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Original Article

Lipo-Prostaglandin E_1 Improves Heart Rate Variability in Patients with Type 2 Diabetes Mellitus

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Abstract: The effect of lipo-prostaglandin E_1 on heart rate variability (HRV) was investigated in 30 hospitalized patients with type 2 diabetes mellitus. After evaluating HRV, patients were divided into Control and Treatment groups. The Control group received the continuation of glycemic control, while the Treatment group received an infusion of 10 μg lipo-prostaglandin E_1/day for seven days in addition to glycemic control. HRV was re-evaluated seven days after the first examination in both groups. The parameters are ; 1) time domain indices consisting of standard deviation of all normal R-R intervals, standard deviation of the means of all 5-min segments of normal R-R intervals, mean of the standard deviation of all normal R-R intervals for all 5-min segments ; 2) frequency domain indices consisting of very low frequency power, low frequency power, high frequency power and total frequency power. There was no significant change in HRV indices between the two examinations in the Control group, whereas there was a significant increase in seven HRV indices between the two examinations in the Treatment group. The present study suggests that lipo-prostaglandin E_1 improves autonomic function in patients with type 2 diabetes mellitus.

Key words: Diabetic autonomic neuropathy, Lipo-prostaglandin E_1, Heart rate variability, Time and spectral analyses, Parasympathetic activity

INTRODUCTION

Among chronic complications of diabetes mellitus, neuropathy is the most prevalent one. It is manifested as a wide variety of clinical symptoms and/or signs ranging from sensori-motor peripheral neuropathy of the extremities to autonomic neuropathy[1)]. Development of autonomic neuropathy in diabetic patients can be particularly life threatening[2,3)]. Several tests have been developed to evaluate autonomic nerve functions[4)]. These tests are designed to measure changes in heart rate induced by standard stimuli, such as deep respiration, postural changes, the Valsalva Maneuver, etc. However, since many of these tests were not reproducible and reliable, spontaneous fluctuations of R-R intervals of electrocardiogram (ECG) over 24-hr[5)] was also analyzed as a means of evaluating autonomic neuropathy. Akselrod *et al.* introduced a spectral analysis of variations of R-R interval of ECG, which allowed to evaluate the interactions between parasympathetic and sympathetic nervous system[6)]. This spectral analysis of heart rate variability (HRV) based on 24-hr ECG turned out to be a useful method[7-14)] for evaluating autonomic neuropathy. A decrease in HRV is considered to reflect autonomic neuropathy with poor prognosis in diabetic

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patients[2,3,8]. However, the treatment of diabetic autonomic neuropathy has not been established. We previously reported that an infusion of prostaglandin $E_1 \cdot \alpha$CD ($PGE_1 \cdot \alpha$CD) for four weeks improves subjective symptoms and vibratory threshold (VT) of patients with diabetic peripheral neuropathy[15]. We also reported that lipo-PGE_1, which is an encapsulated form of PGE_1 in lipid microspheres, ameliorates VT and subjective symptoms in patients with diabetic neuropathy[16]. We speculated that agents like lipo-PGE_1 may also ameliorate diabetic autonomic neuropathy. We therefore designed a clinical study to investigate the effect of lipo-PGE_1 on HRV by time and spectral analyses, which strongly correlate with each other when measured over 24-hr period[17], in patients with type 2 diabetes mellitus.

SUBJECTS AND METHODS

Subjects The present study was performed according to the principles of the Declaration of Helsinki, and full informed consent was obtained from all participants before the study. The Ethical Committee of Yamanashi Medical University approved the study design. All patients were diagnosed as having type 2 diabetes mellitus according to the World Health Organization criteria[18].

