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L/T-type calcium channel blocker reduces non-Gaussianity of heart rate variability in chronic kidney disease patients under preceding treatment with ARB

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Abstract

Introduction: Increased sympathetic nerve activity has been suggested in patients with chronic kidney disease (CKD). Pathologic sympathetic activity can alter heart rate variability (HRV), and the altered HRV has prognostic importance, so that reducing sympathetic activity may be an important strategy. Novel nonlinear HRVs, including deceleration capacity (DC), have greater predictive power for mortality. We have recently proposed an increase in a non-Gaussianity index of HRV, λ_{25s}, which indicates the probability of volcanic heart rate deviations of departure from each standard deviation level, as a marker of sympathetic cardiac overdrive. L/T-type calcium channel blocker (L/T-CCB), azelnidipine, decreases sympathetic nerve activity in experimental and clinical studies.

Methods: In 43 hypertensive patients with CKD under treatment with an angiotensin receptor blocker (ARB), we investigated whether 8-week add-on L/T-CCB treatment could restore HRV.

Results: Means of all normal-to-normal intervals over 24 h (p<0.0001) and DC (p=0.002) increased, and λ_{25s} (p=0.001) decreased regardless of gender, age, renal function or blood pressure, while no significant changes were observed in the other HRVs.

Conclusions: Reduction of λ_{25s} is useful to assess the effect of sympathoinhibitory treatment. Further studies are needed to investigate if the restoration of HRV is directly associated with the improvement of prognosis in patients with CKD.

Keywords

Angiotensin receptor blocker, calcium channel blocker, chronic kidney disease, deceleration capacity, non-Gaussian heart rate variability

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Introduction

Elevated salt-sensitivity of blood pressure (BP) and inappropriate activation of the renin–angiotensin–aldosterone system and the sympathetic nervous system play important roles in high BP and the high incidence of cardiovascular diseases in patients with chronic kidney disease (CKD). Our group has reported the associations of BP with salt-sensitivity[1] and the intrarenal renin–angiotensin–aldosterone system,[2] and it is well known that the sympathetic nervous system can be over-activated in end-stage renal disease[3] and even in the early stage of CKD.[4] In fact, we previously reported abnormal heart rate variability (HRV) in patients undergoing hemodialysis.[5] The pathologic

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sympathetic activity can alter HRV, and the altered HRV has prognostic importance, so that reducing sympathetic activity may be an important strategy in patients with CKD. The conventional HRV measures from 24-h ambulatory electrocardiogram (ECG) are known to provide the indices of autonomic neural functions, and some of them have also been recognized as mortality predictors by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[6] Novel nonlinear HRVs, including heart rate turbulence (HRT),[7] deceleration capacity (DC),[8] and fractal scaling exponents[9] also have greater predictive power than conventional HRV measures for mortality after myocardial infarction.[8–10] Recently, we proposed that the non-Gaussianity index of HRV (λ_{25s}), which indicates the probabilities of volcanic heart rate (HR) deviations of departure from each standard deviation (SD) level, can serve as a marker of sympathetic cardiac overdrive and as a predictor of mortality and morbidity in cardiovascular diseases.[11,12]

Renin–angiotensin system (RAS) inhibitors (i.e. angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ARBs)) are first-line antihypertensive agents for patients with CKD and can assuage sympathetic activity.[13] Most patients with CKD will require two or more antihypertensive agents to achieve the target BP goal, and diuretics and calcium channel blockers (CCBs) are used as second-line antihypertensives.[14] Treatment with diuretics may activate sympathetic activity due to body fluid deprivation, and treatment with amlodipine, L-type dihydropyridine (DHP) CCB, may cause reflex sympathetic stimulation due to excessive and rapid reduction of BP.[15] Meanwhile L/T-type and L/N-type DHP-CCBs, such as benidipine, azelnidipine, cilnidipine and efonidipine, are potent antihypertensives with sympathoinhibitory effects.[16–21] For example, L/T-type calcium channel blocker (L/T-CCB) azelnidipine decreases sympathetic activity in experimental models[17,18] and in clinical study.[19] In patients with hypertension, azelnidipine reduced muscle sympathetic nerve activity and HR while amlodipine increased them.[15,19] In the present study, we therefore investigated whether add-on administration of L/T-CCB restored HRV, including conventional and novel HRV measures in hypertensive patients with CKD under treatment with ARB.

