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Short Communication

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Elevated Serum Growth Differentiation Factor 15 Levels as a Potential Biomarker of the Efficacy of Imeglimin in Individuals With Type 2 Diabetes Mellitus: An Exploratory Study

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Abstract

Background: The aim of the present study was to conduct a prospective observational study to explore the effects of imeglimin on systemic energy metabolism/body composition and to identify potential mitochondria-related biomarkers of the efficacy of the drug in clinical settings.

Methods: In this prospective observational study, 16 participants with type 2 diabetes mellitus in the diabetes clinic of Kyoto University Hospital were enrolled. Individuals were started on imeglimin as monotherapy or add-on therapy.

Results: After 3 months under imeglimin treatment, there was no significant change in basal metabolism or body composition. However, serum levels of growth differentiation factor 15 (GDF15) were higher while those of serum fibroblast growth factor 21 and urine 8-hydroxy-2′-deoxyguanosine were not changed. Additional *in vitro* examination revealed that imeglimin induces GDF15 protein release from human hepatocytes.

Conclusions: Three-month imeglimin treatment increased serum GDF15 levels in clinical type 2 diabetes mellitus patients along with little change in basal metabolism or body composition, suggesting GDF15 as a potential marker for the efficacy of imeglimin.

Keywords: GDF15; Imeglimin; Diabetes mellitus

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Introduction

Imeglimin, a recently developed anti-diabetic drug with a structure similar to that of metformin, is reported to improve mitochondrial function and glycemic control in type 2 diabetes mellitus [1]. Imeglimin and metformin both inhibit hepatic gluconeogenesis and stimulate glucose uptake in peripheral tissues, while imeglimin also enhances glucose-induced insulin secretion [2]. Recent studies indicate that imeglimin may preserve beta-cell mass by maintaining or restoring the functional and structural integrity of their mitochondria [3]. However, mitochondria-related biomarkers of systemic energy metabolism have not been investigated in clinical settings.

Growth differentiation factor 15 (GDF15) and fibroblast growth factor 21 (FGF21) are both recognized as biomarkers for diagnosis and severity assessment of mitochondrial diseases including mitochondrial diabetes mellitus [4]. GDF15, a member of the transforming growth factor beta superfamily, is increased by metformin and may mediate its beneficial effect on body weight and energy balance [5]. FGF21, a member of the FGF19 subfamily, reflects insulin sensitivity and energy expenditure [6]. Interestingly, while increased urine 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels have been reported to indicate mitochondrial oxidative damage in clinical and experimental studies [7], imeglimin has been shown to reduce 8-OHimdG levels in diabetes model mice [7].

In this study, we evaluated the mitochondria-related protein levels of GDF15, FGF21, and 8-OhdG, as well as basal metabolism and body composition, in individuals with type 2 diabetes mellitus under imeglimin treatment.

Materials and Methods

Study protocol

This prospective observational study was carried out in the diabetes clinic of Kyoto University Hospital (Kyoto, Japan). Individuals with type 2 diabetes mellitus who were started on imeglimin as monotherapy or add-on therapy between March

1, 2022, and September 30, 2022, were enrolled. Inclusion and exclusion criteria are shown here (Supplementary Material 1, jocmr.elmerjournals.com/). Clinical information and data including sex, age, duration of diabetes, diabetic retinopathy and nephropathy, laboratory data, and therapeutic agents before, 3, and 6 months after the initiation of imeglimin (2,000 mg/day) treatment, were collected (Supplementary Material 2, jocmr.elmerjournals.com/). Fasting serum GDF15 (DGD150; R&D System, USA) and FGF21 (DF2100; R&D System, USA), urinary 8-OHdG (Nikken seil Co. Ltd., Japan), basal metabolism by indirect calorimeter (MedGem, Microlife Medical Home Solutions, Inc, USA), and body composition by bioelectrical impedance analysis (InBody 720; InBody Japan Inc., Japan) were evaluated before and 3 months after initiation. The protocol of the study was approved by the Kyoto University Graduate School and Faculty of Medicine, Ethics Committee (R3300, trial registration: UMIN000046316) and conforms to the provisions of the Declaration of Helsinki.

