International Journal of
Diabetes & Clinical Diagnosis

Research Article Open Access

Effects of Epalrestat as Aldose Reductase Inhibitor (ARI), Sitagliptin as Incretin-Based Therapy (IBT), or Combined Epalrestat and Sitagliptin on Cardiac Vagal Neuropathy (CVN) in Patients with Type 2 Diabetic Mellitus

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Abstract

Background: Aldose reductase inhibitor (ARI) partially ameliorates cardiac vagal neuropathy (CVN) in patients with diabetes mellitus. Incretin-based therapy (IBT) has neuroprotective properties for neuropathy in mice and rat.

Materials and Methods: Effects of epalrestat as ARI, sitaglipitin as IBT, or combined ARI and IBT on CVN were examined in 42 patients with CVN and type 2 diabetes mellitus. Subjects were divided into 3 groups; group A (n =12) was treated with add-on oral epalrestat; group B (n =18) was treated with add-on oral sitaglipitin; and group C (n = 12) with CVN despite treatment with epalrestat (n=6) for 1 year in group A , sitagliptin (n=5) for 1 year in group B and subcutaneously injected exenatide (n = 1) as IBT for 1 year was treated with add-on combined epalrestat and sitaglipitin, although there were no placebo in all patients with CVN. CVN was defined as maximal coefficient of variance in electrocardiographic beat-to-beat interval (max CV. R-R) of three measurements during deep breathing at rest on ≤2.00%. Since disease duration was ≥20 years in each group, patient had various chronic complications and various treatments.

Results: Mean duration of treatment was 1 year. Max CV.R-R after treatment was significantly (P<0.001) increased after treatment in each group. Nevertheless, 8 (67%) and 5 patients (28%) still had CVN after treatment in groups A and B, respectively, whereas no patients had CVN after treatment in group C. There was significant difference (P <0.002) in magnitude increase of max CV. R-R after treatment between groups using ANOVA with multiple comparison test. No significant differences were observed in other variables before and after treatment between groups.

Conclusion: Sitagliptin may be more effective than epalrestat for CVN in type 2 diabetic patients, and combined epalrestat and sitagliptin may be haven a synergistic effect.

Publication History:

Received: September 26, 2014
Accepted: November 11, 2014
Published: November 13, 2014

Keywords:

Diabetes mellitus (DM), Aldose reductase Inhibitor (ARI), Incretin-based therapy (IBT), Cardiac autonomic neuropathy (CAN), Peripheral neuropathies (PN)

Introduction

Most common peripheral neuropathies (PN) in patients with diabetes mellitus (DM) are chronic sensorimotor distal symmetric polyneuropathy (DPN) and autonomic neuropathy (AN) [1-6]. The autonomic nervous system modulates electrical and contractile activity of the myocardium via interplay of sympathetic and parasympathetic activities.

The cardiac autonomic neuropathy (CAN) of patients with DM is associated with an increased risk of mortality [1-6]. Therefore, it is most studied and is a clinically important form of diabetic AN [1, 5-6]. The American Diabetes Association (ADA) highlighted its significance and addressed it in its guidelines [1, 5]. A recent review suggests that R-R interval as Cardiac Vagal Neuropathy (CVN) may be useful for CAN [6]. It is known that pharmacological interventions included anti-hyperglycemic drugs, anti-oxidants, or anti-hypertensive drugs, as well as aldose reductase inhibitor (ARI) drugs that reduce the activity of polyol pathway of nerves [7-10] have improved actions for CAN.

Recently, Incretin-Based Therapy (IBT) [DPP-4 inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists] has been used as a therapeutic tool for type 2 diabetes (T2DM) [11]. DPP-4 inhibitors increase concentrations and survival of active glucose-dependent insulinotropic polypeptide (GIP) and GLP-1. In addition to this anti-hyperglycemic effect, IBT has neurotropic actions and neuroprotective properties for PN in neurons and neural cells in mice and rat [12-20].

Therefore, the effects of treatment with add-on oral epalrestat as ARI, oral sitagliptin as IBT, or combined epalrestat and sitagliptin on CVN were examined in type 2 diabetic patients with CVN.

Materials and Methods

Subjects

For a randomized trial, 500 patients with DM who regularly visited our clinics on April 1, 2012 were eligible. The analyzed patients with CVN had T2DM. The patients with CVN received add-on drugs (ARI or IBT), while the patients without CVN did not receive drugs (Figure 1). As this study is a randomized trial, all patients with CVN should have treated with placebo drugs [21]. However, some patients had

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Citation: Kamoi K, Sasaki H (2014) Effects of Epalrestat as Aldose Reductase Inhibitor (ARI), Sitagliptin as Incretin-Based Therapy (IBT), or Combined Epalrestat and Sitagliptin on Cardiac Vagal Neuropathy (CVN) in Patients with Type 2 Diabetic Mellitus. Int J Diabetes Clin Diagn 1: 107. doi: http://dx.doi.org/10.15344/2394-1499/2014/107

