Antiproteinuric effect of the calcium channel blocker cilnidipine added to renin-angiotensin inhibition in hypertensive patients with chronic renal disease

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Cilnidipine, a dual L-/N-type calcium channel blocker, dilates both efferent and afferent arterioles and is renoprotective. Our multi-center, open-labeled, and randomized trial compared the antiproteinuric effect of cilnidipine with that of amlodipine in hypertensive patients with kidney disease. A group of 339 patients, already receiving renin–angiotensin system inhibitor treatment, were randomly assigned to cilnidipine or amlodipine. The primary endpoint was a decrease in the urinary protein to creatinine ratio. After 1-year of treatment, systolic and diastolic blood pressures were significantly reduced in both groups which did not differ between them. The urinary protein to creatinine ratio significantly decreased in the cilnidipine compared to the amlodipine group. Cilnidipine exerted a greater antiproteinuric effect than amlodipine even in the subgroup whose blood pressure fell below the target level. This study suggests that cilnidipine is superior to amlodipine in preventing the progression of proteinuria in hypertensive patients when coupled with a renin–angiotensin system inhibitor.

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Based on the accumulating data from a large number of clinical mega-trials,[1–5] it has been established that an inhibitor of the renin–angiotensin system (RAS), such as an angiotensin-converting enzyme (ACE) inhibitor and an angiotensin II type 1 receptor blocker (ARB), has an apparent renoprotective effect. Although the guidelines for the management of hypertension[6–8] recommend that blood pressure (BP) should be strictly controlled in hypertensive patients with kidney disease, adequate BP levels are seldom achieved with only one RAS inhibitor. Actually, combination of two to three antihypertensive drugs is required to decrease BP to target levels, especially in patients with kidney disease.[9] One of the main candidates for a combination with an RAS inhibitor is a dihydropyridine-type calcium channel blocker (CCB), because it reduces BP even in patients who are sometimes considered relatively resistant to antihypertensive drugs.[10]

However, evidence for the renoprotective effect of dihydropyridine CCBs is inconsistent. For example, ramipril had a greater renoprotective effect compared with amlodipine in the African American Study of Kidney Disease and Hypertension (AASK) study.[4] Moreover, the risk of a doubling of the serum creatinine (Cr) was similar between amlodipine and placebo groups in the Irbesartan Diabetic Nephropathy Trial (IDNT).[11] In a recent meta-analysis, the mean change in urinary protein was approximately +2% for dihydropyridine CCBs.[12] On the other hand, the antiproteinuric effect of cilnidipine has been shown in several types of hypertensive animal models[13,14] and hypertensive patients with kidney disease.[15,16] The addition of a dihydropyridine CCB to treatment with an RAS inhibitor is also associated with inconsistent renal effects. The Blood-pressure Control for Renoprotection in Patients with Non-diabetic Chronic Renal Disease Trial (REIN-2) group showed that the addition of felodipine was not of further renal benefit in patients with non-diabetic proteinuric nephropathies and background of ramipril therapy.[17] On the other hand, cilnidipine further reduced urinary albumin in patients with type II diabetic nephropathy under treatment with valsartan.[18]

This inconsistency may be due to the different specificity for calcium channels of each dihydropyridine CCB;[19] there are several variants of calcium channels, such as L, N, T, P/Q, and R.[20,21] Actually, cilnidipine, an L-/N-type dihydropyridine CCB, was shown to suppress renal injury in hypertensive animals and humans[13–16] but amlodipine[4,11] and felodipine,[17] an L-type CCBs, were not. Cilnidipine had a greater renoprotective effect than amlodipine in rat[14] and small-size clinical studies.[16] The blockade of N-type calcium channels is able to inhibit renal sympathetic nerve activity[14] and the resulting efferent arteriolar vasodilation protected the glomeruli through the attenuation of glomerular hypertension.[13] However, because L-type calcium channels do not express in glomerular efferent arterioles,[22] the renoprotective effects of an L-type CCB are expected to be lower than those of an L-/N-type CCB. There is still a lack of clinical trials comparing the renoprotective effects of an L-/N-type and L-type CCBs added to treatment with an RAS inhibitor, which is frequently prescribed for hypertensive disease in clinical practice. Thus, we compared the antiproteinuric effects of cilnidipine with those of amlodipine in hypertensive patients with kidney disease who were already under treatment with an RAS inhibitor.