In vitro GDF15 secretion assay

Imeglimin-induced GDF15 secretion from the human hepatocyte cell line HepG2 was evaluated (Supplementary Material 3, jocmr.elmerjournals.com/).

Statistical analysis

Data are presented as average ± standard deviation. Differences between two groups were analyzed by the unpaired Student's *t*-test, Wilcoxon test or Fisher's exact probability test, as appropriate. P value of < 0.05 was considered statistically significant. JMP Pro®, version 17.0 (SAS Institute Inc., NC, USA) was used to carry out the statistical analysis. A scatter plot was made using JMP Pro® version 17.0.

Results

Characteristics of the subjects

Sixteen individuals with type 2 diabetes mellitus (monotherapy/add-on therapy. n = 2/14) were enrolled (Supplementary Material 2, jocmr.elmerjournals.com/); their profiles are shown in Table 1. After 3 months under imeglimin treatment, glycated hemoglobin (HbA1c) and glycoalbumin (GA) decreased by 0.3% and 1.5%, respectively; the fasting C-peptide index (CPI) and homeostatic model assessment of beta cell function (HOMA-β) increased by 0.7 and 22.9, respectively. No significant changes in homeostatic model assessment for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), lipids, renal function, or liver function were observed. Body fat percentage and resting metabolic rate did not significantly change. GDF15 significantly increased ($P < 0.05$), whereas no significant changes in FGF21 or urine 8-OHdG were observed. The changes in GDF15 during 3-month treatment with imeglimin (ΔGDF15) demonstrated a significant negative correlation with those in GA levels ($r = -0.56$, $P < 0.05$) (Fig. 1), whereas ΔGDF15 had no significant correlation with Δbody mass index (BMI). ΔGDF15 had significant positive correlations with diabetes history, baseline HbA1c and GA levels, respectively, whereas ΔGDF15 showed a significant negative correlation with baseline BMI (Fig. 1). ΔGDF15 tended to be negatively correlated with ΔHbA1c ($r = -0.23$), although the difference was not statistically significant ($P = 0.40$). Interestingly, the nine patients who were taking metformin at baseline tended to have higher ΔGDF15 than the seven patients who were not taking metformin (149.9 ± 155.5 ng/mL vs. 75.2 ± 192.2 ng/mL, $P = 0.42$), although the differences did not reach statistical significance. Clinical follow-up data of 13 of the 16 patients were obtained after 6 months: HbA1c was 0.5% lower and glycoalbumin was 1.5% lower in these patients. Adverse effects during the observation period are shown in Table 1.

Effects of imeglimin in HepG2 cells

Imeglimin-induced GDF15 release from HepG2 cells was evaluated. After 48 h incubation, imeglimin-treated HepG2 cells released a higher concentration of GDF15 compared to that of control, in a dose-dependent manner (Fig. 2).

Discussion

Imeglimin as monotherapy or add-on therapy resulted in improved glycemic control and β-cell function-related indices (fasting CPI and HOMA-β) in a clinical setting. Increased serum GDF15 levels were noted, but there were no significant changes in serum FGF21, urine 8-OHdG, or basal metabolism and body composition.

GDF15 has been reported to be a key molecule in the beneficial effects of metformin on glycemic control and energy metabolism via hepatocytes and intestinal cells [5]. A genome-wide association study suggested that elevated GDF15 expression across various tissues may contribute to improved glycemic control in type 2 diabetes patients taking metformin [8]. Consistent with their similar chemical structure, a recent study found GDF15 mRNA expression in cultured hepatocytes to be notably increased by imeglimin [9]. Moreover, the additional GDF15 protein would be supplied at least partially from hepatocytes via the *in vitro* experiment conducted at the concentration of imeglimin which was close to the serum maximum concentration after the 7-day clinical administration according to the interview form [10].

