the regulation of structure and functions of vascular. RAAS activation leads to hypertension in rats treated with L-NAME. Angiotensin-II a potent vasoconstrictor can activates sympathetic nerve function. Sympathetic hyperactivity can cause vascular remodelling leading to heart failure in a hypertensive rat model. Nitric oxide deficient hypertension causes an imbalance of renin angiotensin aldosterone system.[16]

Treatment with cilnidipine decreases plasma Angiotensin-II level. It is evident from the previous study that cilnidipine is having antioxidant property.[17] and increases plasma NO bioavailability.[18] Cilnidipine attenuates angiotensin II formation by inhibiting renin release and angiotensin converting enzyme (ACE) activity.[19] Cilnidipine inhibits the vicious cycle of renin angiotensin system and oxidative stress in the kidney. It may also decrease the kidney expression of angiotensinogen in the spontaneously hypertensive rats.[20]

Konda *et al.* Observed that cilnidipine doesn't cause any change in Angiotensin II levels, plasma norepinephrine and plasma renin activity. Hence they concluded that cilnidipine, suppressed reflex sympathetic hyperactivity and rennin angiotensin system activation induced by hypotension, by specifically blocking N-type calcium channel. [21]

Similar findings was observed in our study that cilnidipine decreases the level of Angiotensin II in nitric oxide deficient hypertensive rats. Hence, it can be considered that cilnidipine as a regulator of rennin angiotensin system by its inhibitory action of N-type calcium channel in the kidney. These actions of cilnidipine proves the renal protective effects.

CONCLUSION

We conclude that cilnidipine reduce renal damage by reducing proteinuria, improvement in creatinine clearance, and preventing glomerular sclerosis in nitric oxide deficient hypertensive rats. Possibly, through the inhibition of N-type calcium channels and by inhibiting renal Renin Angiotensin System and reducing oxidative stress.

These observations suggest that both L and N- type calcium channel blocker (cilnidipine) could be a better drug for therapeutic purposes in hypertensive patients with chronic renal complications.

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Conflicts of interest

There are no conflicts of interest.

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