Material and methods

Subjects

To be eligible for the study, patients had to fulfill the following criteria: (1) diagnosed with CKD based on Kidney Disease Outcomes Quality Initiative (K/DOQI) criteria[22] of a glomerular filtration rate (GFR) <60 ml/min/1.73 m^2, or GFR ⩾60 ml/min/1.73 m^2 with accompanying proteinuria (defined as >300 mg/gCre); (2) treatment with an ARB (olmesartan) for at least 8 weeks prior to enrollment; (3) office BP >130/80 mmHg (or 125/75 mmHg if proteinuria ⩾1 g/day); and (4) no contraindications for treatment with azelnidipine. Patients were excluded if they had (1) received treatment with CCBs in the 8 weeks before enrollment; (2) myocardial infarction, stroke, or a major surgical procedure in the previous 2 months, significant valvular disease or congenital heart disease, atrial fibrillation or flutter, high-grade heart block or permanent pacemaker implantation, chronic obstructive lung disease, severe hepatic disease, malignant neoplasm, or another physical or mental problem that could limit their normal daily activities; or (3) polycystic kidney disease as the original disease underlying CKD, because this condition is often accompanied by sympathetic hyperactivity, regardless of renal function.[23] All subjects were enrolled after providing informed consent to participate in the study. The study protocol was approved by the Ethics Review Committee of Nagoya City University Graduate School of Medical Sciences and the study was conducted in accordance with the Declaration of Helsinki. In total, 45 consecutive patients with CKD (32 men and 13 women; 59±15 years; body mass index (BMI): 23.6±4.6 kg/m^2) were eligible for the study, but two showed frequent ventricular ectopic beats >10% of the total recorded beats in their 24-h ECG (data-exclusion criteria for HRV analysis as mentioned below) at baseline under the preceding treatment with ARB. Accordingly, 43 patients with CKD (30 men and 13 women; 57±15 years old; BMI: 23.2±4.1 kg/m^2) were studied.

Study protocol

At baseline, which was defined as the time when subjects had taken olmesartan (20–40 (23±8) mg/day) for at least 8weeks, ambulatory 24-h ECG was recorded with a portable recorder (RAC-3103, Nihon Koden, Tokyo, Japan) during usual daily activities. After baseline examinations, subjects received single daily doses of azelnidipine (16 mg/day) in the morning. The BP goal was <130/80 mmHg (<125/75 mmHg if daily proteinuria was ⩾1.0g) for patients with BP above these values. During the 8-week study period, changes in the dosage of olmesartan or additional administration of other antihypertensives were not allowed. When office or home systolic BP fell below 100 or 95 mmHg, respectively, or a patient felt postural dizziness, the dose of antihypertensive agent was decreased and the patient was excluded from the study. Ambulatory 24-h ECG was recorded again after the 8-week add-on L/T-CCB treatment to preceding ARB.

HRV analysis

Ambulatory ECG signals were digitized at 125 Hz and 12 bits with an ECG scanner (DSC-3300, Nihon Koden,

Tokyo, Japan) on which QRS complexes were detected and labeled automatically, and all possible errors in labeling were reviewed and edited manually by experienced technicians. Recordings with a total analyzable length <23.5 h were excluded from the study. Data were also excluded when ventricular and supraventricular ectopic beats were >10% of the total recorded beats. Only normal-to-normal (NN) R-R interval data thus obtained were used for HRV analysis.

