cifically expressed in K-cells plays a critical role in differentiation and GIP production of K-cells in collaboration with Pdx1. It was reported that Rfx6 mRNA expression is regulated by transcription factor neurogenin 3 (Ngn3) in the pancreas of the mouse fetus (30). In another report, no colocalization of Pdx1-expressing cells and Rfx6-expressing cells was found in pancreas of the mouse fetus using immunohistochemistry analysis, and Pdx-1 expression was decreased in the pancreas of Rfx6-deficient mice (20). In this study, Pdx1 mRNA expression levels were changed not only in Rfx6-knockdown STC-1 cells but also in Rfx6-overexpressing STC-1 cells, whereas Rfx6 expression levels were significantly decreased in Pdx1 knockdown STC-1 cells (Figs. 2*E*, 3*C*, and 4*E*). These results suggest that Rfx6 expression is regulated at least in part by Pdx1.

Increased blood GIP levels in obesity have been reported in several studies (7, 14–16). There is a report that healthy human subjects administered high fat food for 2 weeks showed increased plasma GIP levels without developing obesity, suggesting that GIP hypersecretion precedes obesity (31). GIP is released from K-cells into the circulation in response to various nutrients (32–34). Measurement of total GIP and total GLP-1 levels in humans challenged with glucose or meal shows that the postprandial plasma GIP level is greatly augmented when a meal containing abundant fat rather than simple glucose is consumed (35, 36). This suggests that intake of HFD increases GIP secretion and strengthens both direct and indirect effects of GIP on energy accumulation in adipose tissue. We previously reported that both GIP levels after glucose loading and body mass index have a positive correlation in healthy subjects (37). In this study, GIP levels during OGTT were increased in obese GIP-GFP heterozygous mice compared with those in lean GIP-GFP heterozygous mice, even though GIP-GFP heterozygous mice have only one normal *gip* gene, indicating these mice represent a useful model for analysis of the mechanisms involved in the augmented GIP secretion in HFD-induced obesity. A previous study reported that augmentation of GIP secretion in HFD feeding conditions is due to increased K-cell number (38). In that report, agglomerates of Pdx1 and GIP double-expressing cells were found inside the duodenal mucosa of obese rats after HFD feeding. In this study, however, we could not detect agglomerates of K-cells or an increase of K-cell number in the duodenum or upper small intestine of HFD-fed GIP-GFP heterozygous mice by immunohistochemistry and flow cytometry analysis. The reason for this discrepancy could be the difference of species, food composition, and/or duration of the HFD feeding period. In our study, GIP content was significantly increased in the upper small intestine of HFD-fed mice compared with that in CFD-fed mice, and GIP mRNA expression was increased in K-cells of HFD-fed mice. These results suggest that GIP hypersecretion in HFD-induced obese mice is due to increased GIP expression in K-cells. In this condition, the expression levels of Rfx6 and Pdx1 mRNA were significantly increased in K-cells (Fig. 5*K*). As Rfx6 and Pdx1 were found to be important transcriptional factors in producing GIP in K-cells in our results using Rfx6 knockdown and overexpression and in previous *in vitro* studies for Pdx1, an increase in Rfx6 and Pdx1 expressions might well be involved in GIP hypersecretion in K-cells in HFD-induced obese mice.

In conclusion, gene analysis of K-cells isolated from GIP-GFP mice enables identification of the transcription factor Rfx6 that is expressed exclusively in K-cells and is involved in the regulation of GIP expression. We also show that expression of Rfx6 and Pdx1 is up-regulated in the K-cells of HFD-induced obese mice, which suggests that induction of Rfx6 as well as Pdx1 plays a critical role in GIP hypersecretion in HFD-induced obesity.

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RESEARCH ARTICLE

Self-monitoring of blood glucose (SMBG) improves glycaemic control in oral hypoglycaemic agent (OHA)-treated type 2 diabetes (SMBG-OHA study)

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Abstract

Background We conducted a clinical research study to determine the effect of self-monitoring of blood glucose (SMBG) on glycaemic control and the value of a putatively less painful blood sampling technique on SMBG in oral hypoglycaemic agent-treated type 2 diabetes patients; SMBG has not been broadly applied in non-insulin-treated patients in Japan.

