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Vildagliptin Improves Glucose Tolerance and Decreases Plasma Triglycerides in Sprague-Dawley Rats

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Abstract: The number of patients with lifestyle-related diseases, including type 2 diabetes, is increasing. The onset of type 2 diabetes can be prevented by dietary and exercise interventions, as well as drug therapy. Dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 receptor agonists have attracted attention recently as treatments for diabetes, and incretin hormones have been reported to have a protective effect on pancreatic β-cells. It is not clear whether vildagliptin (VIL) can prevent the progression of lifestyle-related disease. Thus, in the present study, Sprague-Dawley rats were fed a high-fat diet with sucrose water (HFDS) to determine whether VIL could inhibit deterioration in glucose tolerance and improve other biomarkers of lipid disorder. Four-month-old male Sprague-Dawley rats were divided into three groups (n=7 in each group); one group was fed a normal diet for 4 months, whereas the remaining two groups were fed the HFDS, with or without VIL for 4 months. When rats were 7 months of age, they were subjected to an intraperitoneal glucose tolerance test (IPGTT); biomarkers of lipid disorder were measured in 8-month-old rats. There was a decrease in the glucose spike in the IPGTT 10 min after loading in the HFDS + VIL group and plasma triglyceride (TG) levels were significantly lower in these rats compared with the HFDS group. The decreased TG levels in HFDS + VIL rats were accompanied by decreases in plasma chylomicron levels. These results suggest that VIL can prophylactically inhibit decreases in pancreatic β-cell function in type 2 diabetes and reduce the risk of cardiovascular disease due to high TG levels. Thus, VIL administration may contribute to the prevention of lifestyle-related disease.

Key words: dipeptidyl peptidase-4 inhibitor, vildagliptin, incretin, chylomicron, impaired glucose tolerance

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Introduction

Type 2 diabetes (T2D) is a serious, progressive disease, and the number of patients with T2D is increasing. The onset of T2D can be prevented by dietary and exercise interventions, as well as drug therapy[1–4]. The pathogenetic mechanism of onset of T2D is a deficiency in insulin secretion caused by pancreatic β-cell dysfunction and a decrease in insulin activity caused by increased insulin resistance. A reduction in the number of pancreatic β-cells is known to result in abnormal glucose tolerance, and it may therefore be possible to prevent the onset of T2D by inhibiting further decreases in β-cells[5–7].

Glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) are hormones secreted by the L- and K-cells of the small intestine, respectively. These hormones participate in serum glucose-dependent insulin secretion and have been reported to protect pancreatic β-cells by inducing them to undergo differentiation and proliferation while inhibiting apoptosis[8–11]. GLP-1 is known to exert inhibitory effects on glucagon secretion through its antiglycemic activity, although the precise mechanism of action has not yet been elucidated. Dipeptidyl peptidase (DPP)-4 inhibitors, as represented by vildagliptin (VIL), inhibit the inactivation of GLP-1 and GIP by DPP-4 and thus allow the two peptides to potentiate the secretion of insulin by β-cells; furthermore, VIL exhibits antihyperglycemic activity[12]. In addition to lowering serum glucose levels, it has been suggested that DPP-4 inhibitors can directly improve endothelial function and protect the myocardium, with the magnitude of the effect increasing with the duration of drug administration[13, 14].

It has also been reported that newer DPP-4 inhibitors have triglyceride (TG)-lowering effects[15, 16]. In vascular diseases such as myocardial infarction, TG-rich remnant cholesterol is present. Therefore, decreasing the TG levels is important in the prevention of the onset of vascular disease[17]. However, the mechanism by which DPP-4 inhibitors prevent the development of T2D is not clear. In the present study, we investigated whether VIL could prevent the deterioration in glucose tolerance and improve other biomarkers of lipid disorder in Sprague-Dawley rats fed a high-fat diet with sucrose water (HFDS).

Materials and methods

Animals and samples

Four-month-old male Sprague-Dawley rats were divided into three groups (n=7 rats in each group): (i) a control group fed a normal diet (ND; CE2; CLEA Japan, Tokyo, Japan); (ii) a group fed HFDS; and (iii) a group fed HFDS and treated with VIL (10 mg/kg per day, p.o.). Rats were fed the different diets from 4 to 8 months of age. The ND comprised 8.9% water, 25.4% protein, 4.4% fat, 4.1% fiber, 6.9% carbohydrate, and 50.3% nitrogen-free extracts, containing 342.2 kcal/100 g. The two HFDS groups were fed a high-fat diet consisting of 8.2% water, 23.4% protein, 11.0% fat, 3.8% fiber, 6.3% carbohydrate, and 46.3% nitrogen-free extracts, containing 378.0 kcal/100 g, with 30% sucrose solution available ad libitum. Rats were housed in a semibarrier system under controlled room temperature (23 ± 1°C), humidity (55 ± 5%),

and lighting (lights on from 06:00 to 18:00 hours) conditions. All studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals of Showa University.

