Original Article

Serum Betatrophin Levels and Clinical Features in Patients With Poorly Controlled Type 2 Diabetes

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Abstract

Background: Betatrophin is a hormone mainly secreted by the liver that influences lipid metabolisms. The main purposes of this study were to investigate the effect of canagliflozin (a sodium glucose transporter 2 inhibitor) on circulating betatrophin levels, and to investigate the correlation of various markers associated with glucose and lipid metabolisms with betatrophin in patients with poorly controlled type 2 diabetes.

Methods: Patients were randomly divided into a control group (n = 15) and a canagliflozin-treated group (n = 15). After hospitalization, the canagliflozin-treated group took 100 mg/day of canagliflozin for 3 days. Blood tests were performed at baseline and after 3 days of treatment.

Results: Canagliflozin treatment for 3 days did not significantly change fasting and postprandial serum betatrophin levels. On the other hand, betatrophin levels had a significant positive correlation with hemoglobin A1c, fasting plasma glucose, and high-density lipoprotein cholesterol levels at baseline.

Conclusions: The current study suggests that short-term treatment by canagliflozin does not influence circulating betatrophin levels, and that betatrophin is positively associated with markers of glycemic control and high-density lipoprotein cholesterol in patients with poorly controlled type 2 diabetes.

Keywords: Betatrophin; Canagliflozin; Type 2 diabetes

Introduction

Betatrophin, also known as an angiopoietin-like protein 8 (ANGPTL8) or lipasin, is a hormone produced mostly in liver in human [1-3]. A previously reported effect of betatrophin on regeneration of pancreatic β cells is currently considered to be negative [4-8]. However, some recent studies show positive association of circulating betatrophin levels with markers of glycemic control in patients with type 2 diabetes [9-11], although the association is not investigated fully in poorly controlled type 2 diabetes with the mean hemoglobin (Hb) A1c levels over 10%. The mechanisms and clinical significances of potentially these associations are yet not apparent fully.

On the other hand, recent clinical studies have also demonstrated the positive associations of circulating betatrophin levels with serum lipids levels in patients with impaired glucose tolerance (IGT) or type 2 diabetes [11, 12], and in healthy subjects [11-13]. Since it is suggested that betatrophin is associated with circulating low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) probably via the respectively different mechanisms [1], it is likely that the change of circulating betatrophin levels by the treatment influences these serum lipids levels. The production of betatrophin in the liver is greatly enhanced under insulin resistance caused by the administration of an insulin receptor blocker [4], and therefore it may be theoretically possible that the agents which can decrease circulating insulin levels, such as sodium glucose transporter 2 (SGLT2) inhibitors [14], increase circulating betatrophin levels, resulting in the change of serum lipids levels. However, it is still unclear whether these agents can influence circulating betatrophin levels in clinical practice.

In the current study, we studied the effect of canagliflozin (an SGLT2 inhibitor) on circulating betatrophin levels in patients with poorly controlled type 2 diabetes by the randomized open-label study design. We also investigated the correlation of betatrophin levels with the various circulating markers associated with glucose and lipid metabolisms at baseline in these patients. Before performing the study, we hypothesized that canagliflozin significantly may increase circulating betatrophin levels, and that betatrophin would positively associated with the markers of glycemic control and serum lipid levels even under the condition of poor glycemic control as this study.

Patients and Methods

Patients

The study, which was named CANARIA (the effect of CANA-
gliflozin (FPG), were 58.7 ± 11.4 years, 25.5 ± 5.0 kg/m², and 181.0 ± 65.6 mg/dL, respectively. There were no significant differences in age, BMI, HbA1c, and FPG between control and canagliflozin-treated groups.

