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An α-glucosidase inhibitor voglibose reduces oxidative stress markers and soluble intercellular adhesion molecule-1 in obese type 2 diabetes

Short tytle: Satoh et al. Voglibose and cardiovascular risk factors.

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Abstract

Objective: Postprandial hyperglycemia and hyperlipidemia are considered risk factors for cardiovascular disease. This study was designed to elucidate whether improving the postprandial state by voglibose, an α-glucosidase inhibitor, leads to the reduction of oxidative stress markers and soluble adhesion molecules in obese type 2 diabetic patients.

Methods and Results: A total of 30 Japanese obese type 2 diabetic patients were randomly assigned and treated for 3 weeks with either diet alone (the control group) or diet plus voglibose (0.9 mg daily) (the voglibose group) (n = 15 each). Analysis of the diurnal metabolic profiles revealed a significant reduction of postprandial hyperglycemia and hyperlipidemia in the voglibose group relative to the control group ($P < 0.05$), despite the similar improvement of body mass index (BMI) and hemoglobin A1c (HbA1c) in both groups. Voglibose also decreased significantly plasma levels of soluble intercellular adhesion molecule-1 (sICAM-1) and urinary excretion of 8-iso-prostaglandin $F_2\alpha$ and 8-hydroxydeoxyguanosine ($P < 0.01$) and C-reactive protein ($P < 0.05$) relative to the control group.

Conclusions: This study represents the first demonstration that voglibose reduces oxidative stress generation and sICAM-1 in parallel with the reduction of postprandial hyperglycemia and hyperlipidemia in obese type 2 diabetic patients.

Introduction

The metabolic syndrome, the coexistence of several risk factors for atherosclerosis, including visceral obesity, hyperglycemia, atherogenic dyslipidemia, and hypertension, has been considered to be a precursor of cardiovascular disease (CVD) [1]. Systemic inflammation and oxidative stress have been postulated to be important pathogenic factors in the development of the metabolic syndrome and thus atherosclerosis [2]. Evidence has accumulated suggesting that the postprandial state including postprandial hyperglycemia and hyperlipidemia contributes to the development of atherosclerosis through oxidative stress generation in diabetes-related metabolic derangements [3].

Recent epidemiological studies such as DECODE/DECODA Study and Funagata Diabetes Study have revealed that serum glucose level 2 h after an oral challenge with glucose or postprandial hyperglycemia is an independent risk factor and is a more powerful predictor of CVD and mortality than the level of fasting plasma glucose (PG) [4,5]. It was suggested that postprandial hyperglycemia is involved in the initiation and promotion of atherosclerosis via multiple mechanisms such as oxidative stress generation, LDL oxidation, and thrombosis activation, and endothelial dysfunction ensues in a setting of meal-induced antioxidant consumption [6]. Thus, antidiabetic agents that are capable of reducing postprandial hyperglycemia are desirable to prevent cardiovascular events associated with diabetes.

The α-glucosidase inhibitors such as acarbose and voglibose are thought to act at the small intestine by competitively inhibiting enzymes that delay the release of glucose from complex carbohydrates, thereby specifically reducing postprandial glucose excursion [7]. They are widely used to reduce postprandial hyperglycemia

and hyperinsulinemia in diabetic patients [7]. It has been demonstrated in the Study To Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) trial that acarbose can delay the development of type 2 diabetes in patients with impaired glucose tolerance (IGT) [8]. The acarbose treatment has been also associated with a significantly lower incidence of CVD, newly diagnosed hypertension, and progression of intima-media thickness (IMT) in IGT subjects [9,10]. Moreover, it was shown by meta-analysis that acarbose can prevent myocardial infarction and CVD in type 2 diabetic patients [11].