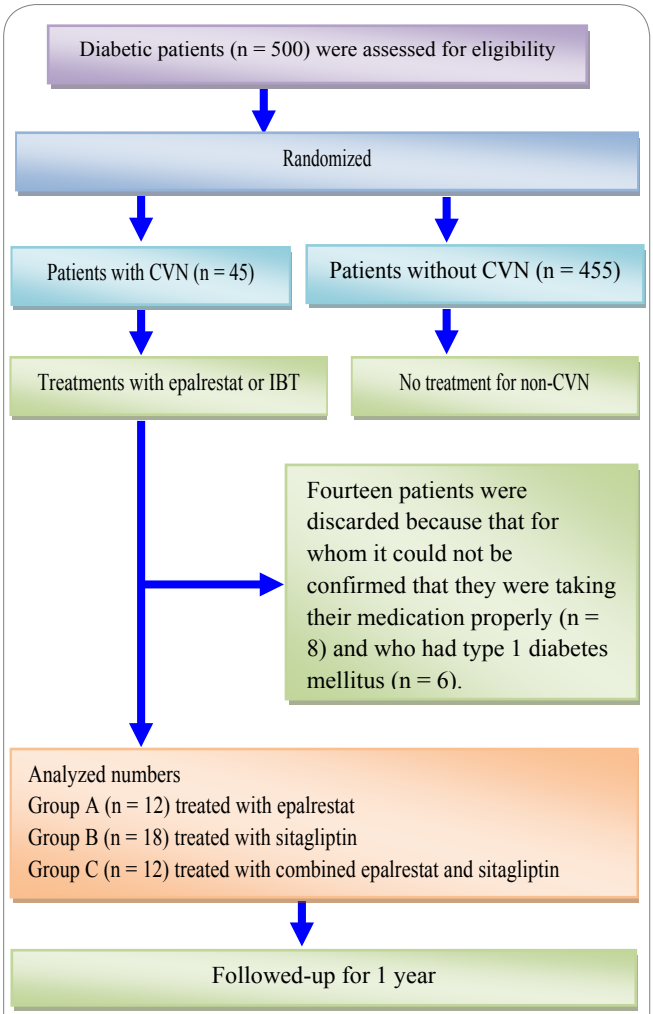

Figure 1: Epalrestat as aldose reductase inhibitor (ARI); CVN, cardiac vagal neuropathy as cardiac autonomic neuropathy (CAN); Sitagliptin, DPP-4 inhibitor as incretin-based therapy (IBT). The "n" shows the number of analyzed patients with CVN and type 2 diabetes mellitus in this study Group A (n= 12) with CVN before treatment was treated with add-on 150 mg/day of oral epalrestat alone as ARI, group B (n= 18) with CVN before treatment was treated with add-on 50 mg/day of oral sitagliptin alone as IBT, and group C (n= 12) with CVN despite treatment with add-on oral epalrestat alone (n = 6) as placebo in group A, oral sitagliptin alone (n= 5) in group B and GLP-1 receptor agonist (n = 1) as IBT for 1 year was treated with add-on combined oral epalrestat and oral sitagliptin. No diabetic patients with CVN have serviced.

received the placebo, but none of all patients with CVN had served as placebo, because that we were afraid of a sudden death owing to CVN [1-6].

DM was diagnosed according to the World Health Organization criteria [22] and guidelines of the Japan Diabetes Society (JDS) [23], which was classified as type 1 diabetes mellitus (T1DM) or T2DM.

The demographic characteristics of participants are presented in Table 1. Average of disease duration from initial discovery in each group was approximately more than 20 years. Therefore, some patients had microvascular disturbances, as well as a history of macrovascular disturbances that included asymptomatic coronary heart disease (CHD), asymptomatic cerebral vascular disease (CVD), and asymptomatic peripheral artery obstruction (PAO).

As microangiopathy, the presence of retinopathy was examined by an ophthalmologist [24], the presence of nephropathy was ascertained based on albuminuria [25-28] or renal anemia, and the presence of neuropathy was determined by the neuropathy assessment described below. Macroangiopathy was defined according to past medical history. Although none of the patients had any symptoms or complaints, they were still checked for any symptoms or complaints related to PN. Furthermore, none of the participants had a history of gastrointestinal disease or impaired liver function.

Study design

Participants of this study (n = 42) were determined based on patients; patients for whom it could not be confirmed that they were taking their medication properly (n = 8) and those with T1DM (n = 6) were excluded. Patients without CVN (n = 455) had received no treatment and no all patients with CVN had served as placebo (Figure 1), although originally, all patients with CVN should have received placebo as part of a randomized study.

The primary outcome was CVN. To determine the effects of drugs on CVN in patients, 42 patients with CVN analyzed were studied. They were divided into three groups, as well as before treatment (group 1) and after treatment (group 2). Group A (n = 12) was treated with add-on ARI, oral epalrestat, 150 mg three times daily for CVN (Table 1). Group B (n =18) was treated with add-on IBT, a DPP-4 inhibitor, oral sitagliptin, 50 mg once daily for DM (Table 1). Finally, group C (n=12) with CVN despite treatment with ARI, oral epalrestat 150 mg three times daily (n=6) for 1 year in group A , and treatment with IBT, oral sitagliptin 50 mg once daily, DPP-4 inhibitor (n=5) for 1 year in group B and subcutaneously injected exenatide 10 ug/time twice daily, GLP-1 receptor agonist (n = 1) for DM with duration 1 year was treated with add-on combined ARI (oral epalrestat 150 mg daily) for CVN and IBT (oral sitagliptin 50 mg daily) for DM (Table 1).