Preparation and biochemical determination of plasma samples

After 4 months feeding of the different diets (i.e. in 8-month-old rats), blood samples were collected from the inferior vena cava under pentobarbital anesthesia (35 mg/kg, i.p.) and mixed with 134 mM EDTA disodium salt solution in a ratio of 50:1 (v/v). Samples were centrifuged at 1750 g for 15 min at 4℃ and the supernatant collected for subsequent analysis. Rats were killed with an overdose of pentobarbital anesthesia, and the liver, visceral fat (VisF) and epididymal fat (EpidF) were isolated and weighed. Plasma total cholesterol (TC), TG, glucose, insulin, and glutamic pyruvic transaminase (GPT) concentrations were determined using commercially available kits (Cholesterol E-test, Triglyceride E-test, Glucose CⅡ-test (Wako Pure Chemical Industries, Tokyo, Japan); Insulin Rat-T ELISA KIT (TMB) (Shibayagi, Gunma, Japan)). Blood HbA1c levels were determined by HbA1c immunoassay (DCA2000 system; Bayer Diagnostics, Elkhart, IN, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-β) were calculated from fasting plasma glucose and insulin levels, respectively[18, 19].

Lipoprotein electrophoresis

Blood samples were collected from the inferior vena cava from pentobarbital (35 mg/kg, i.p.) -anesthetized rats and mixed with 134 mM EDTA disodium salt solution in a ratio of 50:1 (v/v). Samples were centrifuged at 1750 g for 15 min at 4℃ and the supernatant was collected for subsequent analysis. To analyze lipoprotein fractions in the plasma, the samples were subjected to plasma electrophoresis using Lipophor (Joko, Tokyo, Japan).

Intraperitoneal glucose tolerance test

At 7 months of age, rats were subjected to an intraperitoneal glucose tolerance test (IPGTT), which consisted of intraperitoneal glucose loading (1 g/kg body weight) in rats fasted for 16 h. Blood samples were collected from nicks in the tail tip at 0, 10, 20, 30, 60, 90, and 120 min after glucose loading. Blood glucose levels were determined with a commercially available kit (Glucose CⅡ-test; Wako Pure Chemical Industries).

Statistical analysis

All data are expressed as the mean ± SEM. Data were analyzed by the Mann-Whitney *U*-test with Bonferroni correction. $P<0.05$ was considered significant.

Results

Background data in each experimental group

Body weight (BW), liver weight (LW)/BW, visceral fat (VisF)/BW, and epididymal fat

Table 1. Body weight (BW), liver weight (LW), visceral fat (VisF), epididymal fat (EpidF), plasma glucose, insulin, HbA1c, total triglycerides (TG), total cholesterol (TC), and glutamic pyruvic transaminase (GPT) concentrations, homeostasis model assessment of insulin resistance (HOMA-IR), and homeostasis model assessment β-cell function (HOMA-β) in Sprague-Dawley rats fed a normal diet (ND), a high-fat diet with sucrose water (HFDS), or the HFDS plus 10 mg/kg per day, p.o., vildagliptin (VIL) from 4 to 8 months of age

	ND	HFDS	HFDS + VIL
BW (g)	580 ± 14.19	585 ± 10.58*	592 ± 11.96*†
LW/100 g BW	2.61 ± 0.06	6.58 ± 0.11	7.40 ± 0.16
VisF/100 g BW	2.99 ± 0.18	3.34 ± 0.13	3.36 ± 0.24
EipdF/100 g BW	2.18 ± 0.10	2.09 ± 0.08	2.14 ± 0.11
Glucose (mg/dL)	144.29 ± 5.89	171.00 ± 5.96	164.14 ± 6.46
Insulin (ng/mL)	6.12 ± 1.04	6.29 ± 0.89	7.98 ± 1.12
HbA1c (%)	2.64 ± 0.05	2.76 ± 0.07	2.74 ± 0.06
HOMA-IR	55.59 ± 8.63	68.88 ± 9.62	84.38 ± 11.91
HOMA-β	29.02 ± 6.31	21.70 ± 3.52	29.41 ± 4.90
TG (mg/dL)	64.86 ± 4.66	78.57 ± 4.52	44.86 ± 2.80*†
TC (mg/dL)	63.00 ± 4.38	103.86 ± 7.26*	107.57 ± 7.08*
GPT (Karmen)	50.86 ± 9.72	57.71 ± 5.61	58.14 ± 5.44

Data are the mean ± SEM (n=7 rats in each group). *$P<0.05$ compared with the HFDS group (Mann-Whitney U-test). *$P<0.05$ compared with the ND group; †$P<0.05$ compared with the HFDS group (Mann-Whitney U-test with Bonferroni correction).