Statistical analysis

Comparisons between the two groups for age, BMI, HbA1c and FPG were made using an unpaired t-test. The two time points for betatrophin in control or canagliflozin-treated group were compared using a paired t-test. In the correlation analysis of betatrophin with multiple variables, and multiple regression analysis with betatrophin as the dependent variable, these variables excluding aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT) and high-sensitivity C-reactive protein (hsCRP) followed a normal distribution, and the AST, ALT, GGT and hsCRP were log₁₀-transformed because of the skewed distributions. Multiple regression analysis was performed by using the stepwise
forward selection method. All statistical analyses were performed using Ekus eru-Toukei 2012 software (Social Survey Research Information Co., Ltd, Tokyo, Japan). A P value of less than 0.05 was accepted as indicating statistical significance (two-sided).

Results

The results for the change on various markers including glucose, insulin and lipids levels by canagliflozin-treatment for 3 days have been previously presented in detail [15]. The mean values of fasting and postprandial betatrophin in all 30 patients were 0.29 ± 0.21 and 0.25 ± 0.18 ng/mL, respectively. The change in fasting and in postprandial betatrophin levels between day 2 and day 5 in both the control and canagliflozin-treated groups is shown in Figure 1. Although a tendency toward higher levels of fasting and postprandial betatrophin at baseline in the control group compared with the canagliflozin-treated group was found, there were no statistical differences in these betatrophin levels between the control and the canagliflozin-treated groups: 0.36 ± 0.26 vs. 0.22 ± 0.12 ng/mL in fasting serum betatrophin levels (95% confidential interval for the difference: -0.01 to 0.29, P = 0.0688), and 0.30 ± 0.22 vs. 0.20 ± 0.12 ng/mL in postprandial serum betatrophin levels (95% confidential interval for the difference: -0.04 to 0.23, P = 0.1475). The meal test did not significantly change serum betatrophin levels in both groups (Fig. 1a, b). Canagliflozin-treatment did not have an effect on both fasting and postprandial serum betatrophin levels (Fig. 1b). The correlations of fasting serum betatrophin levels with multiple variables, and the results of stepwise analysis with fasting serum betatrophin levels as the dependent variables at baseline in all patients are shown in Table 1. There were significant positive correlations of betatrophin levels with HbA1c, FPG and HDL-C levels. Betatrophin levels also had significant positive association with HbA1c and HDL-C levels in multiple regression analysis.

The correlation of HbA1c and HDL-C levels with betatrophin levels is shown also in Figure 2.

Discussion

In the current study, we investigated the effect of canagliflozin (an SGLT2 inhibitor) on serum betatrophin levels. Because SGLT2 inhibitors decrease insulin levels [14], and because circulating betatrophin levels were remarkably elevated by administration of an insulin receptor blocker resulting in liver insulin resistance [4], we hypothesized that administration of SGLT2 inhibitors, such as canagliflozin, may increase circulating betatrophin levels and therefore may influence serum lipid concentrations. Contrary to our expectations, canagliflozin-treatment did not change both fasting and postprandial serum betatrophin levels, despite the fact that this agent clearly decreased serum insulin levels also in the current study [15]. At the present time, the reason for the result is unknown. However, the result in the current study was that in a short-term observation, therefore it may be possible that this influenced the result at least partially.

Betatrophin levels were not influenced by the meal test in the current study. This finding appears to support the result in a previous animal study where the production of betatrophin in the liver did not change with a normal diet [2], because betatrophin is mainly produced in the liver in humans [3]. In the current study, there was a significant positive correlation of betatrophin with HbA1c and FPG, corresponding with the results of some previous studies in patients with IGT or type 2 diabetes [9-11]. These associations appear to be robust because the positive correlations were evident even in the patients in this study who had mostly poorer glycemic control as reflected by their HbA1c levels (approximately 10%), compared with the previous studies [9-11]. The reason for the potentially positive association between betatrophin and glycemic control as evaluated by HbA1c or FPG remains unclear since the effect of betatrophin on regeneration of pancreatic β cells which was
Table 1. The Correlation of Betatrophin With Multiple Variables, and Stepwise Regression Analysis With Betatrophin as the Dependent Variable