The secondary outcomes were duration (years) of treatment, type of diabetes mellitus, gender, serum creatinine (mg/dL) (Cr), serum total cholesterol (TC) (mg/dL), serum high density lipoprotein cholesterol (HDL) (mg/dL), and serum low density lipoprotein cholesterol (LDL) (mg/dL), HbA1c (%), body mass index (BMI) (kg/m^2), urinary albumin excretion rate (UAER) (mg/gCr), intake of alcohol (g/day), amount of smoking (cigarettes/day), clinic blood pressures (mmHg), duration (years) of DM from discovery, each chronic complications, and kinds of treatments for various diseases, which were known as risk factors for CAN [7-10]. As age and systolic clinic hypertension are also known risk factors for CAN in such patients [29-30], they added in the secondary outcomes. Comparisons were made among groups A, B, and C, and between groups 1 and 2.

The mean duration of treatment with add-on ARI, IBT, or combined ARI and IBT for CVN or DM was about 1 year. All chemical laboratory data, Achilles tendon reflex, and coefficient of variance of 100 consecutively maximal electrocardiographic beat-to-beat intervals (max CV. R-R, %) were obtained in groups 1 and 2 without fasting. Blood pressure was measured in the clinic by the same methods, including the device, device validation, observer, number of measurements, conditions, posture, and cuffs, as described previously [31-32].

Variable	Group A			Group B			Group C		
	Before (Group 1)	After (Group 2)	P value	Before (Group 1)	After (Group 2)	P value	Before (Group 1)	After (Group 2)	P value
Number	12	12	1.000	18	18	1.000	12	12	1.000
Duration of treatment	0	1.0±0.3	0.001	0	1.0±0.3	0.001	0	1.0±0.3	0.001
Type of DM (T1/T2)	0/12	0/12	1.000	0/18	0/18	1.000	0/12	0/12	1.000
Age (years)	72±7	73±7	0.001	71±10	72±10	0.001	72±8	73±8	0.001
Gender (male/female)	5/7	5/7	1.000	8/10	8/10	1.000	3/9	3/9	1.000
BMI (kg/m^2)	24±4	24±4	1.000	22±3	22±3	1.000	24±4	24±5	1.000
HbA1c (%) (NGSP)	7.5±1.4	7.6±1.3	0.690	7.0±0.8	6.9±0.9	0.977	6.9±1.3	6.9±1.8	0.968
Lipid in serum(mg/dl)									
TC	193 ±37	183 ±25	0.324	174 ± 29	177±27	0.668	179±19	173±29	0.414
HDL	54 ±18	53±15	0.653	60±14	57±15	0.426	47±10	49±10	0.121
LDL	112±34	110±27	0.777	94±28	102±20	0.241	109±19	114±24	0.467
TG	151±105	134±90	0.501	111±39	100±22	0.645	124±56	122±49	0.904
Blood pressure(BP)(mmHg)									
Systolic clinic BP	123±15	128±17	0.437	128±16	120±14	0.021	122±15	130±15	0.168
Diastolic clinic BP	63±8	64±14	0.795	70±10	64±12	0.791	66±10	66±9	0.643
Creatinine (Cr) in serum (mg/dl)	1.0±0.4	1.0±0.4	1.000	0.8±0.3	0.9±0.3	0.129	0.8±0.3	0.8±0.3	0.291
Hb in blood (g/dl)	12.5±1.3	13.4±2.1	0.238	13.1±1.2	12.7±7	0.090	12.7±1.4	13.0±1.5	0.392
Urinary albumin excretion rate (mg/g Cr)	277±478	308±448	0.736	165±539	131±310	0.562	128±169	175±258	0.404
Cigarette smoking per day (cig/day)	4.0±12.0	0	0.175	3.0±8	0.6±2.4	0.298	0	0	1.000
Alcohol intake per day (g/day)	7.0±16	3.0±7.0	0.179	3.0±12	0.5±2.1	0.331	0	0	1.000
Duration of diabetes from discovery									
(Year)	25±12			23±9			28±10		
Microangiopathy	12(100%)	10(83%)	0.001	18(100%)	6(33%)	0.001	12(100)	6(50%)	0.021
Neuropathy	12(100%)	9(75%)	0.001	18(100)	5(28%)	0.01	12(100)	0**	0.001
Duration	NC	NC	NC	NC	NC	NC	NC	NC	NC
CVN: Max CV. R-R (%)	1.38±0.25	1.98±1.07	0.020	1.70±0.33	2.68±1.01#	0.002	1.65±0.34	3.64±0.62***	0.0001
≤ 2.0 % in max CV.R-R (%)	12(100%)	8 (67%)	0.001	18(100%)	5 (28%)	0.001	12(100%)	0**	0.001
Heart rate (minute)	82±2	82±2	1.000	82±1	82±1	1.000	82±2	82±2	1.000
DPN	5(47%)	8(67%)	0.688	8(44%)	5(28%)**	0.396	6 (50%)	0**	0.001
Retinopathy	8 (67%)	8(67%)	1.000	5 (28%)	4 (22%)	1.000	5 (42%)	4 (33%)	1.000
NDR/SDR/PPDR/PDR	3/3/0/6	3/3/0/6	1.000	13/2/1/2	14/1/1/2	1.000	7/2/0/3	8/1/0/3	1.000
Nephropathy	10(53%)	11(59%)	0.139	4 (22)	6 (33%)	0.617	6(50%)	5 (42%)	1.000
Normo/Micro/Macro	6/3/3	5/2/5	0.099	14/2/2	12/4/2	1.000	6/3/3	7/3/2	0.302
Renal anemia	0	1 (8%)	1.000	1(6%)	0	1.000	0	1 (8%)	1.000
Macroangiopathy (asymptomatic)	5(42%)	5(42%)	1.000	0	2 (11%)	1.000	5(42%)	5(42%)	1.000
Diabetes treatment	12(100%)	12(100%)	1.000	18(100%)	18(100%)	1.000	6(50%)	10(83%)	1.000
α-GI	0	0	1.000	1(6%)	0	1.000	1(8%)	0	1.000
BG	7(58%)	8(67%)	1.000	10 (56%)	13 (72%)	1.000	8 (67%)	6(50%)	1.000
SU	0	0	1.000	8 (44%)	2 (11%)	0.001	2 (16%)	2 (15%)	1.000
TZD	0	1(5%)	1.000	2 (11%)	2 (11%)	1.000	1(8%)	1(8%)	1.000
DPP-4 inhibitor	0	0	1.000	0	18(100%)*	0.001	5(42%)*	12(100%)**	0.001
GLP-1 receptor agonist	0	0	1.000	0	0	1.000	1(8%)	0	1.000
Insulin	8(67%)	9(75%)	1.000	6 (33%)	2(11%)	1.000	7(58%)	6(50%) ##	1.000
Hypertensive treatment	8(67%)	8(67%)	1.000	14(78%)	14(78%)	1.000	8(67%)	8 (67%)	1.000