Type 2 diabetes is often associated with abnormalities in plasma lipid and lipoprotein profiles, and postprandial hyperlipidemia has been shown to be an independent risk factor and predictor of atherosclerosis and CVD [12]. Since carbohydrates are highly lipogenic precursors, retardation of their digestion by α-glucosidase inhibitors is likely to affect lipid metabolism. Indeed, a single dose of acarbose suppresses postprandial hyperlipidemia as well as postprandial hyperglycemia [13]. However, whether voglibose can reduce postprandial hyperlipidemia has not been tested so far. Furthermore, whether improving the postprandial state by α-glucosidase inhibitors can reduce other risk factors of CVD is also unknown. The aim of this study is to elucidate whether improving the postprandial state by voglibose leads to the reduction of systemic inflammation, oxidative stress markers, and soluble adhesion molecules in obese type 2 diabetic patients.

Methods

Subjects

A total of 30 Japanese obese type 2 diabetic patients (14 men and 16 women, mean age 46.2 ± 2.7 years, mean BMI 32.8 ± 1.2 kg/m^2, mean hemoglobin A1c (HbA1c) 7.7 ± 0.4 %) were recruited in our clinics between April 2002 and June 2004 (Table 1). The patients had stable HbA1c levels (6.5% ≤ HbA1c ≤ 9.5%). The study protocol was approved by the ethical committee on human research in Kyoto Medical Center and Tokyo Medical and Dental University, and all participants gave written informed consent.

Study protocols

The patients were assigned to one of the following treatment groups (a single-blind and run-in period randomization, which patients received); they were treated on hospital basis for 3 weeks with either diet alone (the control group) (7 men and 8 women, mean age 46.0 ± 4.1 years, n = 15) or diet plus voglibose (0.9 mg daily) (the voglibose group) (7 men and 8 women, mean age 46.3 ± 3.6 years, n = 15). Prior to the study, 2 patients in the control group and two in the voglibose group had been treated with sulfonylureas; 1 patient in the control group and one in the voglibose group had been treated with metformin; whereas the remaining 12 patients from each group had only received diet therapy. The administration of sulfonylureas and metformin was continued with fixed dosages throughout the study. Diet therapy consisted of 25 kcal/kg of ideal body weight per day. They consumed 57% of total energy as carbohydrate, 25% as fat, and 18% as protein. In examining the diurnal metabolic profiles, all patients were instructed to maintain the same level of energy intake and physical activity for 3 weeks during hospitalization; they were served with the standard meals for diabetic patients at

0800, 1200, and 1700 throughout the study. They also underwent counseling on dietetics one month before and twice during hospitalization. Patients treated with ACE inhibitors or angiotensin II receptor antagonists were excluded. Lipid lowering medications such as statins and fibrates were also excluded. None received hormone replacement therapy.

At the beginning and at the end of the study, we examined body mass index (BMI), HbA1c, 1,5-anhydro-D-glucitol (1,5-AG), PG, immunoreactive insulin (IRI), C-peptide reaction (CPR), homeostasis model assessment (HOMA-IR) [14], total cholesterol (T-Chol), HDL cholesterol (HDL-C), triglycerides (TG), free fatty acids (FFA), apolipoprotein B (ApoB), apolipoprotein E (ApoE), leptin, adiponectin, C-reactive protein (CRP), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1), and urinary excretion of 8-iso-prostaglandin $F_2\alpha$ (8-iso-$PGF_2\alpha$) and 8-hydroxy-2'-deoxyguanosine (8-OHdG). Prior to and 3 weeks after the voglibose treatment, blood samples were taken before each meal and 90 min after breakfast and lunch to measure PG, IRI, CPR, TG, FFA, ApoB, and ApoE.