Patients with symptoms of sensorimotor peripheral neuropathy were hospitalized for the treatment of neuropathy and recruited to the present clinical study. However, those with neuropathy due to toxic, cancerous or metabolic causes other than diabetes mellitus were excluded from the study. Those who were taking agents, such as vitamins B_1, B_6, B_{12}, E, herbal medicines, other prostanoids, cilostazol, antidepressants, or tranquilizers were also excluded from the study. Finally, 30 patients were recruited for the present study, and numbered in the order of admission for individualization. Those with odd and even numbers were divided into Control and Treatment groups, respectively. However the last two patients were included in the Treatment group to minimize potential effects of background characteristics such as age, sex, known duration of diabetes mellitus and the severity of other microangiopathy between the two groups. Therefore, the Control and the Treatment groups consisted of 14 and 16 patients, respectively.

Study design HRV was examined in all patients after two weeks of hospitalization (1st examination). Patients in the Control group received the continuation of glycemic control, while patients in the Treatment group received an infusion of 10 μg of lipo-PGE_1 (Palux®: Taishou Pharmaceutical Co., Tokyo, Japan) /day for seven days in addition to the continuation of glycemic control, and HRV was re-examined seven days after the initial examination in both groups (2nd examination).

Evaluation of HRV Twenty-four-hr ECG was recorded, and time and spectral analyses were performed using a Model 563K1 Holter Analysis System (Del Mar Avionics, California, USA). Ectopic beats were automatically removed and further edited manually by an expert cardiologist. The total maximal ratio of excluded ectopic beats was 3 %. The analyzed parameters were ; 1) time-domain indices over 24-hr recordings consisting of standard deviation (SD) of all normal R-R intervals (SDNN), SD to mean ratio in % of all normal R-R intervals (CVNN), SD of the means of all 5-min segments of normal R-R intervals (SDANN), mean of the SD of all normal R-R intervals for all 5-min segments (SDNNIDX), the square root of the mean of the sum of the squares of differences between adjacent normal R-R intervals (rMSSD) and %

of difference between adjacent normal R-R intervals that are greater than 50 msec (pNN50); 2) powers of frequency-domain indices consisting of very low frequency (VLF) (0.017–0.05 Hz), low frequency (LF) (0.05–0.15 Hz), high frequency (HF) (0.15–0.5 Hz), total frequency (TF) (0.017–0.5 Hz) and LF/HF ratio. Powers were transformed to natural logarithms (Ln). The frequency domain indices were obtained using fast-Fourier transformation and were computed hour-by-hour from 10-min segments for each hour.

The approximate correspondence of time domain indices and frequency domain indices based on 24-hr ECG recording is reported. Thus, for example SDNN corresponds with TF, SDNNIDX corresponds with the mean of 5-min TF, rMSSD and pNN50 correspond with HF, and SDANN corresponds with ultra low frequency, which is not calculated in this study[17].

Evaluation of diabetic complications Peripheral neuropathy was evaluated by measuring VT at the medial malleolus of the tibia in the 1st and 2nd examinations using an SMV-5 vibrometer[16]. The maximal reliable detectable range of VT by this instrument was 100×10^{-2} gravity (G).

Diabetic retinopathy was diagnosed by expert ophthalmologists at Yamanashi Medical University Hospital and classified as no, simple and proliferative according to Fukuda[19]. Diabetic nephropathy was diagnosed by measuring urinary albumin excretion/day on two different occasions[20]. Those with albuminuria below 30 mg/day, over 30 mg/day but below 300 mg/day and over 300 mg/day were classified as normoalbuminuria, microalbuminuria and clinical albuminuria, respectively. Those who satisfied the conditions described by Yum *et al.* were excluded from the present study[21].

Statistics All continuous variables are expressed as means ± SEM. The two groups were compared using the Mann-Whitney U test. Wilcoxon signed-ranks test was applied to evaluate statistical significance between the 1st and the 2nd examinations in each group. The Kruskal-Wallis test was used to determine the overall significance between the HRV parameters and the severity of retinopathy or nephropathy. The effect of lipo-PGE$_1$ on VT was evaluated by Fisher's exact probability test. A p value below 0.05 was considered statistically significant.