Interestingly, ΔGDF15 showed significant negative correlations with ΔGA in the present study. This finding further suggests GDF15 as a potential biomarker for the efficacy of imeglimin in individuals with type 2 diabetes mellitus. Indeed, it has been reported that GA assesses short-term efficacy of imeglimin more accurately than HbA1c [11]. In the present study, diabetes history, baseline HbA1c and GA levels, and baseline BMI showed significant correlations with ΔGDF15, which may have implications regarding the mechanism un-

Table 1. Profile of the Subjects and Laboratory Data Before, 3, and 6 Months After Initiation of Imeglimin Treatment, and Adverse Effects of Imeglimin During the Study

	Before (n = 16)	3 months (n = 16)	6 months (n = 13)	P value (before vs. 3 months)	P value (before vs. 6 months)
Age (year)	67.5 ± 13.0				
Male (%)	43.8				
Height (cm)	159.1 ± 10.1				
Weight (kg)	64.4 ± 11.0				
BMI (kg/m^2)	25.4 ± 3.2				
Duration of diabetes (year)	11.3 ± 13.1				
Concomitant anti-diabetic medications					
Metformin (%)	56.3				
DPP-4i (%)	37.5				
SGLT2i (%)	25				
GLP-1RA (%)	12.5				
Sulfonylurea (%)	12.5				
Glinide (%)	6.3				
α-glucosidase inhibitor (%)	0				
Complications					
Retinopathy (NDR/NPDR/PPDR/PDR) (%)	87.5/12.5/0/0				
Nephropathy (stage 1/2/3/4/5) (%)	68.5/26.3/5.2/0/0				
Coronary artery disease (%)	12.5				
Body weight (kg)	64.4 ± 11.0	63.4 ± 10.1		0.80	
BMI (kg/m^2)	25.4 ± 3.2	25.0 ± 3.0		0.74	
Waist-hip ratio	0.97 ± 0.04	0.96 ± 0.04		< 0.05	
HbA1c (%)	7.3 ± 0.7	7.0 ± 0.6	6.7 ± 0.4	< 0.01	< 0.001
GA (%)	17.4 ± 3.7	15.9 ± 2.8	15.1 ± 1.9	< 0.001	< 0.05
Fasting CPI	1.6 ± 0.6	2.3 ± 1.5		< 0.01	
HOMA-β	44.0 ± 35.6	66.9 ± 50.6		< 0.05	
HOMA-IR	2.4 ± 1.4	3.2 ± 4.2		0.63	
QUICKI	0.35 ± 0.04	0.34 ± 0.04		0.56	
AST (U/L)	24.3 ± 9.4	24.8 ± 14.3		0.30	
ALT (U/L)	30.6 ± 19.9	27.0 ± 16.1		0.16	
γGTP (U/L)	28.4 ± 15.6	25.3 ± 14.0		0.23	
TG (mg/dL)	143.8 ± 54.5	150.4 ± 66.2		0.62	
LDL-CHO (mg/dL)	98.1 ± 27.1	101.2 ± 30.9		0.49	
HDL-CHO (mg/dL)	57.7 ± 13.3	55.7 ± 12.6		0.41	
Cre (mg/dL)	0.75 ± 0.13	0.79 ± 0.18		0.17	
Serum FGF21 (pg/mL)	794.0 ± 1,236.2	483.1 ± 382.9		0.11	
Serum GDF15 (ng/mL)	308.5 ± 134.0	425.7 ± 171.3		< 0.05	
Urine 8-OHdG (ng/mL)	7.2 ± 3.5	7.0 ± 2.5		0.81	
Type IV collagen (ng/mL)	3.4 ± 0.8	3.3 ± 0.8		0.33	
Ferritin (ng/mL)	93.1 ± 57.7	89.5 ± 58.3		0.32	
CRP (mg/dL)	0.17 ± 0.24	0.17 ± 0.25		0.77	
Skeletal muscle mass (kg)	22.7 ± 5.4	22.7 ± 5.2		0.99	

Table 1. Profile of the Subjects and Laboratory Data Before, 3, and 6 Months After Initiation of Imeglimin Treatment, and Adverse Effects of Imeglimin During the Study - *(continued)*