(EpidF)/BW ratios, HOMA-IR, HOMA-β, and plasma glucose, insulin, HbA1c, TG, TC, and GPT concentrations in the three groups of 8-month-old rats are given in Table 1. There were no significant differences in BW or the EpidF/BW ratio among the three groups. The LW ratio was increased significantly in the HFDS and HFDS + VIL groups compared with the ND group. There was a tendency for an increased VisF/BW ratio in the HFDS and HFDS + VIL groups compared with the ND group, but the differences did not reach statistical significance. In addition, there was a tendency for increased mean plasma glucose levels and decreased HOMA-β in the HFDS compared with ND group. Conversely, there was a tendency for an increased HOMA-IR in the HFDS + VIL group. Compared with the ND and HFDS groups, the mean plasma TG concentrations were significantly lower in the HFDS + VIL group. Mean plasma TC concentrations were significantly higher in the HFDS and HFDS + VIL groups than in the ND group. There were no significant differences in GPT among the three groups.

Table 2. Chylomicron, very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) levels in Sprague-Dawley rats fed a normal diet (ND), a high-fat diet with sucrose water (HFDS), or the HFDS plus 10 mg/kg per day, p.o., vildagliptin (VIL) from 4 to 8 months of age

	ND	HFDS	HFDS + VIL
Chylomicron	100 ± 2.79	96.74 ± 5.82	84.84 ± 2.39*†
VLDL	100 ± 2.62	119.05 ± 5.24*	126.60 ± 5.98*
HDL	100 ± 3.46	93.98 ± 4.22	104.80 ± 4.76

Data are the mean ± SEM (n=7 rats in each group). *P<0.05 compared with the HFDS group (Mann-Whitney U-test). *P<0.05 compared with the ND group; †P<0.05 compared with the HFDS group (Mann-Whitney U-test).
Note, levels in rats in the ND group were set at 100% and values in the HFDS and HFDS + VIL groups are expressed relative to the ND group.

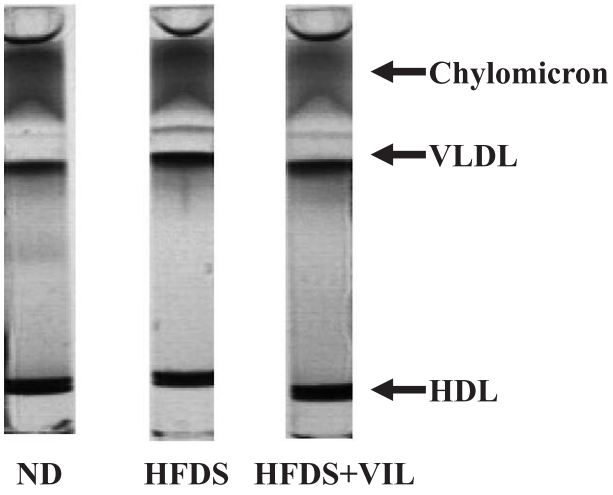

Fig. 1. Results of lipoprotein electrophoresis in rats fed a normal diet (ND), a high-fat diet with sucrose water (HFDS), or the HFDS plus 10 mg/kg per day, p.o., vildagliptin (VIL) from 4 to 8 months of age. VLDL, very low-density lipoprotein; HDL, high-density lipoprotein.

Lipoprotein analysis

The results of electrophoretic lipoprotein analyses and the relative ratios of each fraction are given in Table 2 and Fig. 1. Electrophoresis revealed distinct chylomicron, very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) bands. However, chylomicron levels were significantly lower in the HFDS + VIL group compared with the ND and HFD groups. There was a significant increase in the VLDL fraction in the HFDS and HFDS + VIL groups compared with the ND group. Although there were no significant differences in HDL levels among the three groups, there was a tendency for decreased HDL levels in the HFDS group.