Results

Age, male to female ratio, known duration of diabetes mellitus and the severity of other microangiopathies did not significantly differ between the two groups (Table 1). Moreover, the various HRV indices except rMSSD and Ln HF at the 1st examination did not significantly differ between the two groups (Table 2). There was a significant positive correlation between CVNN and SDNN at the 1st examination ($r = 0.658$, $p = 0.0001$).

There were significant inverse correlations between known duration of diabetes mellitus and SDNN, Ln TF or Ln VLF at the 1st examination (Fig. 1). In addition, Ln TF (Fig. 2) tended to decrease as the severity of retinopathy or nephropathy increased. The values of SDNN, Ln VLF, Ln LF and Ln HF showed similar tendency in relation to the severity of retinopathy or nephropathy (data not shown).

Fig. 3 shows that Ln TF increased in some patients and decreased in others in the Control group between the two examinations, whereas it increased in all patients in the Treatment group. Table 3 shows the effect of lipo-PGE$_1$ on HRV. Although the mean values of some parameters increased at the 2nd examination in the Control group, these parameters did not significantly differ between the two examinations

Table 1. Background Characteristics of the Patients

	Controls (n = 14)	Treatment (n = 16)	p value
Age (yr)	57.4 ± 1.7	56.1 ± 2.5	n.s.
Male/Female ratio	8/6	10/6	n.s.
Known Duration of Diabetes Melitus (yr)	13.6 ± 1.9	12.4 ± 1.7	n.s.
Hb_{A1c} (%) (last 6 months)	9.0 ± 0.3	8.4 ± 0.3	n.s.
Hypertension	3/14 (21 %)	3/16 (19 %)	n.s.
Complication			
Retinopathy			
no	4/14 (29 %)	5/16 (31 %)	n.s.
simple	5/14 (36 %)	6/16 (38 %)	n.s.
proliferative	5/14 (36 %)	5/16 (31 %)	n.s.
Nephropathy			
normoalbuminuria	7/14 (50 %)	5/16 (31 %)	n.s.
microalbuminuria	4/14 (29 %)	9/16 (56 %)	n.s.
clinical albuminuria	3/14 (21 %)	2/16 (13 %)	n.s.
Macroangiopathy (including ASO, IHD, CVA)	1/14 (7 %)	1/16 (6 %)	n.s.

The numerical variables are expressed as the mean ± SEM. Mann-Whitney U test was applied to compare two groups. Abbreviations: yr; years, Hb_{A1c} glycated hemoglobin A_{1c}, ASO; arteriosclerosis obliterans, IHD; ischemic heart disease, CVA; cerebrovascular accidents, n.s.; not significant.

Table 2. Comparison of the Various HRV Indices of the First Examination between the Two Groups

		Controls (n = 14)	Treatment (n = 16)	p value
Variables	Unit			
Time Domain				
CVNN	%	7.5 ±0.4	5.9 ± 0.5	n.s.
SDNN	ms	116 ±10.4	100 ± 10.4	n.s.
SDANN	ms	107 ±10.3	90.6 ± 9.3	n.s.
SDNNIDX	ms	57.2 ±4.7	47.3 ± 5.1	n.s.
rMSSD	ms	44.4 ±2.0	37.9 ± 2.0	0.0258
pNN50	%	16.2 ±1.5	12.7 ± 1.6	n.s.
Frequency Domain				
Ln TF	ms^2	6.7 ±0.1	6.7 ± 0.2	n.s.
Ln VLF	ms^2	5.5 ±0.2	6.0 ± 0.3	n.s.
Ln LF	ms^2	5.4 ±0.1	4.8 ± 0.2	n.s.
Ln HF	ms^2	5.8 ±0.1	4.8 ± 0.2	0.0041
LF/HF		0.7 ±0.1	1.2 ± 0.2	n.s.

