

Dipeptidyl-peptidase IV inhibitor is effective in patients with type 2 diabetes with high serum eicosapentaenoic acid concentrations

Takafumi Senmaru¹, Michiaki Fukui^{1*}, Kanae Kobayashi¹, Hiroya Iwase¹, Shinobu Inada¹, Hiroshi Okada¹, Mai Asano¹, Masahiro Yamazaki¹, Goji Hasegawa¹, Naoto Nakamura¹, Masahiro Iwasaki², Daisuke Yabe³, Takeshi Kurose^{2,3}, Yutaka Seino³

ABSTRACT

Aims/Introduction: Eicosapentaenoic acid (EPA) stimulates glucagon-like peptide-1 (GLP-1) secretion in mice. We investigated the relationship between serum EPA concentrations and the efficacy of dipeptidyl-peptidase IV (DPP-4) inhibitor in patients with type 2 diabetes.

Materials and Methods: Serum EPA concentrations were measured in 62 consecutive patients with type 2 diabetes who were newly given DPP-4 inhibitor as a monotherapy or as an add-on therapy to oral hypoglycemic agents. The dosage of oral hypoglycemic agents was maintained during the observation period. After 24 weeks of treatment with DPP-4 inhibitor, we evaluated the relationships between a decrease in hemoglobin A1c from baseline and serum EPA concentrations, as well as age, sex, body mass index (BMI), hemoglobin A1c at baseline and usage of antidiabetic concomitant drugs.

Results: Hemoglobin A1c was significantly decreased from $8.1 \pm 1.1\%$ to $7.2 \pm 1.0\%$ by DPP-4 inhibitor. A decrease in hemoglobin A1c correlated with BMI ($r = -0.396$, $P = 0.0013$), age ($r = 0.275$, $P = 0.0032$), hemoglobin A1c at baseline ($r = 0.490$, $P < 0.0001$) and log EPA ($r = 0.285$, $P = 0.0246$). Multiple regression analysis showed that BMI ($\beta = -0.419$, $P = 0.0002$), hemoglobin A1c at baseline ($\beta = 0.579$, $P < 0.0001$) and log EPA ($\beta = 0.220$, $P = 0.0228$) were independent determinants of decrease in hemoglobin A1c.

Conclusions: DPP-4 inhibitor is effective in patients with type 2 diabetes with high serum EPA concentrations. (*J Diabetes Invest*, doi: 10.1111/j.2040-1124.2012.00220.x, 2012)

KEY WORDS: Dipeptidyl-peptidase IV inhibitor, Eicosapentaenoic acid, Glucagon-like peptide-1

INTRODUCTION

Glucagon-like peptide-1 (GLP-1), the most potent incretin hormone, stimulates glucose-induced insulin secretion and inhibits glucagon secretion, which consequently leads to a decrease in hepatic glucose production and blood glucose levels¹. In addition to its ability to modulate insulin and glucagon secretion, GLP-1 inhibits gastric emptying and gastric acid secretion², which suppress appetite and energy intake in obese individuals³, and in patients with type 2 diabetes⁴.

GLP-1 is secreted from intestinal L cells in response to ingestion of nutrients, including carbohydrate and lipids⁵. A previous study showed that alpha-linolenic acid (α -LA) promoted GLP-1 secretion through the stimulation of G-protein-coupled receptor (GPR) 120, which is abundantly expressed in the intestine, in mice⁶. Furthermore, a recent study has shown that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), metabolites of α -LA, stimulate endogenous GLP-1 secretion in mice⁷. These reports show that ω -3 polyunsaturated fatty acids (PUFA), including α -LA, EPA and DHA, increase GLP-1 secretion in mice. However, no evidence that ω -3 PUFA ingestion increases GLP-1 secretion is available in humans.

As compared with Americans and Europeans, Japanese eat more fish, which is a major source of EPA and DHA. Hence, serum EPA or DHA concentrations are higher in Japanese than those in Americans or Europeans^{8,9}. Evidence that fish intake prevents atherosclerosis-related cardiovascular disease have been accumulating^{10,11}. A recent study has shown a significant inverse correlation between serum EPA or DHA concentrations and cardiovascular risk¹². Furthermore, some studies showed that fish intake reduced the rates of incidence for diabetes in patients with metabolic syndrome, and reduced the rates of death in patients with type 2 diabetes^{13,14}. However, the direct effects of EPA or DHA consumption on glycemic control in patients with type 2 diabetes have remained uncertain.