RESULTS

Study population

Three hundred and fifty subjects were enrolled for this study and of them 11 were excluded based on the selection criteria (Figure 1). Thus, 339 subjects (these were 90.2% of required sample size with our estimation) were randomly allocated to the cilnidipine group ($n=179$) or amlodipine group ($n=160$). The baseline characteristics of the subjects in the two groups are shown in Table 1 and baseline medication is shown in Table 2. There were no significant differences between the two groups. The final dose was $11.5\pm5.6\,mg\,day^{-1}$ in the cilnidipine group and $5.3\pm2.4\,mg\,day^{-1}$ in the amlodipine group. Twenty subjects from the cilnidipine group and 17 from the amlodipine group were added antihypertensive drugs other than a CCB or an RAS inhibitor during the treatment (cilnidipine group: diuretic $n=12$, α-blocker $n=5$, β-blocker $n=5$; amlodipine group: diuretic $n=14$, α-blocker $n=4$, β-blocker $n=1$). During treatment, 32 subjects from the cilnidipine group (adverse reaction: $n=9$, cardiovascular event: $n=1$, death: $n=2$, lost to follow-up: $n=20$ (discontinuation: $n=13$, moving away: $n=4$, protocol violation: $n=3$)) and 30 subjects from the amlodipine group (adverse reaction: $n=13$, cardiovascular event: $n=4$, death: $n=3$, lost to follow-up: $n=10$ (discontinuation: $n=8$, moving away: $n=2$)) withdrew from the study.

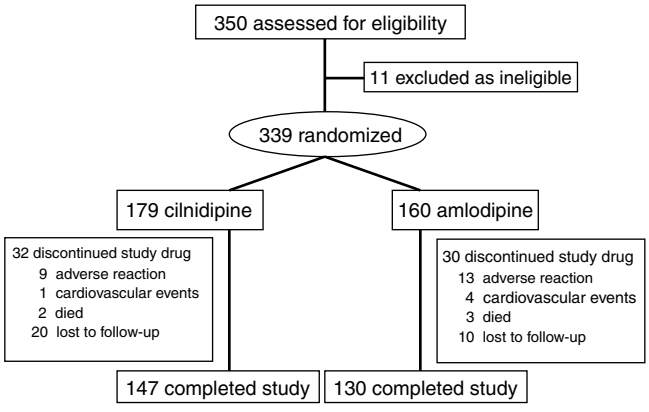

Figure 1 | Flow of participants throughout the study.

Changes in BP and heart rate

Systolic BP was slightly but significantly ($P<0.05$) greater at 2 months of treatment in the amlodipine group than in the cilnidipine group but did not differ at the other time points

Table 1 | Baseline characteristics

Variable	Cilnidipine	Amlodipine	*P*-value
n	179	160	
Age (years)	59.9±13.3	59.3±12.9	NS
Sex (male/female)	121/58	93/67	NS
Causative disease			
Diabetic nephropathy	70	59	NS
Primary renal disease	57	61	
Hypertensive nephrosclerosis	34	26	
Others	18	14	
BP			
Systolic BP (mm Hg)	152.4±14.8	152.4±14.8	NS
Diastolic BP (mm Hg)	86.9±9.6	88.0±9.4	NS
Heart rates (beats per min)	76±12	74±10	NS
Body mass index ($kg\,m^{-2}$)	24.6±3.7	24.8±4.1	NS
Urinary protein/Cr ratio ($mg\,g^{-1}$)	1921±2126	1712±1572	NS
Serum Cr ($mg\,dl^{-1}$)	1.27±0.58	1.29±0.60	NS
Serum total cholesterol ($mg\,dl^{-1}$)	210±44	218±43	NS
Serum HDL cholesterol ($mg\,dl^{-1}$)	56±20	55±16	NS
Serum triglycerides ($mg\,dl^{-1}$)	169±183	182±112	NS
Diabetes mellitus	81 (45%)	67 (42%)	NS
Cerebrovascular disease	11 (6%)	7 (4%)	NS
Ischemic heart disease	9 (5%)	9 (6%)	NS

BP, blood pressure, Cr, creatinine, HDL, high-density lipoprotein; NS, not significant.