The numerical variables are expressed as the mean ± SEM. There were no significant differences in these parameters except rMSSD and Ln HF between the two groups. Abbreviations: n.s.; not significant, ms; millisecond, ms^2; square of millisecond. Mann-Whitney U test was used to compare two groups.

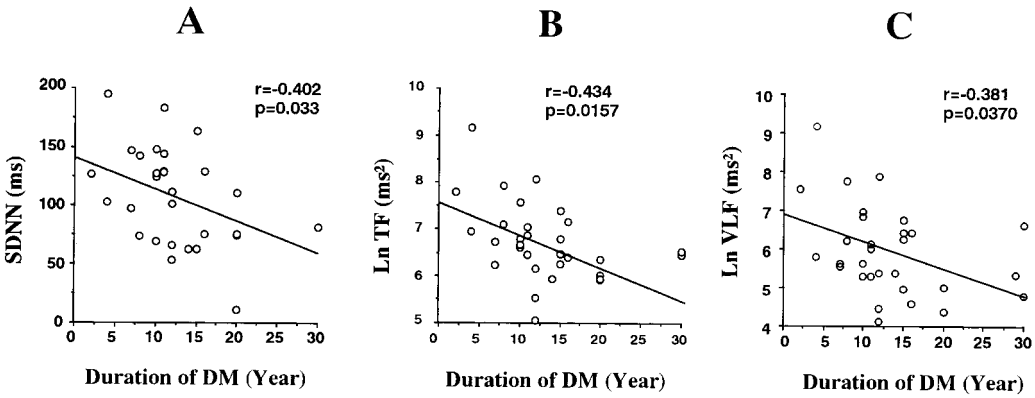

Fig. 1. Correlations between the known duration of diabetes mellitus and SDNN (A), Ln TF (B), or Ln VLF (C).
Abscissas and ordinates indicate known duration of diabetes mellitus in years and SDNN (A), Ln TF (B) or Ln VLF (C), respectively. Data in the 1st examination of all patients were combined. Significant inverse correlations between known duration of diabetes mellitus and SDNN ($r = -0.402$, $p = 0.033$), Ln TF ($r = -0.434$, $p = 0.0157$) or Ln VLF ($r = -0.381$, $p = 0.037$) were observed.