	Before (n = 16)	3 months (n = 16)	6 months (n = 13)	P value (before vs. 3 months)	P value (before vs. 6 months)
Body fat percentage (%)	31.5 ± 8.1	31.7 ± 9.6		0.97	
Resting metabolic rate (kcal)	1,284.0 ± 319.9	1,198.0 ± 216.5		0.45	
Total energy expenditure (kcal)	1,491.0 ± 371.4	1,391.3 ± 251.4		0.44	
Adverse effects of imeglimin during the study					
Metabolic and nutritional disorders		Loss of appetite (6.3%, n = 1/16)			
Gastrointestinal disorders		Diarrhea (18.8%, n = 3/16), nausea (6.3%, n = 1/16), constipation (6.3%, n = 1/16), soft stool (6.3%, n = 1/16), abdominal bloating (6.3%, n = 1/16)			
Laboratory abnormalities		AST elevation (6.3%, n = 1/16)			

BMI: body mass index; DPP-4i: dipeptidyl peptidase 4 inhibitor; SGLT2i: sodium-glucose cotransporter 2 inhibitor; GLP-1RA: glucagon-like peptide-1 receptor agonist; NDR: no diabetic retinopathy; NPDR: non-proliferative diabetic retinopathy; PPDR: pre-proliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy; HbA1c: glycated hemoglobin; GA: glycoalbumin; CPI: C-peptide index; HOMA-β: homeostatic model assessment of β-cell function; HOMA-IR: homeostasis model assessment-insulin resistance; QUICKI: quantitative insulin check index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γGTP: γ-glutamyl transpeptidase; LDL-CHO: low-density lipoprotein cholesterol; HDL-CHO: high-density lipoprotein cholesterol; Cre: creatinine; FGF21: fibroblast growth factor 21; GDF15: growth differentiation factor 15; CRP: C-reactive protein: 8-OHdG: 8-hydroxy-2′-deoxyguanosine.

derlying the differing responses to imeglimin of patients with type 2 diabetes mellitus [12-15]. In fact, participants with longer diabetes history showed significantly higher GA levels at baseline. As ΔGDF15 also had a significant positive correlation with baseline GA, the GA levels of the subjects with longer diabetes history might underlie the relationship between ΔGDF15 and diabetes history noted in this study. Further investigation is required to establish assessment of the efficacy of imeglimin based on GDF15 values.

Additionally, the present study noted no significant change in basal metabolism during imeglimin treatment, which is consistent with a previous report [12]. We also found no significant influence of imeglimin on body composition.

This exploratory study has several limitations including the relatively small number of subjects, which limits the generalizability of the findings. In addition, our 3-month treatment period is insufficient to assess the long-term effects of the drug, particularly in terms of the sustainability of the GDF15 levels and the metabolic changes. In fact, in a previous report [5], the association between change in body weight and change in plasma GDF15 between 0 and 18 months among metformin-treated participants were assessed. In the future, longer-term, direct comparisons between imeglimin and other established treatments for type 2 diabetes are warranted. Furthermore, in-