Table 1: Continued...

Lowering lipid treatment	3 (25%)	5(42%)	0.344	2 (11%)	2 (11%)	1.000	0	4 (33%)	1.000
Lowering uric acid treatment	2 (17%)	2(17%)	1.000	0	0	1.000	0	1 (8%)	1.000
Anti-anemic treatment	3 (25%)	2(17%)	1.000	4 (22%)	4 (22%)	1.000	0	0	1.000
Cardiac protectant treatment	1 (8%)	0	1.000	1 (6%)	1 (6%)	1.000	0	1 (8%)	1.000
Anti-coagulation treatment	3(25%)	3(25%)	1.000	1 (6%)	1 (6%)	1.000	0	2 (16%)	1.000
Anti-neuropathy treatment	0	19(100%)	0.001	0	0**	1.000	6(50%)*, ##	12 (100%) *,##	0.001
Others treatment	1 (8%)	1 (8%)	1.000	0	0	1.000	2(16%)	1 (8%)	1.000

Table 1: Data are expressed as means ± SD. Each value was collected in the clinic without fasting. DM, diabetes mellitus; T1, type 1; T2, type 2; BMI, body mass index; HbA1c, glycohemoglobin A1c; NGSP, National Glycohemoglobin Standardization Program: TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; Hb, hemoglobin; NC, not clear; DPN, chronic sensorimotor distal symmetric polyneuropathy; NDR, non-diabetic retinopathy; SDR, simple diabetic retinopathy; PPDR, pre-proliferative retinopathy; PDR, proliferative retinopathy; Normo, normoalbuminuria; Micro, microalbuminuria; Macro, macroalbuminuria; α-GI, alpha-glucosidase inhibitor; BG, biguanide; SU, sulfonylurea; TZD, thiazolidinedione; ARI, aldose reductase inhibitor such as epalrestat; DPP-4 inhibitor as sitagliptin, incretin-based therapy (IBT); CVN, cardiac vagal neuropathy as cardiac autonomic neuropathy (CAN).
Number in parenthesis represents the percent ratio of each variable to patients participated in each group. CVN was defined as less than 2.00 % in 100 consecutive maximal CV. R-R intervals (max CV. R-R) from usual breathing to deep breathing at rest for measurement on electrocardiography at all times for three measurements if max CV. R-R was less than 2.00 %.
Difference in the means of each variable between groups 1 and 2 was statistically evaluated by dependent chi square with McNemar's test analysis or paired Student t test with or without Welch's correction (P value). Difference in the mean of each variable between groups A, B and C was statistically evaluated by independent chi square analysis or unpaired Student t test with or without Welch's correction (*P<0.05 and **P<0.01 vs. each variable in group A, and #P<0.05 and ##P<0.01 vs. each variable in group B). Also, the difference in magnitude of max CV. R-R among groups A, B and C was statistically evaluated by one ANOVA with multiple comparison test (***P<0.05 and #P < 0.001 vs. in group A, respectively).

At baseline, patients in groups A and B had been treated with various therapies except ARI or IBT, whereas patients in group C had been treated with various therapies with add-on ARI or IBT (Table 1). All patients received diet and exercise therapy for hyperglycemia. Furthermore, patients who did not require insulin for hyperglycemia received oral pharmacotherapy. The oral pharmacotherapy consisted of α-glycosidase inhibitor (α-GI), sulfonylurea (SU), biguanide (BG), thiazolidinedione (TZD), or combinations of these agents (Table 1). Patients who required insulin for hyperglycemia had received insulin analogues using multiple daily injections (MDI) or twice-daily injections, or combinations of these agents. Insulin therapy consisted of subcutaneous injections of long-acting insulin analogues prior to sleep and bolus subcutaneous injections of rapid-acting insulin analogues in MDI, or subcutaneous injections of mixed insulin analogues twice a day. Additionally, some patients were treated with anti-hypertensive drugs, anti-dyslipidemic drugs, or other drugs for various other diseases.

Neuropathy assessment

PN as neuropathy was defined by subjective symptoms, signs, and the following laboratory findings. Subjective symptoms of PN, which were assessed by interview at the time of medical examination both before and after treatments, comprised burning pain, electrical or stabbing sensations, paresthesiae, hyperesthesia, or deep aching pain, or dizziness when standing and shortness of breath with activity [1-6]. The Achilles tendon reflex was also tested both before and after treatment confirmed DPN.