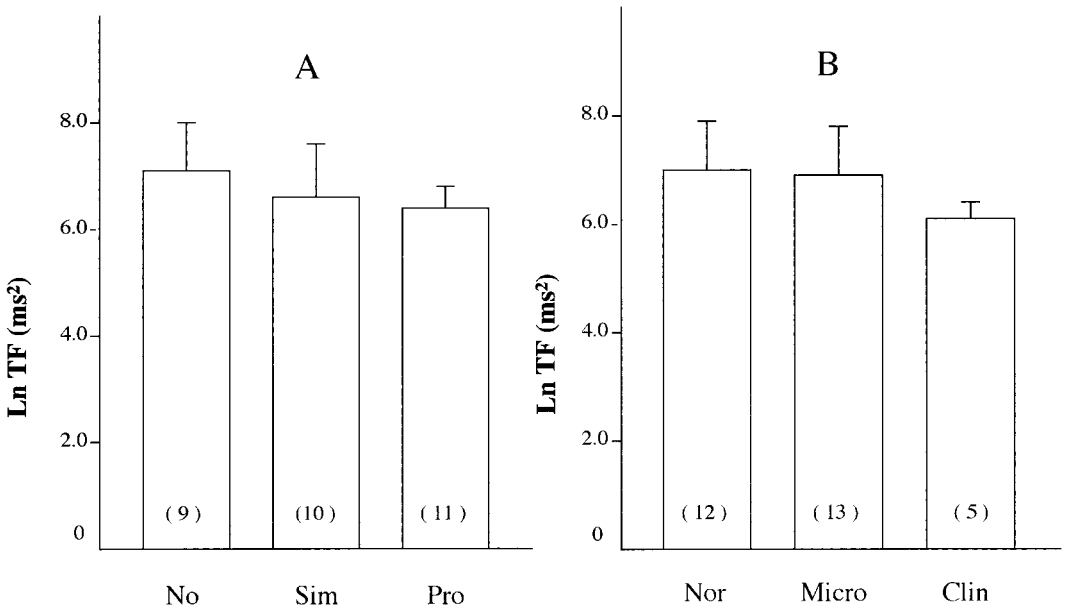

Fig. 2. Ln TF in relation to the severity of retinopathy (A) or nephropathy (B).
Data from the 1st examination of both groups were combined and subgrouped based on the severity of retinopathy (A) and nephropathy (B).
Abbreviations: No, no retinopathy; Sim, simple retinopathy; Pro, proliferative retinopathy; Nor, normoalbuminuria; Micro, microalbuminuria; Clin, clinical albuminuria. Although there were no significant differences, Ln TF tended to decrease as the severity of retinopathy or nephropathy progressed.

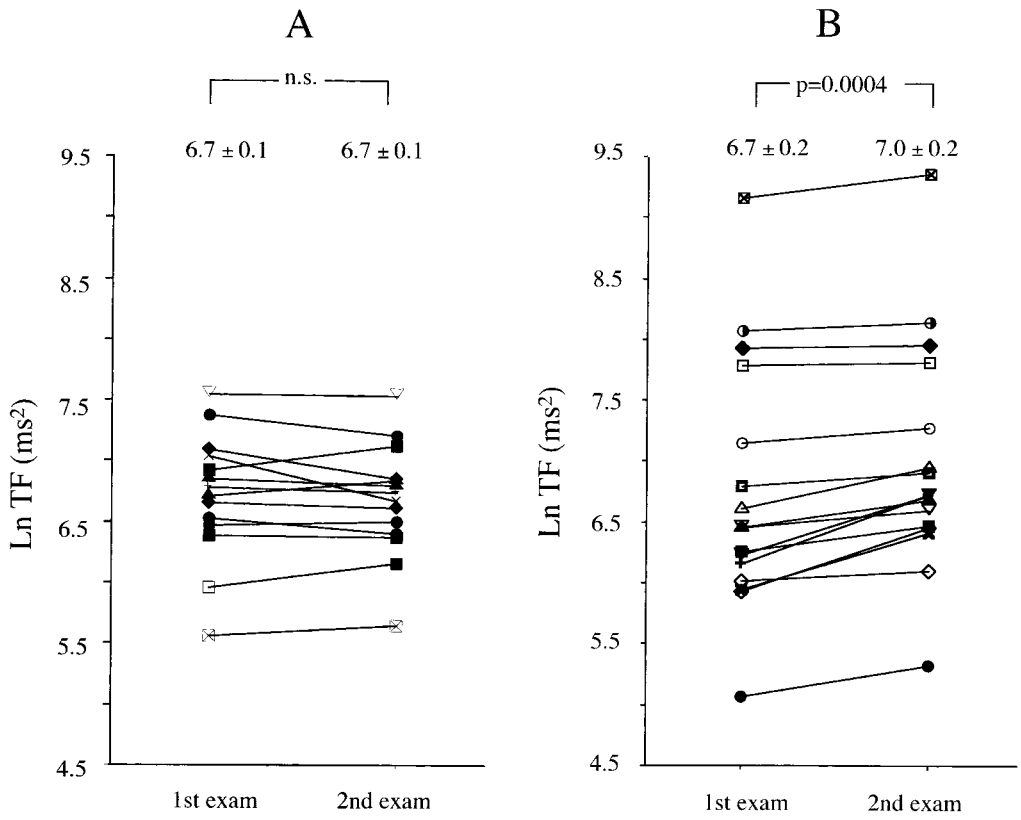

Fig. 3. Changes in Ln TF of each individual between the two examinations.
Each symbol represents Ln TF value of each individual in the Control (A) and in the Treatment (B) groups between the 1st and the 2nd examinations. The values on top indicate means ± SEM, which are identical to the values in Table 3. Ln TF significantly increased ($p = 0.0004$) between the two examinations in the Treatment group, while no significant difference in the Control group.
Abbreviations: exam; examination, n.s.; not significant.