Plasma parameter measurements

For plasma separation, each blood sample was immediately transferred to chilled siliconized glass tubes containing EDTA (1 mg/ml), and centrifuged at 4 °C. Plasma samples were frozen and stored at -70 °C until assay. HbA1c, PG, CPR, T-Chol, HDL-C, TG, FFA, ApoB, and ApoE levels were measured according to the standard procedures. Serum 1,5-AG concentrations were determined by an established enzymatic method using a 1,5-AG clinical test kit (Lana-1,5-AG, Nippon Kayaku, Tokyo, Japan). IRI was measured by enzyme immunoassay using a commercially available kit (Tosoh, Tokyo, Japan). Plasma concentrations of

leptin and adiponectin were determined using the respective radioimmunoassay kits (Linco Research, St. Charles, MO) [15]. Plasma levels of CRP were measured by the latex-enhanced assay using particle-enhanced technology performed on the Behring BN nephelometer (Dade Behring) [15]. sICAM-1 and sVCAM-1 were measured using commercially available immunoassays from Research and Diagnostic systems (Minneapolis, MN) [16].

Urinary parameter measurements

A morning urine sample was collected from each patient and stored frozen at -70 °C using N_2 gas. Urine samples were centrifuged at 10000g for 10 min, and after proper dilution the supernatant was used for the determination of 8-iso-$PGF_2\alpha$ using an ELISA method using a kit from Cayman Laboratories (Ann Arbor, MI) [17]. 8-OHdG was measured by a competitive ELISA kit (8-OHdG Check, Japan Institute for the Control of Aging, Fukuroi, Shizuoka, Japan) [18].

Statistical analysis

Data are presented as the mean ± SE, and $P < 0.05$ was considered statistically significant. The student's two-tailed *t* test was used for baseline comparison between the two groups, and comparison of differences between the means within each group before and after the study. In this study, the diurnal metabolic profiles within each group before and after the study were assessed by the student's two-tailed *t* test and ANOVA. All statistical analyses were performed using the Stat View program version 5.0 for Windows (SAS Institute Inc.).

Results

Baseline characteristics of the study subjects

There were no significant differences between the control and voglibose groups in age, BMI, HbA1c, 1,5-AG, PG, IRI, CPR, HOMA-IR, T-Chol, HDL-C, TG, FFA, ApoB, and ApoE prior to the study (Table 1). The two groups also did not differ significantly in leptin, adiponectin, CRP, sICAM-1, sVCAM-1, and urinary excretion of 8-iso-$PGF_2\alpha$ and 8-OHdG at baseline (Table 1).

Effects of voglibose on glucose and lipid metabolism

According to the student's two-tailed *t* test, the changes in BMI and HbA1c in both the control and voglibose groups during the study were not significant (ΔBMI, $P = 0.367$; ΔHbA1c, $P = 0.477$). In the control group, PG, IRI, CPR, HOMA-IR, HDL-C, TG, FFA, ApoB, and ApoE remained unchanged (Table 1). After the voglibose treatment, PG, IRI, CPR, HOMA-IR, T-Chol, TG, ApoB, and ApoE were markedly decreased relative to the control group, although BMI, HbA1c, and 1,5-AG were similarly reduced in both groups (PG, T-Chol, TG, ApoB, and ApoE, $P < 0.01$; IRI, CPR, and HOMA-IR, $P < 0.05$) (Table 1). In this study, FFA tended to be decreased but did not reach statistical significance in the voglibose group (Table 1).

Effects of voglibose on plasma leptin and adiponectin concentrations

Plasma leptin concentrations decreased significantly in both the control and voglibose groups (control group, $P < 0.05$; voglibose group, $P < 0.01$). However, plasma adiponectin concentrations were unchanged in both groups (Table 1). Neither gender nor the administration of sulfonylureas had any impact on the above parameters in both groups (data not shown).

Effects of voglibose on systemic inflammation, oxidative stress markers, and

soluble adhesion molecules

CRP, sICAM-1, and urinary excretion of 8-iso-$PGF_2\alpha$ and 8-OHdG were significantly decreased in the voglibose group relative to the control group (sICAM-1, 8-iso-PGF2α, and 8-OHdG, $P < 0.01$; CRP, $P < 0.05$), whereas CRP, sICAM-1, and urinary excretion of 8-iso-$PGF_2\alpha$ and 8-OHdG remained unchanged in the control group (Table 2). In this study, there was no significant change in sVCAM-1 in both the control and voglibose groups.