GLP-1 is rapidly inactivated by the enzyme named dipeptidyl-peptidase IV (DPP-4)¹⁵. DPP-4 inhibitor increases endogenous active GLP-1 levels through inhibition of DPP-4 enzyme

¹Department of Endocrinology and Metabolism, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto, ²Division of Metabolism and Clinical Nutrition, and ³Division of Diabetes, Clinical Nutrition and Endocrinology, Kansai Electric Power Hospital, Osaka, Japan

*Corresponding author: Michiaki Fukui Tel: +81-75-251-5505 Fax: +81-75-252-3721 E-mail address: sayarinapm@hotmail.com

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activity, leading to increased circulating insulin levels and decreased blood glucose levels. The circulating GLP-1 levels were reported to be lower in patients with type 2 diabetes than in non-diabetic patients¹. Therefore, DPP-4 inhibitor might be more effective in increasing circulating GLP-1 levels in patients with type 2 diabetes if they have low GLP-1 levels.

Based on these lines of evidence, we hypothesized that DPP-4 inhibitor would be effective in patients with type 2 diabetes with high serum ω -3 PUFA concentrations. In the present study, therefore, we investigated the relationship between serum EPA or DHA concentrations and a decrease in hemoglobin A1c in patients with type 2 diabetes prescribed DPP-4 inhibitors.

MATERIALS AND METHODS

Patients

Serum EPA and DHA concentrations were measured in 62 consecutive patients with type 2 diabetes recruited from the outpatient clinic at Kyoto Prefectural University of Medicine, Kyoto, Japan, who were newly given DPP-4 inhibitor as a monotherapy or as an add-on therapy to oral hypoglycemic agents (OHA). Patients with advanced renal dysfunction (serum creatinine concentration was equal to or more than 2.0 mg/dL) were excluded from the present study. Sitagliptin at a daily dose of 50 mg, which was the standard dose in Japanese patients, was continuously given once daily during the observation period. The prior diet, exercise program and dosage of OHA in the study patients were maintained during the observation period. After 24 weeks of treatment with DPP-4 inhibitor, we evaluated the relationships between a decrease in hemoglobin A1c from baseline and serum EPA or DHA concentrations, as well as age, sex, body mass index (BMI), hemoglobin A1c at baseline and usage of antidiabetic concomitant drugs. Type 2 diabetes was diagnosed according to the report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus¹⁶. Approval for the study was obtained from the local research ethics committee, and informed consent was obtained from all patients. The study was carried out in accordance with the Declaration of Helsinki.

Biochemical Analyses

Serum EPA and DHA concentrations were measured at SRL Inc., Tokyo, Japan. Serum total cholesterol, high-density lipoprotein cholesterol and triglyceride concentrations were assessed using standard enzymatic methods. Hemoglobin A1c was assayed using high-performance liquid chromatography and was expressed as a National Glycohemoglobin Standardization Program unit as recommended by the Japan Diabetes Society¹⁷.

Statistical Analysis

Means, medians or frequencies of potential confounding variables were calculated. Skewed variables, such as EPA and DHA, are presented as the median (interquartile range), and continuous variables are presented as the mean \pm standard deviation (SD). Paired *t*-test was carried out to assess the statistical significance of difference in hemoglobin A1c between baseline and

after DPP-4 inhibitor treatment, and unpaired *t*-test was carried out to assess the statistical significance of difference in a decrease in hemoglobin A1c between groups using Stat View software (version 5.0; SAS Institute, Cary, NC, USA). Because EPA and DHA showed skewed distributions, logarithm (log) transformation was carried out before performing correlation and regression analysis. The relationship between log EPA, log DHA or log (EPA + DHA) and a decrease in hemoglobin A1c was examined by linear regression analysis. In multiple regression analysis to assess the effects of various factors on decrease in hemoglobin A1c, we included independent variables that were significantly correlated with a decrease in hemoglobin A1c in the univariate analyses. A *P*-value <0.05 was considered statistically significant.

RESULTS

Characteristics of the 62 patients with type 2 diabetes enrolled in the present study are shown in Table 1. Mean hemoglobin A1c at baseline was $8.1 \pm 1.1\%$. The median (interquartile range) serum EPA concentration was $62.6 \mu\text{g/mL}$ (39.5–88.0) and the median (interquartile range) serum DHA concentration was $139.1 \mu\text{g/mL}$ (109.4–165.7). Among 62 patients, 16 patients were given DPP-4 inhibitor as a monotherapy and 46 patients were given it as an add-on therapy (34 sulfonylurea, 25 metformin, 8 pioglitazones, 5 alpha-glucosidase inhibitor).

Mean hemoglobin A1c was significantly decreased from $8.1 \pm 1.1\%$ at baseline to $7.2 \pm 1.0\%$ at 24 weeks after administration of DPP-4 inhibitor. Correlation between log EPA, log DHA or log (EPA + DHA) and other variables are shown in Table 2. Log EPA and log (EPA + DHA) correlated with a decrease in hemoglobin A1c. Log DHA tended to correlate with a decrease in hemoglobin A1c, but it did not reach statistical significance.