Table 2 | Baseline medication

Medication	Cilnidipine	Amlodipine	*P*-value
RAS inhibitor			
ARB	114	98	NS
ACE inhibitor	41	45	
ARB+ACE inhibitor	24	17	
Diuretics	45	45	NS
β-Blocker	16	17	NS
α-Blocker	17	16	NS
Central sympatholytic agent	1	1	NS

ACE, angiotensin-converting enzyme; ARB, angiotensin II type 1 blocker; NS, not significant; RAS, renin-angiotensin system.

(Figure 2). Diastolic BP did not differ between the two groups during the treatment. In the last month of treatment, systolic (cilnidipine; 133.1±15.6 mm Hg, amlodipine; 134.5±16.6 mm Hg, NS) and diastolic (75.6±8.7 vs 77.9±9.4 mm Hg, NS) BP did not differ either. Subjects with a BP <140/90 mm Hg accounted for 69.3 and 62.3% in the cilnidipine and amlodipine groups (NS), respectively. Subjects with a BP <130/85 mm Hg accounted for 36.9 and 37.7% (NS), respectively; BP level of this subgroup was also similar between cilnidipine and amlodipine groups (systolic BP: 120.5±6.8 vs 118.9±6.5 mm Hg, diastolic BP: 71.3±7.7 vs 73.2±6.1 mm Hg, NS, respectively). Heart rate did not differ either (cilnidipine; 73.9±11.8 beats per min, amlodipine; 73.6±11.7 beats per min, NS).

Changes in urinary protein/Cr ratio

In the cilnidipine group, the urinary protein/Cr ratio was significantly lower in the last month of the study than in the amlodipine group (1308.6±121.2 vs 1881.1±188.8 mg g^{-1}: s.e.m., $P<0.05$) (Figure 3). When the percent change from baseline was calculated, it was significantly lower in the cilnidipine group at 3 months of treatment or later. After 12 months of treatment, the urinary protein/Cr ratio had decreased in the cilnidipine group (−14.4±5.6%: s.e.m.) but not in the amlodipine group (+13.9±7.7%: s.e.m.), showing a significant ($P<0.01$) difference between the two groups. Even in subgroups with different baseline, urinary protein/Cr ratio, age, sex, or BP after the 12 months of treatment, cilnidipine significantly suppressed the urinary protein/Cr ratio compared with amlodipine (Figure 4). In a subgroup with primary renal disease, cilnidipine was also more antiproteinuric than amlodipine. In a subgroup with diabetic nephropathy, although the percent changes in urinary protein/Cr ratio did not differ between the two groups (Figure 4), its absolute value was lower in the cilnidipine group than in the amlodipine group (1160.5±171.6 vs 2405.0±447.1 mg g^{-1}: s.e.m., $P<0.05$) after 1 year of treatment, while the baseline urinary protein/Cr ratio did not differ (2243.3±330.4 vs 2137.5±268.3 mg g^{-1}: s.e.m., NS). However, in the subgroup with hypertensive nephrosclerosis, the antiproteinuric effect was

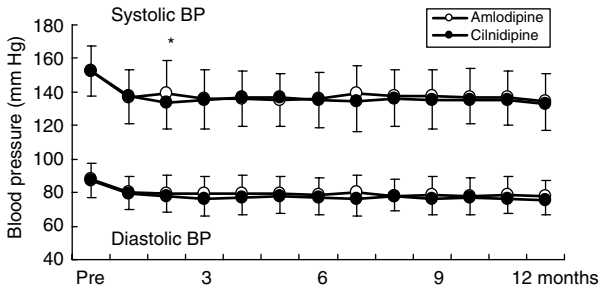

Figure 2 | Changes in systolic and diastolic BP. BP was almost the same in the cilnidipine (closed circles) and the amlodipine (open circles) groups except for a slight difference in systolic BP at one point. *$P<0.05$, cilnidipine vs amlodipine groups.