(Table 3). On the other hand, SDNN, SDANN and SDNNIDX and Ln VLF, Ln LF, Ln HF and Ln TF increased significantly during the study period in the Treatment group (Table 3). However, CVNN, rMSSD, pNN50 and LF/HF ratio did not significantly differ between the two examinations (Table 3).

We analyzed the effect of lipo-PGE_1 according to the number of patients whose VT ameliorated more than 50 % of the 1st examination. Lipo-PGE_1 showed a significant effect on VT by Fisher's exact probability test (Table 4).

DISCUSSION

We evaluated the effect of lipo-PGE_1 on VT using Fisher's exact probability test and observed a significant effect as we previously reported[16]. However, VT is a subjective sign of sensorimotor peripheral neuropathy. We therefore investigated the effect of lipo-PGE_1 on HRV, which is an objective and a stable index of autonomic nerve function[22].

Although we did not evaluate HRV in non-diabetic healthy individuals in this study, many of our patients had lower SDNN values than

Table 3. The Effect of Lipo-PGE$_1$ on Time and Frequency Domain Indices of HRV

		Control group (n = 14)			Treatment group (n = 16)		
		1st exam	2nd exam	P value	1st exam	2nd exam	P value
Variables	Units						
Time Domain							
CVNN	%	7.4 ± 0.4	7.3 ± 1.4	n.s.	5.9 ± 0.5	6.4 ± 0.4	n.s.
SDNN	ms	116 ± 10.4	124 ± 10.6	n.s.	100 ± 10.4	111 ± 10.5	0.0019
SDANN	ms	107 ± 10.3	116 ± 10.2	n.s.	90.6 ± 9.3	100 ± 10.1	0.0279
SDNNIDX	ms	57.2 ± 4.7	57.1 ± 3.4	n.s.	47.3 ± 5.1	52.2 ± 4.4	0.0464
rMSSD	ms	44.4 ± 2.0	46.4 ± 2.8	n.s.	37.9 ± 2.0	39.3 ± 1.9	n.s.
pNN50	%	16.2 ± 1.5	16.7 ± 1.4	n.s.	12.7 ± 1.6	12.8 ± 1.6	n.s.
Frequency Domain							
Ln TF	ms^2	6.7 ± 0.1	6.7 ± 0.1	n.s.	6.7 ± 0.2	7.0 ± 0.2	0.0004
Ln VLF	ms^2	5.5 ± 0.2	5.4 ± 0.2	n.s.	6.0 ± 0.3	6.3 ± 0.3	0.0006
Ln LF	ms^2	5.4 ± 0.1	5.3 ± 0.1	n.s.	4.8 ± 0.2	5.0 ± 0.2	0.0494
Ln HF	ms^2	5.8 ± 0.1	5.8 ± 0.1	n.s.	4.8 ± 0.2	5.0 ± 0.2	0.0200
LF/HF		0.7 ± 0.1	0.6 ± 0.1	n.s.	1.2 ± 0.2	1.1 ± 0.2	n.s.

The numerical variables are expressed as the mean ± SEM. Abbreviations: exam; examination, n.s.; not significant, ms; millisecond, ms^2; square of millisecond. Wilcoxon signed-ranks test was applied to compare the 1st and the 2nd examinations in each group.