We calculated the conventional HRV measures recognized as mortality predictors by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology,[6] which included the means of all NN intervals over 24 h (MNN), SD of all NN intervals over 24 h (SDNN), SDs of the averages of NN intervals in all 5-minute segments during 24 h (SDANN), square root of the mean square of differences between adjacent NN intervals (RMSSD), HRV triangular index (HRVTI), the power of high-frequency (HF, 0.15–0.40 Hz), low-frequency (LF, 0.04–0.15 Hz), very-low-frequency (VLF, 0.003–0.04 Hz) and ultra-low-frequency (ULF, ⩽0.003 Hz) components, and the LF-to-HF ratio (LF/HF).[6] We also calculated newly recognized mortality predictors. Scaling exponents were calculated by detrended fluctuation analysis[9] and were defined separately for the short term (4–11 beats) and long term (>11 beats) as α1 and α2, respectively. DC was calculated by Bauer's signal processing technique of phase-rectified signal averaging.[8] In brief, the technique gives separate characterizations of deceleration-related and acceleration-related modulations to distinguish conceptually between vagal and sympathetic factors that affect HRV, and quantifies them as DC and acceleration capacity, respectively.[8] HRT describes sinus rhythm cycle-length perturbations after isolated premature contractions (PVCs), and the physiologic pattern of HRT consists of brief HR acceleration (quantified by turbulence onset (TO)) and subsequent HR deceleration (quantified by the turbulence slope (TS), Appendix 1).[7,10] Normal HRT is defined by negative TO and TS >2.5 ms/beat, and the HRT patterns are classified as normal (category 0), partially abnormal (category 1), and abnormal (category 2). Of note, HRT was measured only when >5 isolated PVCs suitable for analysis were obtained in 24-h ECG. Finally, we calculated non-Gaussianity index of HRV, λ_{25s}. The non-Gaussianity index is used to detect intermittency of HR increment (Appendix 2).[12,24,25] This index has been derived from a method for analyzing multi-scale statistics of complex fluctuations, originally used for characterizing intermittency of hydrodynamic turbulence. The background and a mathematical description have been described elsewhere.[24,25] λ_{25s} indicates probabilities of the volcanic HR deviation of departure from each SD level, and a larger value of λ_{25s} means that the observed distribution of the HRV has fatter tails and a sharper peak compared with the normal Gaussian distribution, which displays no broad base or fat tails (supplementary Figure 1). Our study group[25] has developed an estimation method for the non-Gaussianity index (λ_s) based on the q-th order absolute moment of a time series, and also postulated that the accuracy of estimated λ_s for typical non-Gaussian time series (data points, $n \approx 10^5$) is much higher when using a positive value of q close to zero, as compared with relatively large values of q (>2). Thus, in this study, we calculated the λ_s based on the 0.25th order moment (q=0.25) to emphasize the center part of probability density function and to reduce the effects of large outliers caused by ectopic beats. Recently, we have reported that an increase in non-Gaussianity of HRV at time scale of 25 seconds (λ_{25s}) is associated exclusively with increased cardiac mortality risk independent of clinical risk factors and other HRVs in patients with a history of acute myocardial infarction (AMI).[12] We therefore used λ_{25s} to characterize the non-Gaussian nature of HRV.

The control data for the HRV analysis were obtained from 43 age-, gender-, and BMI-matched persons, who underwent 24-h ambulatory ECG for the evaluation of chest discomfort under no medication of antihypertensive agents, but were proven not to have any cardiac and kidney diseases nor hypertension (n=43). For ethical reasons, ARB and L/T-CCB were not started in these 43 persons.

BP and albuminuria

Office BP and HR were determined as averages of two readings in two visits at baseline and in two visits after 8-week add-on L/T-CCB treatment. BP and HR were measured with the validated automated oscillometric sphygmomanometer (MPV3301, Nihon Koden, Tokyo, Japan) after subjects had been seated for at least 5 min. At each visit, spot urine and blood samples were collected to calculate the ratio of urinary albumin to urinary creatinine (mg/gCre) and the estimated GFR (eGFR) using the Japanese equation for eGFR:[26]

$$eGFR = 194 \times \left[serum\ creatinine\ concentration,\ mg/dL\right]^{-1.094} \times \left[age,\ yrs\right]^{-0.287} \times \left[1\ for\ male;\ 0.739\ for\ female\right].$$

Statistical analysis

Results are expressed as means ± SD, or as median (interquartile range, IQR) according to their distribution. Data distributions were tested using the Kolmogorov–Smirnov test, and variables that were not normally distributed were analyzed after log transformation. Differences in parameters between baseline and ARB plus L/T-CCB combination treatment were examined by Student t-test for paired samples or by Wilcoxon signed-rank test, as appropriate.