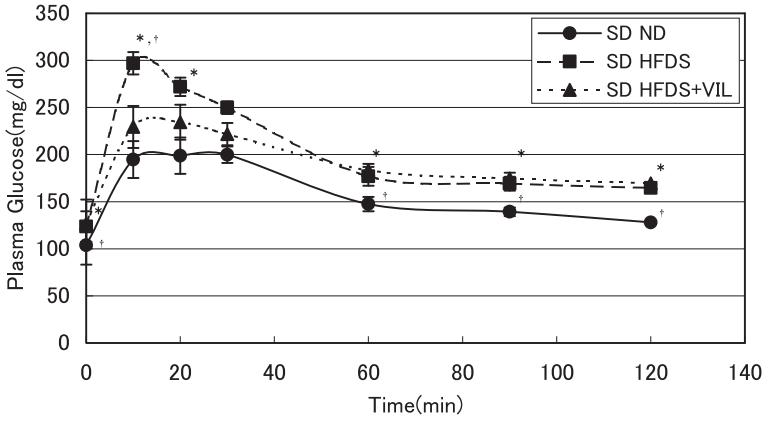

Fig. 2. Plasma glucose levels following the intraperitoneal glucose tolerance test (IPGTT) in rats fed a normal diet (ND), a high-fat diet with sucrose water (HFDS), or the HFDS plus 10 mg/kg per day, p.o., vildagliptin (VIL) from 4 to 8 months of age. Data are the mean ± SEM (n=7 rats in each group). $^{*}P<0.05$ compared with the ND group; $^{\dagger}P<0.05$ compared with the HFDS + VIL group (Mann-Whitney U-test).

IPGTT

Mean plasma glucose levels in each group after the IPGTT are shown in Fig. 2. There was an increase in plasma glucose levels in all three groups after glucose loading. However, at 0, 60, 90, and 120 min after loading, the increase in plasma glucose was significantly greater in the HFDS and HFDS + VIL groups than in the ND group. At 10 min after loading, the HFDS + VIL group showed a significant decrease in the plasma glucose spike compared with the HFDS group.

Discussion

In the present study, BW changes were not observed in rats administered VIL. Elevated levels of GLP-1, which acts on the central nervous system and suppresses appetite, can lead to weight loss[20, 21]. Weight changes do not appear to occur in humans taking DPP-4 inhibitors[22]. However, in the present study, the LW/BW ratio was increased in the group of rats receiving VIL, probably due to the accumulation of fat in the liver with the increase in GIP activity. GIP is known to promote lipoprotein lipase-induced uptake of fat into fat cells[23]. Conversely, GLP-1 inhibits fat accumulation in the liver through activation of AKT phosphorylation in liver cells, insulin signaling, and expression of peroxisome proliferator-activated receptor-α. Previous studies have reported incretin hormone-induced inhibition of fat accumulation in the liver[24, 25]. Therefore, the increase in the LW/BW ratio in the present study may have been due to differences in signal intensity from GIP and GLP-1 in response to VIL administration. It is also possible that the effects of VIL differ in rats and humans. Additional research is needed to clarify this point.

The mean HOMA-IR, which serves as an indicator of insulin resistance, tended to increase in rats administered VIL, and this may be related to the increased LW/BW ratio because fat accumulation in the liver contributes to increased insulin resistance[26, 27]. In the IPGTT, a

significant reduction in the glucose spike was seen in the HFDS + VIL group 10 min after administration of the glucose load compared with the HFDS group. The HFDS + VIL group also exhibited a tendency for an increase in HOMA-β, an index of insulin secretion. These results suggest that VIL can prophylactically inhibit the decrease in pancreatic β-cell function in T2D.

α-Glucosidase inhibitors (α-GIs) are currently used in Japan for the treatment of impaired glucose tolerance. The Study to Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) trial research group reported that acarbose administration reduces the risk of developing diabetes by 31% for every 3.3 years[4]. It has also been reported that voglibose administration can reduce the risk of developing diabetes similar to acarbose[28]. Activated GLP-1 concentrations were found to increase significantly with miglitol or acarbose administration[29]. The prevention of diabetes by α-GIs is possibly mediated via incretin hormones. The results obtained in the present study are analogous to those in previous studies of α-GIs, because VIL also increased GLP-1 concentrations. Additional trials in humans are needed to determine whether VIL can protect against the development of T2D.

Postprandial hyperglycemia is related to the development of vascular lesions[30, 31]. Moreover, reducing postprandial hyperglycemia in patients with impaired glucose tolerance reduces the incidence of cardiovascular disease[32, 33]. Lowering postprandial hyperglycemia with DPP-4 inhibitors is useful in preventing the complications associated with diabetes, and DPP-4 inhibitors are also useful in ameliorating impaired glucose tolerance.

In the present study, there was no change in plasma TC levels in rats administered VIL. However, TG levels decreased significantly in the HFDS + VIL group compared with the ND and HFDS groups. Based on our electrophoretic lipoprotein analyses, chylomicron levels were also significantly reduced in the HFDS + VIL versus the ND and HFDS groups. It is possible that VIL inhibits chylomicron synthesis. Inhibition of chylomicron synthesis in the small intestine by incretin has been suggested in various studies[15, 16, 34, 35]. High TG levels contribute to the risk of cardiovascular disease[17] ; therefore decreasing TGs is important in reducing that risk.

In the present study, VIL prevented the increase in glucose tolerance and decreased plasma TG levels in HFDS-fed Sprague-Dawley rats. These results suggest that VIL administration may contribute to the prevention of lifestyle-related disease.

Conflict of interest

The authors have declared no conflict of interest.

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