CVN owing to a disturbance of vagal nerves (parasympathetic dysfunction) was ascertained by standardized cardiovascular methods as max CV. R-R [1-6, 10] using electrocardiography (FCP-7431 or FCP-7541, Fukuda Denshi, Co. Tokyo, Japan) in popular in Japan in a room with a stable temperature and with the participant lying in a resting supine position for 10 min. The change of max CV.R-R from usual breathing to deep breathing at rest was used to evaluate CVN [6, 10]. The lower limit of normal range was >2.00% [7-10]. Although measurement of max CV.R-R is generally accepted as a useful test of cardiac parasympathetic function [7-10], measurement of max CV. R-R had a poor reproducibility for unknown reasons [1-6]. Therefore, the max CV. R-R ≤2.00% at all times over three measurements in patients with a value ≤2.00% at the first measurement was defined as CVN based on the guidelines [1-6, 9].

Other assay methods

Participants were examined using the same assay methods reported previously [24-28, 31-32]. All chemical laboratory data were obtained without fasting. Only one specimen was used to assess urinary albumin status.

Albumin concentration in random spot urine was measured by latex agglutination photometric immunoassay method. Microalbuminuria and macroalbuminuria were defined as UAER ≥30 mg/gCr and ≥300 mg/gCr, respectively [24-28].

Serum levels of Cr and triglyceride (TG) (mg/dL) were measured by enzyme color methods using commercial kits. Serum levels of TC, HDL, and LDL were measured by direct methods using commercial kits [24-28]. The normal ranges without fasting in serum were Cr ≤1.0 mg/dL, TC 120-200 mg/dL, HDL 40-90 mg/dL, LDL 80-120 mg/dL and TG 60-300 mg/dL.

HbA1c (JDS) as an indication of glycemic control was measured by high-performance liquid chromatography [24-28], and the value was expressed as the National Glycohemoglobin Standardization Program (NGSP) equivalent, as per the guidelines of the Japan Diabetes Society [23]. Blood HbA1c (NGSP) less than 6.5% was considered within the normal range.

Alcohol and cigarette smoking intake assessment

Daily alcohol and smoking intakes were assessed by interview at the

time of medical examination in groups 1 and 2.

Blood pressure assessment

Clinic blood pressure was measured by the same method reported previously [31-32]. Briefly, clinic blood pressure was measured once initially by the patients themselves at the clinic during the day; blood pressure of the left arm was measured after a 5-min rest in a sitting position using an automatic device based on cuff-oscillometric method that generates a digital display of both systolic and diastolic blood pressures. All devices met the criteria set by the Association for the Advancement of Medical Instrumentation. A standard arm cuff was used to obtain clinical blood pressure. Hypertension was defined as an elevated blood pressure (systolic clinic blood pressure >140 mmHg, diastolic clinic blood pressure >90 mmHg) [33].

Statistical methods

Results are expressed as means ± SD. Differences in means of each variable between groups 1 and 2 were evaluated statistically by the dependent chi square with McNemar's test or the paired Student's t-test with or without Welch's correction, and the differences in means of each variable between groups were also evaluated statistically by independent chi square or unpaired Student's t-test with or without Welch's correction. To show the difference in magnitude of increase in the max CV.R-R among three groups of treatment, one way ANOVA with multiple comparison tests was performed. Two-tailed values of $p < 0.05$ were considered significant.

Analysis was performed using GraphPad Prism version 6.03 (GraphPad Software Inc, San Diego, CA) and Statistical Package of Biosciences for Windows version 9.66 (ComWorks Co, Tokyo, Japan).

Results

Baseline

First, 31 participants of 45 patients with CVN (of 500 patients with diabetes mellitus) had add-on ARI for CAN or IBT for DM (received intervention). However, the number of analyzed patients with CVN (groups A, B, and C) was 42 (Figure 1). Namely, fourteen patients were excluded.

Second, the 42 analyzed patients (groups A, B, and C) with CVN had T2DM. Means of age, duration of DM from discovery, BMI, HbA1c, TC, HDL, LDL, TG, Cr, and UAER at baseline in the patients were 72 ± 9 years, 25 ± 10 years, 23 ± 4 kg/m^2, 7.2% ± 1.1%, 181 ± 30 mg/dL, 55 ± 15 mg/dL, 103 ± 28 mg/dL, 125 ± 70 mg/dL, and 0.9 ± 0.3 mg/dL, and 187 ± 439 mg/gCr, respectively. Means of systolic and diastolic clinic pressures, intake of alcohol, amount of smoking, and max CV.R-R were also 125 ± 15 mmHg, 65 ± 7 mmHg, 122 ± 10 mmHg, and 68 ± 8 mmHg, 3 ± 12 g/day, 2 ± 8 cig/day, and 1.59 ± 0.33%, respectively. The duration of neuropathy in the patients was unclear, because that there was no symptom for neuropathy. Overall, 23 patients (55%) and 11 patients (26%) had microangiopathy (except CVN) and macroangiopathy, respectively. On microangiopathy, there were 18 patients with retinopathy (7 simple, 1 preproliferative, and 10 proliferative), 16 with nephropathy (8 microalbuminuria, 8 macroalbuminuria, and 4 renal anemia), and 19 with neuropathy. On macroangiopathy, there were 7 patients with CVD, 6 patients with CHD, and 1 patient with POA. For anti-diabetic treatment, 33 patients (79%) were treated with oral pharmacotherapy, and 31 (74%) required insulin therapy. Additionally, 12 patients (29%) were treated with anti-dyslipidemia drugs, 37 (88%) required anti-hypertensive drugs, and 14 were treated with other drugs.