Table 1. Clinical characteristic of patients and the changes with 8-week add-on treatment with L/T-CCB, azelnidipine.

	ARB	ARB+L/T-type CCB	*p*-value
Proteinuria mg/gCre	845 (420–2210)	490 (185–1235)	0.0002
(geometric mean)	(950 ± 4)	(480 ± 4)	<0.0001
eGFR, ml/min/1.73 m^2	50 ± 25	49 ± 25	0.09
Systolic BP, mmHg	139 ± 14	121 ± 14	<0.0001
Diastolic BP, mmHg	82 ± 11	71 ± 8	<0.0001
Heart rate, rpm	77 ± 12	73 ± 14	0.1

Values are expressed as the mean ± SD, or median (IQR) (*n*=43).
ARB: angiotensin receptor blocker; CCB: calcium channel blocker.

Correlations among quantitative variables were evaluated by the least-squares method. Relationships between the changes in the variables were analyzed by linear regression through the origin. The effects of baseline characteristics on the changes in HRV measures, significantly altered by add-on L/T-CCB treatment, were examined by repeated measures analysis of variance (ANOVA). The model incorporated baseline demographic variables, including age, sex, and baseline values of eGFR and systolic BP. Forward stepwise multiple regression analysis was conducted to compare the contribution of the change in HRVs, which showed significant correlation with systolic BP at baseline, to the change in systolic BP. Statistical analyses were performed using SPSS Statistics 22 (IBM Corp., NY, USA); $p<0.05$ was considered to be significant.

Results

Baseline

At baseline under ARB treatment, the mean±SD or median (IQR) for proteinuria, eGFR, systolic BP, diastolic BP, and HR were 845 (420–2210) mg/gCre (geometric mean±SD, 950±4 mg/gCre), 50±25 ml/min/1.73 m^2, 139±14 mmHg, 82±11 mmHg, and 77±12 rpm, respectively (Table 1). HRVs at baseline are shown in Table 2. No significant difference was observed between baseline HRVs and their control values. Among HRVs, only DC correlated significantly with eGFR (r=0.36, p=0.02). Baseline systolic BP correlated directly with λ_{25s} (r=0.43, p=0.004) and α2 (r=0.47, p=0.001), and inversely with DC (r=−0.41, p=0.006) and LF/HF (r=−0.35, p=0.02), but did not show significant relationships with other HRVs. Only 10 patients met precondition (>5 isolated PVC) to determine HRT categories (Appendix 1).[7,10] Five of these patients had normal HRT (Category 0); one had partially abnormal (category 1); and four patients had abnormal HRT (category 2).

Effects of add-on azelnidipine

None of the participants experienced their office or home systolic BP of <100 or 95 mmHg, respectively, or felt postural dizziness to discontinue the study treatment. The addition of L/T-CCB to ARB treatment decreased systolic BP, diastolic BP and proteinuria (Table 1). However, eGFR and HR were not changed significantly by treatment with L/T-CCB.

HRVs during add-on of L/T-CCB to ARB treatment are shown in Table 2. MNN and DC increased and λ_{25s} decreased significantly (Figure 1). SDNN, SDANN, HRVTI, total power, ULF, VLF, LF, LF/HF, β and α1 increased; and RMSSD, HF and α2 decreased, but these trends were not significant. MNN during combination therapy with L/T-CCB and ARB was higher than the control value (p=0.03), but no significant difference was observed between other HRVs and the control values. HRT during ARB treatment was analyzable in 10 patients (Appendix 1).[7,10] Therefore, our study cannot provide statistical power to examine the change in HRT. Change in systolic BP correlated positively with the change in λ_{25s} (r=0.41, p=0.006), and inversely with the change in DC (r=−0.41, p=0.006), but did not correlate with the changes in LF/HF (r=−0.23, p=0.1) or α2 (r=−0.07, p=0.7). The change of DC correlated inversely with that of λ_{25s} (r=−0.38, p=0.01, Figure 2). When analyzed by stepwise multiple regression analysis (R^2=0.17, p=0.006), the main determinants of change in systolic BP was the change in λ_{25s} (β=0.67, F=8.5), rather than the change in LF/HF, α2 or DC.