Effects of voglibose on the diurnal metabolic profiles

Both at the beginning and at the end of the study, PG increased significantly after breakfast and lunch relative to those before breakfast and lunch in both the control and voglibose groups ($P < 0.01$). PG was significantly suppressed before breakfast and after breakfast and lunch during 3-week treatment of voglibose ($P < 0.01$). There were no appreciable changes in the control group (Fig 1A). IRI and CPR increased significantly after breakfast and lunch in both groups (after breakfast, $P < 0.01$; after lunch, $P < 0.05$). After 3-week treatment of voglibose, IRI before and after breakfast and lunch was significantly decreased ($P < 0.05$) (Fig 1B). CPR before and after breakfast and lunch was also significantly reduced by the voglibose treatment (before and after breakfast, $P <0.05$; before and after lunch, $P<0.01$) (Fig 1C). The voglibose treatment also significantly reduced TG at all points examined (before and after breakfast and before lunch and dinner, $P < 0.01$; after lunch, $P < 0.05$), whereas there were no appreciable changes in the control group (Fig 1D). FFA was also significantly reduced after breakfast and before dinner in the voglibose group ($P < 0.01$) (Fig 1E), whereas it was unchanged in the control group. ApoB and ApoE were significantly suppressed at all points examined in the voglibose group, although there were no appreciable changes in

the control group (Fig 1F, 1G). ANOVA revealed that in the voglibose group, all the parameters in the diurnal metabolic profiles were significantly improved after 3-week treatment (CPR, TG, ApoB, and ApoE, $P < 0.01$; PG, IRI, and FFA, $P < 0.05$), although there were no significant changes in the control group.

Discussion

Recent epidemiological studies have demonstrated that postprandial hyperglycemia is an independent risk factor and a more powerful predictor of CVD than fasting PG [4,5]. Currently, α-glucosidase inhibitors, fast-acting and short-duration insulin secretagogues, and rapid-acting human insulin analogues have been widely used to suppress postprandial hyperglycemia. This may be more efficient for reducing the risk of CVD in diabetic patients. Indeed, the STOP-NIDDM trial showed that acarbose treatment is associated with a significantly lower incidence of CVD, newly diagnosed hypertension, and progression of IMT in IGT subjects [9,10]. It was also shown by meta-analysis that acarbose can prevent myocardial infarction and CVD in type 2 diabetic patients [11]. Furthermore, it was shown that voglibose reduces the progression of IMT in Japanese patients with type 2 diabetes [19]. Here we investigated whether improving the postprandial state by voglibose leads to the reduction of systemic inflammation, oxidative stress markers, and soluble adhesion molecules in obese type 2 diabetic patients.

This study is the first demonstration that treatment with voglibose for 3 weeks reduces CRP, sICAM-1, oxidative stress markers such as urinary excretion of 8-iso-$PGF_2\alpha$ and 8-OHdG in parallel with improving postprandial hyperglycemia in obese type 2 diabetic patients. Although HbA1c was reduced similarly in both the control and voglibose groups during the study, postprandial PG after breakfast and lunch were decreased in the voglibose group ($P < 0.01$). These observations suggest that voglibose is capable of reducing oxidative stress markers and sICAM-1 by improving postprandial hyperglycemia. Since oxidative stress markers and increased plasma levels of soluble adhesion molecules have been

associated with the development of CVD [20], it is conceivable that treatment with voglibose may lead to the reduction of the risk of CVD in obese type 2 diabetes. It has been demonstrated *in vitro* that intermittent high glucose enhances oxidative stress generation and ICAM-1 and VCAM-1 expression in cultured human umbilical endothelial cells (HUVECs), where it can induce a marked increase in cellular apoptosis [21,22]. Furthermore, there are several reports showing that reactive oxygen species induces expression of adhesion molecules in endothelial cells both *in vivo* and *in vitro* [23,24]. In this study, sVCAM-1 was not reduced by the voglibose treatment, which is consistent with a previous report that only serum levels of sICAM-1, and not sVCAM-1, is elevated in diabetic patients without macroangiopathy [25]. These findings, taken together, suggest that improving postprandial hyperglycemia by voglibose may reduce oxidative stress generation in the vasculature, thereby leading to the reduction in inflammatory response in endothelial cells and eventually in vascular injuries associated with diabetes.