The data of all variables at baseline in each group (A, B, and C) were shown in Table 1.

In group A, all patients had T2DM. There was duration of neuropathy unclearly. Ten patients (83%) had microangiopathy except CVN (8 with retinopathy, 10 with nephropathy and 5 with neuropathy). Five patients (42%) had macroangiopathy (4 with CVD and 1 with CHD). For anti-diabetic treatment, 7 patients (58%) were treated with oral pharmacotherapy, and 8 (67%) required insulin therapy. Additionally, 8 patients (67%) were treated with anti-hypertensive drugs, 3 (25%) required anti-dyslipidemic drugs, and 9 (75%) were treated with other drugs.

In group B, all patients had T2DM. No patients were treated with ARI or IBT. Eleven one (61%) patients had microangiopathy except CVN (5 with retinopathy, 4 with nephropathy, and 6 with neuropathy) before treatment. There was unclear duration of neuropathy. None of patient had macroangiopathy. For anti-diabetic treatment, 11 patients (61%) were treated with oral pharmacotherapy, and 9 (50%) required insulin therapy. Additionally, 14 patients (78%) were treated with anti-hypertensive drugs, 2 (11%) required anti-dyslipidemic drugs, and 6 (33%) were treated with other drugs (Table 1).

In group C, all patients had T2DM. There was unclear duration of neuropathy. Eight patients (67%) had microangiopathy except CVN (5 with retinopathy, 6 with nephropathy and 6 with neuropathy). Five patients (42%) had macroangiopathy (3 with CVD and 3 with CHD). For anti-diabetic treatment, six patients (50%) were treated with hypoglycemic pharmacotherapy, including add-on IBT, and 7 (58%) required insulin therapy. Additionally, 8 patients (67%) were treated with anti-hypertensive drugs, 0 required anti-dyslipidemic drugs, and 8 (67%) were treated with other drugs.

The mean HbA1c was significantly higher than normal in each group ($P < 0.001$). The mean max CV. R-R was less than 2.00% in all groups. All patients had no anemia and no hypertension, and no significant differences were evident in other variables between each group.

Outcomes of endpoints after treatment

Primary outcome

In all patients in all groups, there was significantly ($p < 0.0001$) difference in the magnitude of increased max CV.R-R between groups 1 and 2. Table 1 and Figure 2 show the primary outcome (CVN) in each group.

In group A, although there was no significant difference in heart rate between groups 1 and 2, the mean max CV. R-R was significantly higher in group 2 than in group 1. However, the max CV. R-R was more decreased in some patients in group 2 than in group 1, and 8 patients in group 2 still had CVN. The prevalence of patients with persistent CVN in group 2 was 67%, indicating that more than half of the patients still had CVN after treatment.

In group B, although there was no significant difference in heart rate between groups 1 and 2, the mean max CV. R-R was significantly higher in group 2 than in group 1 or group A. Five patients in group 2 still had CVN, although there was no patient as shown in whom value

of CV R-R was decreased in group 2, which was different from group A. The prevalence of patients with persistent CVN in group 2 was 28%, which was significantly ($p = 0.02$) lower than in group A.

In group C, although there was no significant difference in heart rate between groups 1 and 2, the mean max CV. R-R was significantly higher in group 2 than in group 1, and the mean increase in CV.R-R was significantly higher than in group A or B. CVN recovered completely in all patients in group 2. The 6 patients treated with epalrestat in group 1 (the prevalence of patients was 50%) (Table 1) had increased in value of max CV.R-R after treatment with combined epalrestat and sitagliptin for CVN and DM (3.2±0.4% in group 2 from 1.6±0.5 % in group 1).

ANOVA with multiple comparison test showed there was a significant ($p < 0.002$) difference in magnitude change after treatment of the mean max CV. R-R between groups.

In group A, more patients were on hypoglycemic drugs in group 2 than in group 1. The drugs consisted of BG, TZD or insulin. However, there was a tendency in increased HbA1c as the glycemic control after the treatment (Table 1), although no significant differences were observed. The numbers of patients treated with anti-dyslipidemic drugs or various other drugs including epalrestat were increased, especially ARI drug was significantly increased, whereas the numbers of patients treated with anti-anemia and cardioprotectant were less. No significant differences were observed in other variables except age between groups 1 and 2.

In group B, the mean of systolic clinic blood pressure was significantly lower in group 2 than in group 1, although no hypertension was seen in the patients before or after treatment. For anti-diabetic treatment, the percentage of patients treated with add-on sitagliptin increased significantly to 100%, whereas there was a tendency for the numbers