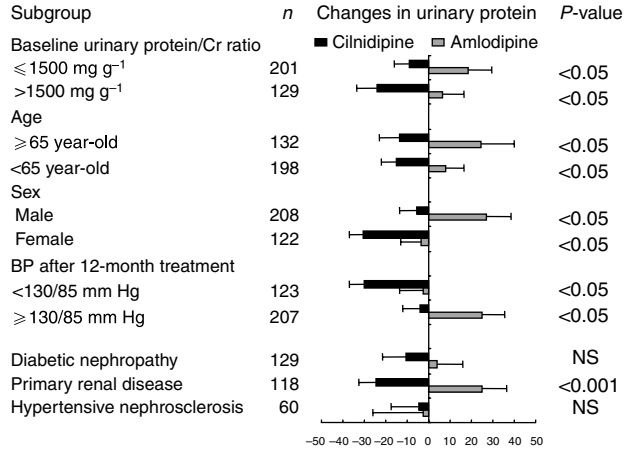

Figure 4 | Changes in urinary protein/Cr ratio in subgroups with different baseline urinary protein/Cr ratio, age, sex, BP after 12 months of treatment, or etiology of kidney disease. Results are expressed as the mean±s.e.m. The antiproteinuric effect was superior in the cilnidipine group compared with the amlodipine group even in the different subgroups.

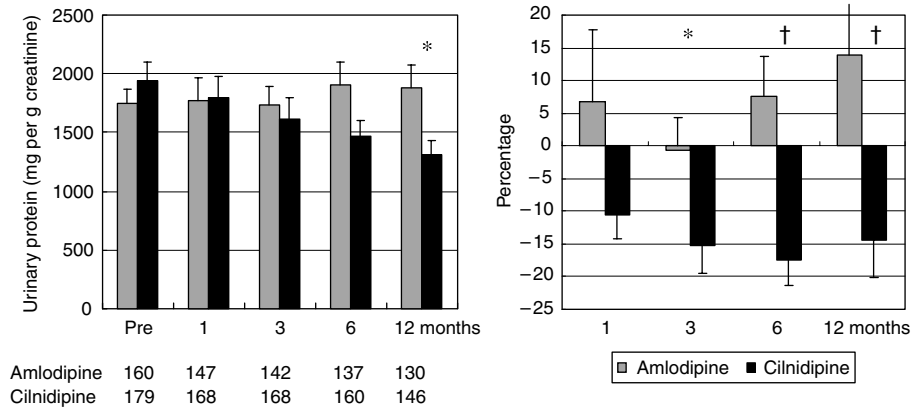

Figure 3 | Changes in urinary protein/Cr ratio during the treatment period. Results are expressed as the mean±s.e.m. The urinary protein/Cr ratio was suppressed in the cilnidipine group but not in the amlodipine group. *$P<0.05$, †$P<0.01$, cilnidipine vs amlodipine groups.

not different between cilnidipine and amlodipine, probably due to the small number of subjects ($n = 60$, totally).

Changes in serum Cr

The serum Cr was slightly increased in both groups, but after 1 year of treatment, the level was similar in the two groups (1.37 ± 0.72 vs $1.45 \pm 0.83\,mg\,dl^{-1}$).

Cardiovascular events and death

The occurrence of cardiovascular disease was two times greater in the amlodipine group but the difference between the two groups was not statistically significant (Table 3). There was no case of cardiovascular death in the cilnidipine group, while two subjects died in the amlodipine group (sudden death and rupture of aortic aneurysm), but the difference was not significant. The all cause mortality rate did not significantly differ.