Effects of baseline clinical characteristics

Repeated measures ANOVA revealed no significant effects of gender, age, or baseline values of eGFR or systolic BP on the decrease in λ_{25s}, or on the increase in MNN and DC with add-on L/T-CCB treatment (Table 3).

Discussion

This study is the first study to report the effects of sympatholytic L/T-type CCB, azelnidipine, on conventional and novel HRVs in clinical practice. We observed that add-on administration of L/T-CCB increases MNN and DC, and decreases λ_{25s} in patients with CKD under ARB treatment regardless of their gender, age or baseline eGFR or BP. Clinical studies have reported that beta-blockers were

Table 2. Changes in heart rate variabilities with 8-week add-on administration of azelnidipine in hypertensive patients with chronic kidney disease under treatment with olmesartan.

	Control	ARB	ARB+ L/T- CCB	*p*-value*
Time domain measures				
Mean NN interval (ms)	817 ± 142	835 ± 112	872±125†	<0.0001
SDNN (ms)	136 ± 47	130 ± 38	133 ± 37	0.3
SDANN (ms)	123 ± 44	119 ± 37	124 ± 37	0.2
RMSSD (ms)	25 (19–38)	25 (16–34)	24 (19–31)	0.1
Time domain geometric measures				
HRVTI (ms)	36 ± 13	32 ± 10	34 ± 10	0.2
Frequency domain measures				
Total power	9.20 ± 0.68	9.03 ± 0.69	9.09 ± 0.63	0.5
ULF [ln(ms^2)]	8.94 ± 0.70	8.79 ± 0.71	8.86 ± 0.66	0.5
VLF [ln(ms^2)]	6.96 ± 0.73	6.79 ± 0.74	6.81 ± 0.77	0.8
LF [ln(ms^2)]	5.91 ± 1.00	5.61 ± 1.20	5.65 ± 1.14	0.7
HF [ln(ms^2)]	4.99 ± 1.35	4.79 ± 1.17	4.77 ± 1.06	0.9
LF/HF	2.96 (1.63–4.16)	2.48 (1.53–3.88)	2.58 (1.64–4.05)	0.4
Spectral exponent β	1.36 ± 0.15	1.35 ± 0.13	1.36 ± 0.13	0.6
Nonlinear measures				
Scaling exponent α1	1.19 ± 0.28	1.15 ± 0.28	1.17 ± 0.23	0.5
Scaling exponent α2	1.12 ± 0.05	1.13 ± 0.06	1.13 ± 0.06	0.9
DC (ms)	6.71 ± 2.32	6.17 ± 1.84	6.55 ± 1.85	0.002
λ_{25s}	0.56 ± 0.17	0.56 ± 0.15	0.50 ± 0.12	0.001

Values are expressed as the mean ± SD, or median with interquartile range (n=43). ARB: angiotensin receptor blocker; CCB: calcium channel blocker. Abbreviations for HRV measures are explained in the text. *p-values were for comparison between ARB and ARB+L/T-CCB; †No significant difference was observed between baseline HRVs and their control values; and MNN during combination therapy with L/T-CCB and ARB was higher than the control value (p=0.03), but no significant difference was observed between other HRVs and the control values.