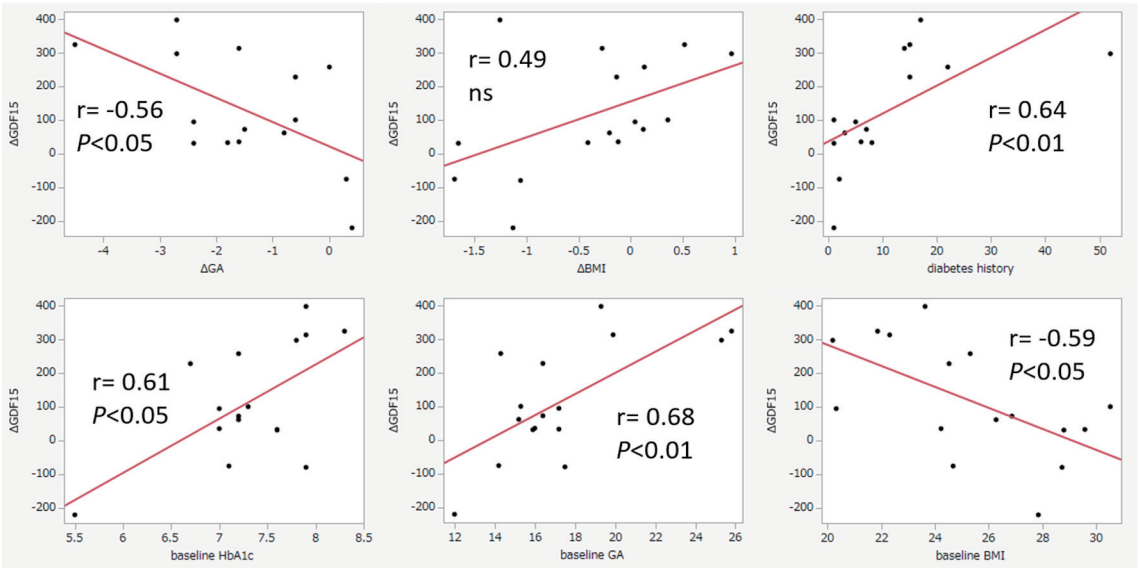

Figure 1. Correlation between Δgrowth differentiation factor 15 (GDF15) and Δglycoalbumin (GA), Δbody mass index (BMI), diabetes history, baseline HbA1c, baseline GA, baseline BMI. Correlation between ΔGDF15 and each parameter is shown as "r". ΔGDF15 vs. each parameter, *P < 0.05. ns: not significant; HbA1c: glycated hemoglobin.

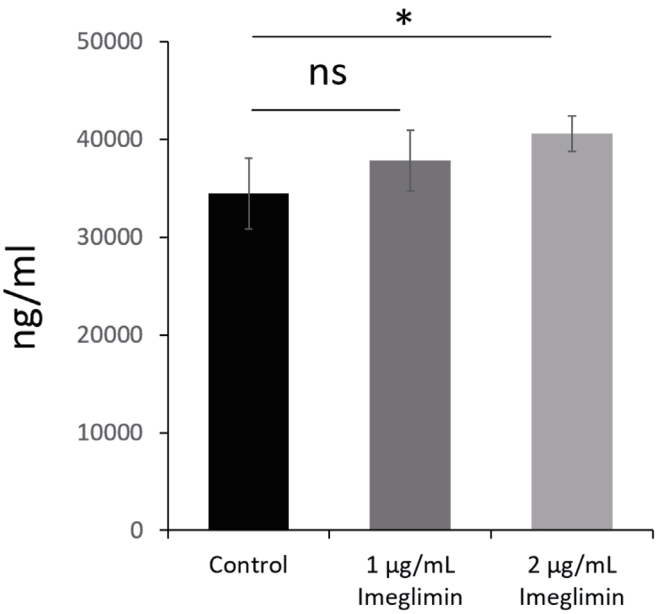

Figure 2. GDF15 released from imeglimin-treated HepG2 cells. Control (n = 3), 1 μg/mL imeglimin-treated (n = 3), 2 μg/mL imeglimin-treated HepG2 cells (n = 3). Data are mean ± SD. Control vs. imeglimin-treated, *P < 0.05, ns: not significant; SD: standard deviation.

clusion of patients with various degrees of renal or hepatic impairment would provide insights into the broader applicability of imeglimin.

In conclusion, 3-month imeglimin treatment increased serum GDF15 levels in clinical type 2 diabetes mellitus patients along with little change in basal metabolism or body composition, suggesting GDF15 as a potential marker for the efficacy of imeglimin.

Supplementary Material

Suppl 1. Selection and exclusion criteria.

Suppl 2. Time course of the study. Three participants were transferred to a family clinic between 3 and 6 months.

Suppl 3. Cell culture and *in vitro* GDF15 secretion assay.

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Conflict of Interest

None to declare.

Informed Consent

The written informed consent was obtained from the subject.

Author Contributions

NW contributed to data collection, analysis, discussions of this study and wrote the manuscript. TM contributed to planning, design, data collection, analysis, discussions, and editing the manuscript. MF contributed to performing *in vitro* experiments. KS (Kentaro Sakaki), SO, YS, KA, AO, SN, EJ, MM, RF contributed to collecting clinical data. KS (Kenichiro Shide) and KW contributed to planning and discussions. DY and NI contributed to discussions and editing the manuscript. NH contributed to planning, design, discussions and editing the manuscript. All authors contributed to the article and approved the submitted version.

Data Availability

The data supporting the findings of this study are available from the corresponding author on reasonable request.

Abbreviations

GDF15: growth differentiation factor 15; FGF21: fibroblast growth factor 21; 8-OhdG: urine 8-hydroxy-2′-deoxyguanosine; GA: glycoalbumin; CPI: C-peptide index

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