Adverse reactions

Adverse reactions were observed in 20 subjects of the cilnidipine group and in 19 patients of the amlodipine group (Table 4). About half of the adverse reactions in each group (cilnidipine $n = 10$, amlodipine $n = 10$) were considered to relate to the study drugs. Severe adverse reactions were observed in two subjects treated with cilnidipine (exacerbation of renal failure and metastatic lung cancer) and in four treated with amlodipine (general malaise, pleural effusion, lung cancer, and carcinomatous peritonitis).

Table 3 | Cardiovascular events and death

	Cilnidipine	Amlodipine
Cardiovascular disease	1	3
Angina pectoris	1	0
Myocardial infarction	0	1
Abdominal aortic rupture	0	1
Sudden death	0	1
Stroke	2	4
Cerebral infarction	2	3
Transient ischemic attack	0	1
All cause mortality	2	3
Cardiovascular death	0	2
Non-cardiovascular death	2	1

Table 4 | Adverse reactions

	Cilnidipine	Amlodipine
Hypotension	0	2
Palpitation	3	0
Edema	2	1
Hot flushes	0	1
Headache	0	1
Malaise, fatigue	1	1
Skin reaction	2	1
Abnormal laboratory data	6	0
Worsening of liver function	3	1
Worsening of kidney function	2	1
Others	9	12
Total number of patients[a]	20	19

[a]Five patients with cilnidipine and one patient with amlodipine had two or more adverse reactions.

DISCUSSION

In this study, we firstly demonstrated that cilnidipine was more beneficial than amlodipine as additional medication for hypertensive patients who had kidney disease associated with significant proteinuria (urinary protein/Cr ratio $\geqslant 300\,mg\,g^{-1}$) and who were under treatment with an RAS inhibitor. Our results further confirm those of previous reports showing that cilnidipine had greater antiproteinuric effects than amlodipine,[14,16] and those of a study showing that the combination therapy with cilnidipine and an ARB ameliorated urinary albumin excretion more potently than ARB monotherapy.[18] In this study, moreover, cilnidipine was more beneficial than amlodipine even under treatment of an RAS inhibitor. As a dihydropyridine CCB is frequently prescribed as secondary choice for patients with hypertensive disease treated with an RAS inhibitor, the present results should have an impact on the practical management of hypertension associated with kidney disease; it is suggested that cilnidipine, a dual L-/N-type CCB, rather than an L-type CCB should be recommended to be 'second agents' in hypertensive patients with significant proteinuria under treatment with an RAS inhibitor.

In addition to RAS inhibition, strict BP control is considered to play an important role in preventing the progression of kidney disease.[6–8] In this study, although systolic and diastolic BPs did not differ as a whole, slight lower systolic BP levels were observed in cilnidipine group at 2 month of the treatment. However, this insubstantial difference of systolic BP for the short-time period may not affect urinary protein. Also, in the previous report,[23] 24 h ambulatory BP measurement showed no significant difference in the reduction in any of the BP parameters between cilnidipine and amlodipine, associated with similar decrease in office BP. In this study, required BP goal was not attended as a whole. However, even in subjects whose BP was controlled at $<130/85$ mm Hg, the antiproteinuric effect of cilnidipine was greater compared with that of amlodipine (Figure 4) despite the comparable BP reduction in both groups, suggesting that the beneficial effect of cilnidipine may be beyond its BP lowering effect. Therefore, even if the target BP is achieved, the choice of an appropriate secondary dihydropyridine CCB might be critical for hypertensive patients with kidney disease under treatment with an RAS inhibitor.