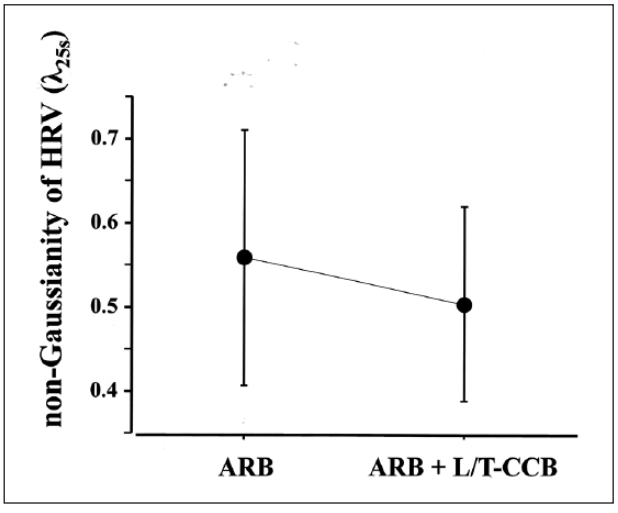

Figure 1. Non-Gaussianity index λ_{25s} before and during the treatment with ARB+ L/T-type CCB.
Add-on treatment with sympatholytic L/T-type CCB, azelnidipine, decreases λ_{25s} in patients with CKD under ARB treatment (values are shown in Table 2, p=0.001).
ARB: angiotensin receptor blocker; CCB: calcium channel blocker; CKD: chronic kidney disease.

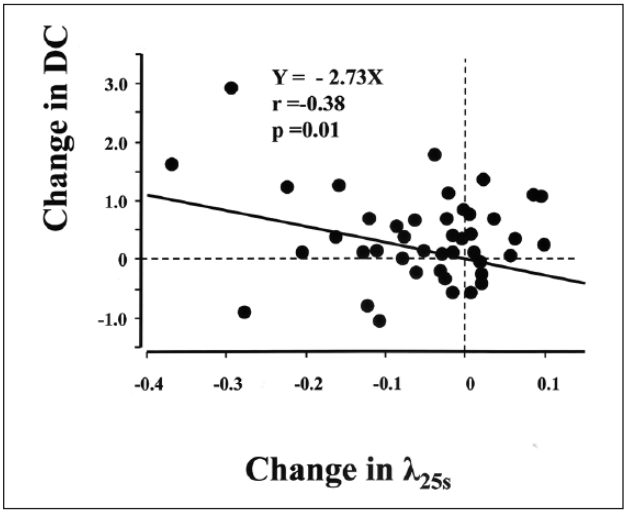

Figure 2. Relationship between the change in non-Gaussianity index λ_{25s} and the change in deceleration capacity (DC).
Inverse correlation was found between the changes in λ_{25s} and DC.

associated with lower λ_{25s}, supporting λ_{25s} as a marker of sympathetic nerve activity.[11,12] In fact, our study found that sympatholytic L/T-type CCB attenuated λ_{25s}. On the other hand, decreases in conventional HRV measures, such as SDNN, HRVTI, ULF, VLF, LF and HF have been thought to reflect cardiac vagal dysfunction.[6] Nonlinear HRV measures, such as DC, scaling exponent α_1, and spectral exponent β are related to complex interplays between vagal and sympathetic functions,[8,9,27,28] and abnormal HRT

Table 3. Repeated measures ANOVA of the effects of baseline clinical characteristics on the changes in MNN, DC, and λ_{25s}.

Factors	Significance of effect (p values)		
	Increase in MNN	Increase in DC	Decrease in λ_{25s}
Gender	0.9	0.9	0.3
Age	0.4	0.8	0.4
Baseline eGFR	0.6	0.8	0.4
Baseline SBP	0.7	0.9	0.3

Data are *p*-values for the effect of factors in repeated measures ANOVA models including gender, age, baseline eGFR, and baseline SBP as explaining factors on the changes in MNN, λ_{25s} or DC during add-on treatment with L/T-CCB, azelnidipine.

reflects the impairment of cardiac vagal reflex through the baroreceptor reflex mechanism.[7,10]

As well as for assessment of cardiac autonomic functions, the analysis of HRV has been developed to predict the risk for mortality and adverse cardiovascular events. A variety of HRV measures have been proposed as predictors of increased risk for sudden cardiac death and all-cause mortality in patients after AMI[7–9,12] and in those with heart failure[11] and end-stage renal disease.[5,28] For example, it was reported that lower values of SDNN, HRVTI, VLF, LF and LF/HF ratio were significant predictors of all-cause mortality in 446 survivors of AMI with a depressed left ventricular function.[9] In contrast to these measures, non-Gaussianity index of λ_{25s} is a unique measure of HRV. An increase, but not a decrease, in λ_{25s} is associated with an adverse prognosis in patients with cardiac diseases.[11,12] Furthermore, the predictive power of λ_{25s} is independent of those of the other HRV measures.[11,12] In the present study, λ_{25s} significantly decreased with L/T-CCB, while most HRV measures (attributable to vagal function) tended to increase but their increase was not significant. This observation is consistent with the fact that azelnidipine assuages sympathetic nerve activity rather than vagal nerve activity.