Table 1 | Clinical characteristics of patients with type 2 diabetes at baseline

Characteristics	
<i>n</i>	62
Sex (male/female)	30/32
Age (years)	63.2 ± 11.6
Age at onset of diabetes (years)	52.3 ± 13.4
Duration of diabetes (years)	10.6 ± 8.3
Body mass index (kg/m^2)	24.1 ± 4.0
Hemoglobin A1c (%)	8.1 ± 1.1
Systolic blood pressure (mmHg)	127 ± 18
Diastolic blood pressure (mmHg)	71 ± 12
Total cholesterol (mmol/L)	4.74 ± 0.80
Triglycerides (mmol/L)	1.03 ± 0.33
High-density lipoprotein cholesterol (mmol/L)	1.42 ± 0.39
Diabetic therapy (diet/oral hypoglycemic agents)	16/46
Eicosapentaenoic acid ($\mu\text{g/mL}$)	62.6 (39.5–88.0)
Docosahexaenoic acid ($\mu\text{g/mL}$)	139.1 (109.4–165.7)

Data are *n*, mean \pm SD or median (interquartile range).

Table 2 | Correlation between log eicosapentaenoic acid, log docosahexaenoic acid or log(eicosapentaenoic acid + docosahexaenoic acid) and other variables

	Log EPA		Log DHA		Log(EPA + DHA)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age	0.169	0.1833	0.222	0.0836	0.210	0.1010
Age at onset of diabetes	0.167	0.2283	0.313	0.0232	0.261	0.0619
Duration of diabetes	0.083	0.5516	-0.127	0.3705	-0.026	0.8537
Body mass index	-0.228	0.0702	-0.091	0.4822	-0.166	0.1990
Hemoglobin A1c at baseline	-0.022	0.8631	-0.001	0.9924	-0.034	0.7930
Systolic blood pressure	-0.112	0.3810	0.181	0.1598	0.016	0.9038
Diastolic blood pressure	-0.058	0.6492	0.098	0.4520	-0.003	0.9788
Total cholesterol	-0.154	0.2811	-0.115	0.4344	-0.149	0.3103
Triglyceride	-0.165	0.2481	0.221	0.1280	0.065	0.2773
High-density lipoprotein cholesterol	0.198	0.1173	0.096	0.4605	0.141	0.2773
Decrease in hemoglobin A1c	0.285	0.0246	0.206	0.1077	0.260	0.0411

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Table 3 | Multiple regression analysis on decrease in hemoglobin A1c

	β	<i>P</i>
Age	0.078	0.4500
Body mass index	-0.419	0.0002
Hemoglobin A1c at baseline	0.579	<0.0001
Antidiabetic concomitant drugs	0.012	0.9144
Log (eicosapentaenoic acid)	0.220	0.0228

Presence of antidiabetic concomitant drugs (no = 0, yes = 1).

A decrease in hemoglobin A1c correlated with BMI ($r = -0.396$, $P = 0.0013$), age ($r = 0.275$, $P = 0.0032$), hemoglobin A1c at baseline ($r = 0.490$, $P < 0.0001$), log EPA ($r = 0.285$, $P = 0.0246$) and log (EPA + DHA) ($r = 0.260$, $P = 0.0411$). There were no significant differences in decrease in hemoglobin A1c between male and female patients ($1.0 \pm 0.8\%$ vs $1.0 \pm 0.9\%$, $P = 0.9758$). Decrease in hemoglobin A1c was greater in patients treated with antidiabetic concomitant drugs compared with that in patients without those ($1.1 \pm 0.9\%$ vs $0.6 \pm 0.4\%$, $P = 0.0342$). In the multiple regression analysis to examine the effects of variables on the decrease in hemoglobin A1c, we included the following independent variables, which were significantly correlated with a decrease in hemoglobin A1c in the univariate analyses: age, BMI, hemoglobin A1c at baseline, usage of antidiabetic concomitant drugs and log EPA. Multiple regression analysis showed that BMI, hemoglobin A1c at baseline and log EPA were independent determinants of a decrease in hemoglobin A1c (Table 3).

DISCUSSION

In the present study, serum EPA concentrations correlated with a decrease in hemoglobin A1c. This correlation remained significant after adjustment for age, BMI, hemoglobin A1c at baseline and usage of antidiabetic concomitant drugs.

The median (interquartile range) serum EPA concentration was 62.6 $\mu\text{g/mL}$ (39.5–88.0; the mean serum EPA concentration was $68.6 \pm 34.1 \mu\text{g/mL}$), which was almost consistent with previous reports of the EPA concentration in general Japanese^{9,18}. Serum EPA concentrations increase with age in apparently healthy Japanese¹⁸. In the present study, however, there was no significant correlation between log EPA and age in patients with type 2 diabetes.