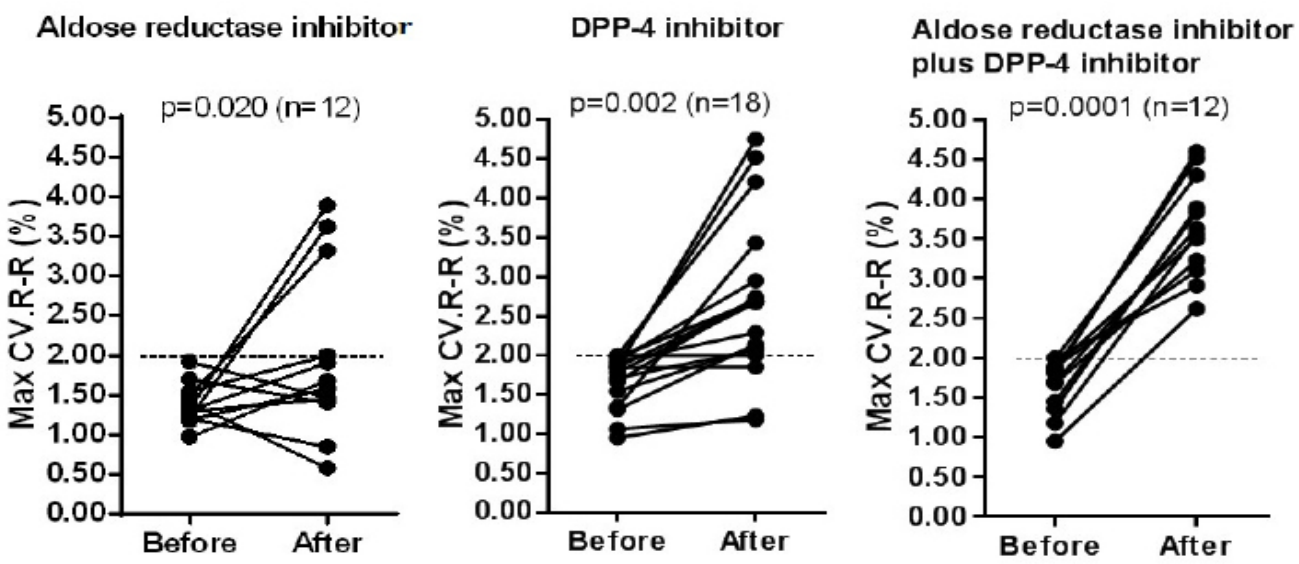

Figure 2: Changes in maximal CV. R-R with cardiac vagal neuropathy (CVN) before (group 1) and after treatment (group 2) with oral epalrestat 150 mg daily alone as aldose reductase inhibitor (ARI) in group A (n = 12) at the left side, before and after treatment with oral sitagliptin 50 mg daily alone, DPP-4 inhibitor as incretin-based therapy (IBT) in group B (n=18) at the center, and before and after treatment with the combination of ARI (oral epalrestat) and IBT (oral sitagliptin) with CVN in group C (n=12) at the right side.
The broken line represents the low limit of the normal range in 100 consecutive maximal CV. R-R intervals (max CV.R-R) from usual breathing to deep breathing at rest for measurement on electrocardiography. CVN was defined as max CV. R-R less than 2.00% at all times during three measurements in diabetic patients. The CVN defined by max CV.R-R was represented as a kind of cardiac autonomic neuropathy (CAN).
A total of 42 patients with CVN and with type 2 diabetic patients (T2DM) were studied. They were divided into three groups (A, B, and C). Group A (n = 12) was treated with add-on oral epalrestat 150 mg daily alone as ARI, group B (n=18) was treated with add-on oral sitagliptin 50 mg daily alone, DPP-4 inhibitor as IBT, and group C (n= 12) with CVN despite treatment with oral epalrestat alone (n = 6) for 1 year in group A, oral sitagliptin alone (n = 5) for 1 year in group B and subcutaneously injection of GLP-1 receptor agonist (n =1) as IBT for 1 year was treated with add-on combined oral epalrestat and oral sitagliptin. The mean duration of each treatment was 1 year.

Secondary outcome

In the secondary outcome, there was no significant difference in various variables and in prevalence of patients with various chronic complications between groups 1 and 2 in patients in all groups. Further, there was no significant difference in the number of patients treated with pharmacotherapy for various diseases, but the numbers of patients treated with anti-neuropathy drugs including epalrestat increased significantly ($P < 0.0001$) more in group 2 than in group 1. Table 1 shows the secondary outcomes of other variables in each group.

of patients treated with α-GI decreased and those treated with SU or insulin were decreased significantly ($P< 0.01$). There was a tendency in decreased HbA1c as the glycemic control after the treatment (Table 1), although no significant differences were observed. There was no significant difference in other variables except age between groups 1 and 2.

In group C, no significant difference in including 6 patients treated with epalrestat was found in blood pressure between before and after treatment. Chronic complications consisting of microvascular diseases were decreased more in group 2 than in group 1. Furthermore, the

number of patients treated with oral pharmacotherapy for various diseases including epalrestat and sitagliptin increased significantly more in group 2 than in group 1, whereas there was a tendency for the numbers of patients treated with oral α-GI or subcutaneously injected insulin for hyperglycemia to be decreased. There was similar to level of HbA1c as the glycemic control after the treatment including 6 patients with epalrestat (Table 1), although no significant difference was observed. There were no significant differences in other variables except age between groups 1 and 2.

In all groups, no significant differences were found in the other variables except durations of diabetes from discovery and of treatment with various drugs (Table 1). The means of age, duration of diabetes and duration of the treatment in each group were more than 70 years, 20 years and 1 year, respectively.

Discussion

Although the study may require randomized and controlled trials, the present study was not a pure randomized trial and not a pure controlled trial. If I'm forced to say, the 50% patients with CVN in group C had ARI, which had served as placebo. Further, all patients had old age and T2DM without obesity and with normal renal function.

The majority had been suffering from DM for prolonged period; therefore, they had various complications and had received various types of pharmacotherapy. Although all patients had no hypertension, treatment with add-on oral sitagliptin alone resulted in a significant small decrease in clinic systolic blood pressure, similar to a previous report [34]. This effect seems to be mediated through vasodilatation, as well as renal sodium handling-associated natriuresis [34]. Alterations in the prevalence of chronic complications other than CVN after treatments were similar to the reports described previously [1-6].