Use of a short-acting DHP-CCB is associated with increased risk of a cardiovascular event, probably due to reflex activation of sympathetic nerve activity caused by excessive and rapid reduction of BP.[29–31] Even among long-acting DHP-CCBs, there is controversy whether amlodipine can stimulate sympathetic nerve activity and increase HR.[32–34] In contrast, RAS inhibitors including ARBs are known to reduce sympathetic neural activity,[13] which is recognized as an advantage of them over DHP-CCB. Azelnidipine, a highly lipid-soluble L/T-type DHP-CCB, has been reported to reduce sympathetic nerve activity.[15,17–19,35] Direct measurement of sympathetic nerve activity during treatment with azelnidipine has also been studied in experimental model. Shokoji et al.[17] implanted a renal nerve electrode in spontaneously hypertensive rats, and found that the sympathoinhibitory effects of azelnidipine were associated with baroreflex inhibition. Konno and colleagues[18] reported that azelnidipine could reduce oxidative stress in the rostral ventrolateral medulla (RVLM; the vasomotor center determining sympathetic outflow) through the inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and upregulation of Cu/Zn- and Mn-superoxide dismutase (SOD). Treatment with azelnidipine also increased endothelial nitric oxide expression levels in the brain, heart and aorta.[35] In a recent study of hypertensive patients, azelnidipine reduced muscle sympathetic nerve activity while amlodipine increased it.[19] Furthermore, combined treatment with an ARB and azelnidipine has been reported to confer greater benefits for prevention of cardiovascular events in patients with CKD than treatment with high-dose olmesartan alone.[36] We therefore hypothesized that the combined use of azelnidipine further attenuates sympathetic nerve activity even in patients under ARB treatment. This hypothesis is supported by the reduction of λ_{25s} in our study. Prospective studies are desirable to confirm the direct associations between the reduction in λ_{25s} with sympatholytic agents and prognostic improvement in patients with CKD.

As mentioned above, HF, RMSSD and DC have been lumped together as a marker of vagal nerve activity. In the present study, however, the increase in DC with add-on treatment with L/T-CCB was accompanied by no significant changes in HF or RMSSD. Change in systolic BP correlated inversely with the change in DC, but did not correlate with the changes in HF or RMSSD. In a previous cohort study of 281 patients with end-stage renal disease (median follow-up, 87 months), we also observed that a decrease in DC predicts increased risk for all-cause mortality, while HF and RMSSD did not.[28] In addition, we previously observed that DC correlated inversely with λ_{25s},[12] and in the present study the change of DC correlated inversely with that of λ_{25s}. On the basis of these findings, we speculate that DC is not a simple measure of vagal activity but a product of complex interplay between sympathetic and vagal nerve activities.

The limitations of the study include the small number of subjects, and the lack of a placebo-treated group. No significant difference was observed between HRVs during ARB treatment and their control values. RAS inhibitors have been known to reduce sympathetic neural activity,[13] but we could not determine whether preceding treatment with ARB had lowered λ_{25s} before the initiation of azelnidipine.

Although experimental studies have suggested that azelnidipine decreases sympathetic nerve activity via an antioxidant effect in the RVLM,[18] we could not clarify the mechanisms of the reduction in λ_{25s} with azelnidipine.

Conclusion

In conclusion, 8-week add-on administration of L/T-type CCB, azelnidipine, increases MNN and DC, and reduces λ_{25s} during daily activities in hypertensive CKD patients under treatment with ARB. The pathologic sympathetic activity can alter HRV, and the altered HRV has prognostic importance, so that reducing sympathetic activity would be an important strategy in patients with CKD. The reduction of λ_{25s} is useful to assess the effect of sympathoinhibitory agents. Further studies are needed to investigate if the increases in MNN and DC, and the reduction in λ_{25s} with medical treatment, are directly associated with the improvement of prognosis in these patients.