DPP-4 inhibitor increases endogenous active GLP-1 levels through prevention of GLP-1 degradation, leading to an increase in circulating insulin levels and a decrease in blood glucose levels. Because of the pharmacological action of DPP-4 inhibitor on insulin secretion, DPP-4 inhibitor is effective in type 2 diabetic patients with impaired insulin secretion, which is a characteristic of Japanese patients with type 2 diabetes. Indeed, as compared with American and European patients with type 2 diabetes, Japanese patients with type 2 diabetes might be higher responders to DPP-4 inhibitor^{19,20}. Interestingly, the present study has shown a strong correlation between BMI and decrease in hemoglobin A1c, suggesting that DPP-4 inhibitor is more effective in lean patients who generally have impaired insulin secretion, rather than in obese patients with insulin resistance. This is consistent with a recent study reporting the association of BMI with efficacy of the DPP-4 inhibitor, sitagliptin²¹.

Previous studies showed that DPP-4 inhibitor was effective as an add-on therapy to some antidiabetic drugs, including metformin²², pioglitazone²³ and sulfonylurea²⁴. The profile of diabetic therapy before DPP-4 inhibitor administration was as follows: 16 used diet, 15 received sulfonylurea monotherapy, 12 received metformin monotherapy, eight received sulfonylurea and metformin, five received sulfonylurea and pioglitazone, two received sulfonylurea and alpha-glucosidase inhibitor, and four took three drugs. We analyzed the correlations between serum EPA concentrations and a decrease in hemoglobin A1c in patients with DPP-4 inhibitor monotherapy, DPP-4 inhibitor add-on therapy

to sulfonylurea, to metformin, and to sulfonylurea and metformin. Log EPA tended to correlate with a decrease in hemoglobin A1c only in patients with DPP-4 inhibitor add-on therapy to metformin, although it did not reach statistical significance ($r = 0.526$, $P = 0.0796$). There were no significant correlations between log EPA and decrease in hemoglobin A1c in other treatment groups. It seems to be difficult to show these results because of the small sample size in each treatment group.

In basic studies, it has been shown that GLP-1 secretion is induced by ω -3 PUFA administration^{6,7}. Hirasawa *et al.*⁶ have shown that GPR120 functioned as a receptor for unstructured long-chain fatty acids, and that the stimulation of GPR120 with α -LA promoted GLP-1 secretion *in vitro* and *in vivo*. Furthermore, GPR120 has been shown to be abundantly expressed in the intestine both in humans and mice. Morishita *et al.*⁷ have shown that the intracolonic administration of EPA stimulated GLP-1 secretion *in vivo*. However, the mechanism of GLP-1 secretion by ω -3 PUFA has been less clear. In the present study, furthermore, serum DHA concentrations did not show a significant association with the efficacy of DPP-4 inhibitor, although they tended to positively correlate with a decrease in hemoglobin A1c. Further studies are required to clarify the mechanism of ω -3 PUFA-stimulated GLP-1 secretion. Taken together with these previous studies, and the ability of DPP-4 inhibitor to increase endogenous active GLP-1 levels, the present result that serum EPA concentrations correlated with the efficacy of DPP-4 inhibitor might provide indirect evidence that EPA has a potential to promote GLP-1 secretion in humans.

Limitations of the present study included a small sample size and a short observation period. In addition, we did not have data for GLP-1 and insulin concentrations. Therefore, the direct link between serum EPA concentration and GLP-1 or insulin concentrations could not be assessed. Furthermore, because ingested fatty acids are generally absorbed within the small intestine, EPA might be expected to act on GPR120 inadequately after oral administration. There are no reports of a linear relationship between EPA intake and serum EPA concentrations. However, serum EPA concentrations positively correlated with EPA intake assessed by a self-administered diet history questionnaire²⁵ in a subgroup of patients with type 2 diabetes ($n = 40$, $r = 0.419$, $P = 0.0066$). Therefore, we hope that serum EPA concentrations could be a marker of EPA intake. To our knowledge, this is the first report of the relationship between serum EPA concentrations and the efficacy of DPP-4 inhibitor in patients with type 2 diabetes, and suggests new avenues for research into incretin therapies. Large interventional studies are required to better assess the contributions of EPA to the efficacy of DPP-4 inhibitor in patients with type 2 diabetes.

In conclusion, DPP-4 inhibitor is effective in patients with type 2 diabetes with high serum EPA concentrations. The present study would contribute to the future strategies for anti-diabetic therapies.

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All authors declare no conflict of interest.

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