CVN was a kind of CAN. CAN have been contributed to morbidity, mortality and reduced quality of life for persons with DM [1-6]. In the present study, CVN was partially reversed by treatment with add-on oral epalrestat alone, which has been demonstrated in previous reports [7-10]. CVN was also partially reversed by treatment with oral sitagliptin alone; however, the effect of sitagliptin may be superior to that of epalrestat treatment. Furthermore, treatment with combined epalrestat and sitagliptin for CVN may be more effective than treatment with epalresta or sitagliptin alone, since the treatment with combined epalrestat and sitagliptin resulted in complete recovery from CVN, even though no significant difference was found in other variables between before and after treatment. To the best of our knowledge, this is a first time in the world that treatment with add-on oral sitagliptin alone may have been found to be superior to treatment with oral epalrestat alone for CVN and that treatment with combined epalrestat and sitagliptin may have a synergic effect for CVN in type 2 diabetic patients.

In general, diabetic PN is associated with decreased large motor fiber function and altered small C fiber structure in both myelinated and unmyelinated fibers [1-10], which was closely associated with alterations of dorsal root ganglion (DRG) cells, Schwann cells, and Purkinje cells. In such fibers, PN is due to decreased activities of Na+/K+-ATPase, myoinositol, diacylglycerol, and protein kinase C via attenuation of cAMP levels [12-13] by the polyol pathway [9-10,12-13], Wnt pathway [35-36] or other causes [3,14-18,20-21,37-40]. Both GLP-1 receptors were detected in DRG [18] and in Schwann cells [38], and GIP receptors were detected in Purkinje cells [39] and have a neurite outgrowth of the nerve fibers via a promoter of nerve growth factor [14,16], as well as a significant role in increasing the activity of Na+/K+-ATPase [38] by phosphorylation through signal-regulated kinase levels [38] and by the Wnt pathway [36] via cyclic AMP in the fibers, which protects the structure and function of the nerve fibers [13, 16, 19].

It is known that sitagliptin increases cyclic AMP via GIP and GLP-1 [15,37,40-41]. Accordingly, as no significant difference was observed in magnitude of HbA1c as a measure of glycemic control and in the prevalence of patients with chronic complications before and after treatment in each group, the mechanism underlying treatment with epalrestat for CVN is due to reduction of activity in the polyol pathway of nerve fibers [9, 19, 30, 42], whereas the mechanism underlying treatment with sitagliptin for CVN may be due to activation of nerve growth factor [13, 16], Na+/K+-ATPase [17, 38] or Wnt pathway [35-36] via increased cyclic AMP [17, 19, 37, 41], and may be by supporting neuronal survival [9,13,17,19] in the nerve fibers. Treatment with combined epalrestat and sitagliptin for CVN may be due to greater activations of nerve growth factor, Na+/K+-ATPase, and the Wnt pathway.

Although usually, oral sitagliptin had a tendency to improve the glycemic control in patients with T2DM [28], the reason why it was insignificant was not clear. The patients in this study may require more dose of insulin, but we did not increase the dose, because that we were afraid of hypoglycemia owing to increased dose of insulin, or AN may be related to be glycemic control [43], although we examined CVN alone, but not AN.

The Limitation of this Study

First, this study was that a diagnosis of CVN as CAN was made solely by measuring the max CV.R-R on the electrocardiogram. The diagnosis of CAN should usually be based on the results of a battery of autonomic tests rather than one single test [1-5]. In the present study, other laboratory examinations such as Valsalva test, a tilt table test, pupillary light reflex, microvibration tests or sudomotor tests were not performed. AN is also associated with bladder and/or sexual dysfunction, including loss of penile erection and/or retrograde ejaculation [1,4]; however, these findings were not confirmed in the present study.

Second, even though this trial was randomized, it was altered. The number of patients not only with CVN but also treated with add-on ARI as placebo for CVN was small, and no all patient with CVN had served as placebo, because that we were afraid of a sudden death of the patients with CVN [1-6], although what the placebo treatment for CVN was remained as a question [21]. Therefore, the conclusions may be limited.

Third, a time course of such treatments for CAN was not examined. Therefore, the duration of initial recovery after such treatments in patients with T2DM is not known.

Fourth, as it was not present that patient had painful complains, the effects of ARI, IBT or combined ARI and IBT on the painful neuropathy in patients with T2DM could be not examined specifically.

Fifth, although the effects of ARI, IBT or combined ARI and IBT on CVN in patients with T1DM were of interest, there were few patients with T1DM treated with ARI, IBT or combined ARI and IBT. Therefore, it was not possible to perform statistical analyses.

Conclusion

In summary, the present results suggest that sitagliptin may be more

effective than epalrestat for CVN in patients with T2DM, and treatment with combined epalrestat and sitagliptin may have a synergistic effect for CVN. Since IBT is currently used in patients with T2DM, the findings may be significant in the management of DM.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

Kamoi K. designed the research (project conception, development of overall research plan and study), conducted the experiments, collected and analyzed data, performed statistical analysis and wrote the paper. Sasaki H. provided essential comments.

Acknowledgements

The authors would like to thank the clinical laboratory technicians for their assistance, Prof (Aichi Medical University), Dr. J Nakamura and Emeritus President of Chubu Rosai Hospital (Japan Labour Health and Welfare Organization), Dr. N Hotta of their valuable comments.

Trial Registration

ClinicalTrials.gov (NCT01545024)

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