It is well known that both postprandial hyperglycemia and atherogenic dyslipidemia (impairment of postprandial chylomicron and very low density lipid protein (VLDL) metabolism) are present in type 2 diabetic patients [26]. Furthermore, postprandial hyperlipidemia has been reported to be an independent risk factor of CVD [27] and a predictor of carotid IMT in type 2 diabetic patients [12]. In this study, we found that voglibose reduces significantly fasting TG in obese type 2 diabetic patients, which is consistent with previous studies that TG in diabetic patients is reduced by long-term treatment with acarbose [13,28]. Voglibose also reduced postprandial FFA, and TG, ApoB and ApoE at all the points examined in the diurnal profile. In this context, Ceriello *et al.* demonstrated that the impact of combined postprandial hyperglycemia and hyperlipidemia on

oxidative stress generation and soluble adhesion molecules is greater than that of postprandial hyperglycemia and hyperlipidemia independently [23] and suggested an independent and cumulative effect of postprandial hyperglycemia and hypertriglyceridemia on endothelial dysfunction in type 2 diabetic patients [29]. We observed a drastic reduction in remnant-like lipoprotein particle (RLP)-cholesterol during the voglibose treatment (unpublished observation), which is consistent with a report by Yoshino *et al.* that RLP-cholesterol tends to be decreased in diabetic subjects after 3-month treatment of acarbose [30]. Doi *et al.* also showed *in vitro* that RLPs can increase expression of ICAM-1 and VCAM-1 in HUVECs through oxidative stress generation [31]. These observations, taken together, suggest that reduction of both postprandial hyperglycemia and hyperlipidemia by voglibose is responsible for the reduction of oxidative stress markers and sICAM-1 in obese type 2 diabetic patients.

There are several potential mechanisms whereby voglibose treatment can improve diabetic dyslipidemia in this study. The reduction of hypertriglyceridemia by voglibose may be due at least in part to a slower rate of hepatic uptake of dietary carbohydrates, key precursors of *de novo* lipogenesis [32]. Furthermore, voglibose improved postprandial hyperinsulinemia; it may also reduce diurnal insulin secretion by lowering postprandial hyperglycemia and insulin resistance. Because hepatic VLDL secretion is stimulated during chronic hyperinsulinemia and insulin resistance [33], a decrease in postprandial IRI may also contribute to the decrease in TG after the voglibose treatment. The marked reduction in diurnal ApoB and ApoE by voglibose may be related to the improvement of postprandial hyperinsulinemia and insulin sensitivity [34], thereby potentially contributing to the reduction of the risk of CVD [35].

To assess the pathophysiologic implication of the postprandial state as a risk of CVD, most of the previous studies employed challenge meals such as oral glucose tolerance test or high fat meal. Since we used the standard meals for diabetic patients, the data of this study may be more physiologically relevant. In examining the diurnal metabolic profiles, all patients were instructed to maintain the same level of energy intake and physical activity for 3 weeks during hospitalization. Although the standard meals were regularly served during hospitalization, glucose monitoring throughout the day should be desirable to achieve accurate diet loading.

In conclusion, this study represents the first demonstration that voglibose decreases oxidative stress generation and sICAM-1 by improving postprandial hyperglycemia and hyperlipidemia in obese type 2 diabetic patients, thereby potentially leading to the reduction of the development of atherosclerosis and CVD.

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