| Simple | Multiple |
|--------|----------|
| R      | P        | β      | P        |
| Age (years) | -0.3471 | 0.0602 | -0.2685 | 0.0869 |
| BMI (kg/m²) | -0.1322 | 0.4862 | - | - |
| FPG (mg/dL) | 0.3890 | 0.0370* | - | - |
| HbA1c (%) | 0.5501 | 0.0016* | 0.3451 | 0.0331* |
| SBP (mm Hg) | -0.0463 | 0.8079 | - | - |
| DBP (mm Hg) | 0.2710 | 0.1474 | - | - |
| TG (mg/dL) | 0.0517 | 0.7862 | - | - |
| HDL-C (mg/dL) | 0.4754 | 0.0079* | 0.4616 | 0.0041* |
| LDL-C (mg/dL) | -0.0736 | 0.6991 | - | - |
| Cr (mg/dL) | -0.1780 | 0.3466 | - | - |
| Insulin (µU/mL) | 0.0409 | 0.8330 | - | - |
| UA (mg/mL) | -0.0279 | 0.8835 | - | - |
| U-CPR (µg/day) | -0.1494 | 0.4392 | - | - |
| Log₁₀-hsCRP (mg/L) | -0.0898 | 0.6434 | - | - |
| Log₁₀-AST (U/L) | 0.2760 | 0.1398 | - | - |
| Log₁₀-ALT (U/L) | 0.3333 | 0.0719 | 0.246 | 0.1244 |
| Log₁₀-GGT (U/L) | -0.0212 | 0.9113 | - | - |

R: Pearson’s correlation coefficient; β: standard partial regression coefficient; P: P value. P < 0.05 is defined as statistical significance (*). For hsCRP, AST, ALT and GGT, the data was log₁₀-transformed due to the skewed distribution. BMI: body mass index; FPG: fasting plasma glucose; HbA1c: hemoglobin A1c; SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; Cr: creatinine; UA: uric acid; U-CPR: urinary C-peptide immunoreactivity; hsCRP: high-sensitivity C reactive protein; AST: aspartate transaminase; ALT: alanine transaminase; GGT: gamma-glutamyl transpeptidase. Multiple regression analysis with betatrophin as the dependent variable was performed using stepwise forward selection method, and R² was 0.5691. The independent variables tested initially were all those used in simple regression analysis. Used cut-off F value was 2.

Figure 2. The correlation of betatrophin with HbA1c (a) and HDL-C (b). HbA1c: hemoglobin A1c; HDL-C: high-density lipoprotein cholesterol.
previously reported [4] is currently considered to be negative [5-8].

Betatrophin also had a significant positive correlation with HDL-C in accordance with the results of some previous studies [11, 12], although these associations were not found in other reports [19]. This positive association may be reasonable because it is suggested that betatrophin may be an important regulator of HDL-C [20]. On the other hand, in the current study, betatrophin did not correlate with LDL-C and TG, whose positive associations with betatrophin have been also reported [19]. It should be noted that the mechanisms for these associations may be respectively different although the detailed mechanisms are not yet apparent fully [1]. Speculatively it may be possible that poor glycemic control in the patients in this study influenced the potential associations of LDL-C or TG levels with betatrophin levels. Our results might suggest that the treatment of elevating betatrophin may be beneficial in the aspect of increasing HDL-C, which is generally considered to possess anti-atherogenic effects [21], especially in patients with poorly controlled type 2 diabetes.

In the current study, the numbers of the patients in both the control and the canagliflozin-treated groups were small, and the treatment time was for merely 3 days, which are the limitations of this study.

In conclusion, in patients with poorly controlled type 2 diabetes, canagliflozin-treatment for 3 days did not significantly change fasting and postprandial serum betatrophin levels. Circulating betatrophin levels had a positive correlation with HbA1c, FPG and HDL-C levels in these patients. For understanding these findings, a future study with larger numbers of patients and longer observation period is needed.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

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