Table 4. The Effct of Lipo-PGE$_1$ on Vibratory Threshold (VT)

Effect on VT	Controls (n = 14)	Treatment (n = 16)	p value
Decrease	3	12	
No change	11	4	0.0092
Increase	0	0	

VT was measured with SMV-5 vibrometer. Decrease was defined when the VT decreased more then 50 % or became detectable range from more than 100×10^{-2} gravity (G). Increase was defined when the VT increased more then 50 % or became more than 100×10^{-2} gravity (G). Fisher's exact probability test was applied.

those of HRV in healthy individuals reported by Umetani *et al.*[23]. Since known duration of diabetes mellitus inversely correlated with HRV at the 1st examination, which agrees to the previous reports[7,11,12], HRV can be a useful parameter of diabetic complication. Although there were no significant differences in the background characteristics between the two groups, rMSSD and Ln HF showed significant difference between the two groups. We think that this discrepant observations may suggest that these HRV parameters do not necessarily deteriorate evenly during the process of diabetes mellitus.

Some HRV indices increased during the study period in the Control group. We previously reported that VT improved by glycemic control alone[24]. In the present study, those whose VT improved in the Control group (Table 4) coincided with those whose Ln TF values increased (Fig. 3). So, we speculate that the increase in some HRV indices may be attributable, at least in part, to the effect of glycemic control. On the other hand, the increase in HRV indices in the Treatment group indicates that lipo-PGE$_1$ signif-

icantly increased three time domain and all four frequency domain indices. We think that significant differences in rMSSD and Ln HF in the 1st examination between the two groups do not undermine the efficacy of lipo-PGE_1 on HRV, because these parameters were lower in the Treatment group.

CVNN has long been used as a parameter of diabetic autonomic neuropathy[25]. Although we confirmed that CVNN is also a useful parameter of HRV because it correlated with SDNN at the 1st examination , the results in Table 3 suggest that these HRV parameters do not necessarily change synchronously especially during an acute treatment period. However, we speculate that CVNN and other parameters may also show significant change if patients are treated over a longer period.

HF component reportedly reflects parasympathetic activity[6,17]. On the other hand, LF component, the physiological correlate of which is not yet so clear as HF component, is considered to reflect sympathetic activity modified by parasympathetic activity[6,17]. The results of the present study thus suggest that lipo-PGE_1 improves parasympathetic activity in diabetic patients . We also think that lipo-PGE_1 improves the balance between sympathetic and parasympathetic activities, probably more strongly with regard to the latter, because it decreased LF/HF ratio.

On the other hand, lipo-PGE_1 also significantly affected VLF, physiological correlates of which remain unknown. Speculations regarding the physiological correlates with VLF include fluctuations in activity of the renin-angiotensin system[6], body temperature regulation[26] or peripheral vasomotor activity[26]. Recent reports suggest that VLF is a better predictor of prognosis than LF or HF in patients with acute myocardial infarction[27]. We therefore believe that VLF is also an independent and important index of HRV and speculate that it may be more important component than LF or HF in HRV. From this standpoint, we speculate that the effect of lipo-PGE_1 on HRV is also favorable to an unknown physiological correlate that might determine long term prognosis in patients with diabetic autonomic neuropathy.

Recently, Kontopoulos *et al.*[28] reported the efficacy of quinapril upon diabetic autonomic neuropathy by evaluating HRV. Although the drug and the treatment periods are different, we think that the results of the present study are similar to those of Kontopoulos *et al.*[28] with regard to time and frequency domain indices of HRV.

Since lipo-PGE_1 affected HRV parameters within a week, we speculate that the major effect of lipo-PGE_1 on HRV may be due to the improvement in circulation. However, we observed that cyclic AMP increases nitric oxide production and Na^+, K^+-ATPase activity in human neuroblastoma SH-SY5Y cells[29]. So, we think that it is possible that the effectiveness of lipo-PGE_1 in diabetic autonomic neuropathy is mediated to some extent by direct action on peripheral nerve. This remains to be clarified in the future.

In conclusion, the present open study indicate that lipo-PGE_1 improves HRV in diabetic patients, which suggests an improvement in autonomic neuropathy. A large scale and longer period of double blind study of lipo-PGE_1 infusion on HRV in diabetic patients is warranted.

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