Declaration of conflicting interests

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Supplemental material

The online figures are available at http://jra.sagepub.com/supplemental

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Appendix 1. Heart rate turbulence

HRT describes fluctuations of the sinus rhythm cycle length after PVCs.[7] The early acceleration and late deceleration of sinus rate after PVC are quantified by two parameters: the TO and TS. Turbulence onset was calculated as follows:[7,10]

$$\mathrm{TO} = \left[\left(\mathrm{RR}_1+\mathrm{RR}_2\right) - \left(\mathrm{RR}_{-2}+\mathrm{RR}_{-1}\right)\right] / \left(\mathrm{RR}_{-2}+\mathrm{RR}_{-1}\right) \times 100\ (\%)$$

where RR_{-2} and RR_{-1} are the two R–R intervals immediately preceding PVC coupling interval, and RR_1 and RR_2 are the two R–R intervals immediately following the compensatory pause. TS is defined as the maximum positive regression slope assessed over any five consecutive sinus rhythm R–R intervals within the first 15 sinus rhythm R–R intervals after the PVC. In normal subjects, the initial brief acceleration of sinus rate after the PVC is characterized by a negative TO, and the subsequent rate deceleration is characterized by a positive TS.[7,10] Thereafter, the response patterns are classified as normal (category 0), partially abnormal (category 1), and abnormal (category 2).[7,10] HRT is measurable only when >5 isolated PVCs suitable for analysis are obtained in 24-h electrocardiography.

Appendix 2. Non-Gaussianity index λ

Following four steps show the estimation procedure of the non-Gaussianity index of HRV at time scale "s" (λ_s).[11] In step 1, time series of NN R–R intervals are resampled at equally spaced time intervals with Δt=250ms (4 Hz) using cubic spline interpolation, yielding interpolated time series $\{b(t)\}$. After subtracting average interval b_{ave}, integrated time series $\{B(t)\}$ are obtained by integrating $\{b(t)\}$ over the entire length.

$$B(t) = \sum_{i=1}^{t/\Delta t} \{b(i\Delta t) - b_{ave}\}$$

In step 2, the local trend of $\{B(t)\}$ is eliminated by third-order polynomial that is fit to $\{B(t)\}$ within moving windows of length 2s, where s is the scale of analysis. In step 3, intermittent deviation $\Delta sB(t)$ is measured as the increment with a time lag "s" of the detrended time series $\{B^*(t)\}$. For instance, in a window from T−s to T+s, the increments are calculated as

$$\Delta sB(t) = \left\{B\left(t + s/2\right) - f_{fit}(t + s/2)\right\} - \left\{B\left(t - s/2\right) - f_{fit}(t - s/2)\right\}$$

where $T-s/2 \leqslant t < T+s/2$ and $f_{fit}(t)$ is the polynomial representing the local trend of $\{B(t)\}$, of which the elimination assures the zero-mean probability density function in the next step. In step 4, $\{\Delta_s B\}$ are standardized to have zero mean and unit variance. Then, the non-Gaussianity index λ_s is estimated as

$$\lambda_s = \sqrt{\frac{2}{q(q-2)}\left[\ln\left(\frac{\sqrt{\pi}\langle|\Delta_s B|^q\rangle}{2^{q/2}}\right) - \ln\Gamma\left(\frac{q+1}{2}\right)\right]}$$

where $\langle|\Delta_s B|^q\rangle$ denotes an estimated value of the q-th order absolute moment of $\{\Delta_s B\}$. In our analysis, the parameters in the above equation are set as $s = 25$ s and $q = 0.25$. The estimated value of λ_s can be used to characterize deviation from Gaussianity (normality of the observed distribution). If the λ_s is close to zero, the observed probability distribution of $\{\Delta_s B\}$ is close to a Gaussian distribution. On the other hand, a larger value of λ_s means that the observed distribution has fatter tails and a sharper peak in comparison with the Gaussian distribution (supplementary Figure 1).