The greater antiproteinuric effect attained with cilnidipine compared with amlodipine may be due to the N-type calcium channel blockade achieved with cilnidipine, but not with amlodipine, and the resultant sympathetic nerve inhibition.[14] Supporting this hypothesis, non-hypotensive doses of moxonidine, an agent that reduces sympathetic nerve activity, could ameliorate not only albuminuria but also structural damage in subtotally nephrectomized rats,[24] suggesting a potential role of the increased sympathetic nerve

activity in progression of renal failure. There is a consistent intimate relationship between the sympathetic nervous system and urinary protein excretion in patients with chronic kidney disease.[25] For example, moxonidine reduced urinary albumin in patients with essential hypertension.[26] A number of relatively small clinical studies have suggested that non-dihydropyridine CCBs, which attenuated sympathetic nerve activity, reduce urinary protein more markedly than dihydropyridine CCBs, which do not exert a sympatho-inhibitory effect, although both types of CCBs similarly decrease BP.[27,28] Accordingly, cilnidipine, a dual L-/N-type CCB, has been reported to have a greater renoprotective effect than amlodipine, an L-type CCB,[14,16] which is compatible with the result of this study. These findings are supported by experimental studies indicating that cilnidipine-induced N-type calcium channel blockade inhibits renal sympathetic nerve activity,[14] inducing a reduction of glomerular hypertension through a vasodilation of efferent arterioles,[13] but that an L-type CCB does not effectively reduce glomerular hypertension because of the absence of L-type calcium channels in the efferent arterioles.[22] Taken together, these results strongly support the hypothesis that the antiproteinuric effect of cilnidipine might be account for the inhibition of sympathetic nerve activity through the blockade of N-type calcium channels in chronic kidney disease patients with the increased sympathetic activity.[25,26] On the other hand, an L-type CCB may rather cause sympathoactivation through a direct vasodilator effect.

Several clinical studies demonstrate that proteinuria is a predictor of subsequent progression of kidney disease; for example, in multivariate analysis of data from the AASK study,[29] baseline proteinuria correlated to decline of glomerular filtration ratio independently. Importantly, proteinuria has been recently recognized to be one of cardiovascular risk factors. In the Framingham cohort, proteinuria showed a three-fold increase of the mortality rate and was strongly associated with other risk factors for cardiovascular disease.[30] In a sub-analysis in the Systolic Hypertension in Europe (Syst-Eur) trial,[31] proteinuria was a significant predictor of total mortality and all cardiovascular end points. Experimental and clinical data converge to indicate that, in chronic kidney disease, proteinuria reduction protects against renal and cardiovascular failure.[32] Fosinopril, which decreased urinary albumin excretion, exhibited much greater reduction of cardiovascular events during the 4-year follow-up than calculated reduction using Framingham risk score, but pravastatin, which did not affect urinary albumin, inhibited cardiovascular events comparably to calculated reduction.[33] Thus, antiproteinuric antihypertensive drugs are considered to be more beneficial regarding the cardiovascular as well as renal outcome in patients with kidney disease.

In this study, 24-h urine collection could not be carried out, because it may be too inconvenient and cumbersome for outpatients to give their cooperation to this study. Although 24 h urine collection is a gold standard that is suggested to be a best practice,[34] several investigators demonstrated that urinary protein/Cr ratio in spot urine collection is well correlated with daily urinary protein excretion.[35–38] Actually, systematic review by Price *et al.*[39] suggested that the protein/Cr ratio on a random urine specimen could be useful in clinical practice. Also, the Work Group of the Kidney Disease Outcomes Quality Initiative of the National Kidney Foundation recommended first morning urine collection or random spot urine collection to monitor proteinuria in patients with established kidney disease.[40] Actually, in clinical practice, 24 h urine collection cannot be often accepted by patients with mild to moderate kidney disease. Therefore, urinary protein/Cr ratio from spot urine collection may be a parameter to meet clinical practice, although there are some limitations in spot urine collection.

Sample size and period of treatment are an additional limitation of our study. Although the cardiovascular risks of patients were mild to moderate, the number of patients was relatively small ($n = 339$, totally) and this study was short (1 year). Thus, cardiovascular events were too rare and the changes in serum Cr were too slight to sufficiently evaluate the influence of cilnidipine. Actually, the occurrence of cardiovascular events and death in the cilnidipine group was approximately half that of the amlodipine group, but the difference was not statistically significant. Also, the changes in serum Cr were not significantly different between the two groups. Small sample size, moreover, causes a limitation of subgroup analysis such as diabetic nephropathy and hypertensive glomerulosclerosis (Figure 4). In addition, diagnosis of cardiovascular event depended on judgment of individual investigators, which was also a limitation on evaluation of cardiovascular events in this study.

In conclusion, the addition of cilnidipine rather than amlodipine ameliorated urinary protein excretion in hypertensive patients with kidney disease who were already under treatment with an RAS inhibitor. Therefore, combination therapy with cilnidipine and an RAS inhibitor may be recommended for these patients.

MATERIALS AND METHODS

Participants

This study was a multicenter, open-labeled, randomized trial conducted in 35 hospitals (Appendix) in Japan from June 2002 to July 2006. This study was approved by the Institutional Review Board of The University of Tokyo Clinical Research Center and by the review board of all the other hospitals, and it was undertaken in accordance with the Declaration of Helsinki Principles. Written informed consent was obtained from all subjects. The enrollment criteria of the subjects included (1) urinary protein/Cr ratio $\geqslant 300\,mg\,g^{-1}$ (average of two consecutive measurements during a 4-week period before the treatment), (2) serum Cr $\leqslant 3.0\,mg\,dl^{-1}$, (3) systolic and diastolic BP $\geqslant$ 130/85 mm Hg, and (4) treatment with an ARB or an ACE inhibitor during 2–3 months or more before the administration of cilnidipine or amlodipine. The exclusion criteria were (1) age younger than 20 years or older than 80 years; (2) hypertensive emergency; (3) severe heart failure, severe arrhythmia, angina, and myocardial infarction within 6 months of the start of the trial; (4) stroke within 6 months of the start of the trial; (5)

severe diabetes mellitus, which required hospitalization because of extremely high plasma glucose or complications such as diabetic ketoacidosis; (6) pregnancy; and (7) history of severe side effects of a CCB, an ARB or an ACE inhibitor. Required sample size was estimated as 376 that significant difference could be detected when the difference of both groups was 10% (statistical power: 80%, two-sided level of significance: 5%) considering the previous report.[15]

Interventions

The subjects were randomly allocated to two groups, cilnidipine (5–20 mg daily) or amlodipine (2.5–7.5 mg daily) in combination with an ARB or an ACE inhibitor. Randomization was done according to the assignment in sealed envelops for each subject to indicate the grouping of subjects, which were send to investigators. The monitoring investigator (KA), who did not treat study subjects and did not know the profile of the subjects before the start of trial, monitored randomization in order of the entry of the subjects in each institute. The dose of an ARB or an ACE inhibitor was not changed during the treatment period. The target BP was $<130/85$ mm Hg. BP measurement was carried out according to the Japanese Society of Hypertension Guidelines for Management of Hypertension.[8] If cilnidipine or amlodipine plus an RAS inhibitor failed to reduce BP to the target level, a third drug (other than an RAS inhibitor or a CCB) was added.

Outcome measures

The primary end point was changes in the urinary protein/Cr ratio from the pretreatment period to 1, 3, 6, and 12 months of treatment. Urinary protein was measured by Pyrogallol Red method. At each time point, treatment compliance was checked at the outpatient clinic. The key secondary outcomes were cardiovascular events, which depended on judgment of individual investigators, and death. In addition, urinary electrolytes, serum Cr, blood biochemistry, blood cells counts, electrocardiography, and chest X-ray were examined at 12 months of treatment.

Statistical analysis

Data were analyzed according to randomized treatment assignments of participants regardless of their subsequent medication status (intention-to-treat analysis) and expressed as the mean $\pm$ s.d. The mean values in the two groups were compared by unpaired *t*-test. Analysis of variance with repeated measurements and subsequent multiple comparison test (Dunnett) were applied to test the effect of treatment on BPs, heart rate, and urinary protein/Cr ratio. We compared the occurrence of cardiovascular events and death between the two groups using χ^2 test. Statistical significance was set at $P<0.05$.

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