Methods One hundred thirty-seven subjects were recruited for the 24-week, prospective, comparison study and randomized into three groups: 46, no SMBG group; 46, fingertip group; and 45, palm group. The primary endpoint was change in HbA_{1c}. The secondary endpoints were SMBG compliance, dropout rate, treatment changes, and patient's and physician's satisfaction.

Results Six subjects in the fingertip group (13.2%) and one subject in the palm group (2.2%) were dropped because of pain. A_{1C} level of all subjects at 24-week was decreased more in the fingertip (−0.23%) and palm (−0.16%) groups than that in the no SMBG group (+0.31%) ($p < 0.05$). SMBG compliance was higher in the fingertip group (2.17 times/day) than that in the palm group (1.65 times/day) ($p < 0.05$). A_{1C} level of treatment-unchanged subjects was decreased more in the fingertip (−0.25%) and palm (−0.21%) groups than that in the no SMBG group (+0.30%) ($p < 0.05$). SMBG compliance was higher in the fingertip group (2.24 times/day) than that in the palm group (1.65 times/day) ($p < 0.05$). Patient's questionnaire showed that 84.1% of the fingertip group and 90.2% of the palm group were satisfied with SMBG. Physician's satisfaction was higher in the palm group (94.0%) than that in the fingertip group (80.0%) ($p < 0.05$).

Conclusion SMBG is beneficial for glycaemic control, and palm blood sampling is a useful procedure for oral hypoglycaemic agent-treated type 2 diabetes.

Keywords type 2 diabetes; palm puncture; self-monitoring of blood glucose (SMBG)

Introduction

Self-monitoring of blood glucose (SMBG) is a tool for patients with diabetes to detect patterns of blood glucose control. Results of SMBG can be useful to make adjustments in diet, physical activity, and medications in order to achieve glycaemic targets, preventing hypoglycaemic events. In addition, it has been reported that SMBG is associated with decreased diabetes-related morbidity and all-cause mortality in insulin-treated patients as well as in insulin-naïve

type 2 diabetic subjects [1]. However, the efficacy of SMBG for glycaemic control requires three or more daily blood testing for patients using multiple insulin injections [American Diabetes Association (ADA) level of evidence = A] [2]. In patients treated less frequently with insulin injection or oral hypoglycaemic agent (OHA) or diet alone, SMBG is recommended for achieving glycaemic goals by expert consensus and clinical experience (ADA level of evidence = E). Considering that the number of type 2 diabetes (T2D) patients is now growing rapidly all over the world, self-management will have to play an increasingly larger role in the treatment of diabetes [3].

Six randomized control trials (RCTs) of 36 full text articles indicated that the overall effect of SMBG was a statistically significant decrease of 0.39% in A_{1C}, compared with the control groups [4]. A meta-analysis of 1307 non-insulin-treated patients with T2D showed a decrease in A_{1C} level by 0.42% in SMBG-performed patients compared with SMBG-not-performed ones [5]. Three RCTs with 1000 patients indicated that improvement in glycaemic control with SMBG tended to be seen in studies with initial A_{1C} above 8% [6]. On the other hand, it was reported that the link from SMBG to improved glycaemia in non-insulin-requiring T2D is less definitive [7]. O'Kane *et al.* reported that SMBG had no effect on glycaemic control but was associated with higher scores on a depression subscale [8]. Tengblad *et al.* showed that the use of SMBG also was not associated with improved glycaemic control in any therapy category of patients with T2D in primary care [9]. Evidence from a meta-analysis of 2552 patients was not convincing for a clinically meaningful effect of clinical management of non-insulin-treated T2D [10].

However, ROSES trial showed that after 6 months, mean HbA_{1c} reduction was $1.2 \pm 0.1\%$ in the intervention group and $0.7 \pm 0.2\%$ in the control group, with an absolute mean difference between groups of -0.5% [11]. Most recently, Malanda *et al.* reviewed the efficacy of SMBG on glycaemic control in patients with T2D who are not using insulin and concluded that there was a statistically significant reduction of A_{1C} level with SMBG of -0.3% (95% CI -0.4 to -0.1, 2324 participant, nine trials) compared with that of the control group [12]. Thus, SMBG may be helpful for glycaemic control in non-insulin-treated T2D patients.

In Japan, SMBG is not broadly applied in non-insulin-treated T2D because it is not covered by health insurance. In addition, patients are often reluctant to perform SMBG because it looks painful at blood sampling. Partly for these reasons, the efficacy of SMBG on glycaemic control in Japanese patients with T2D is still undetermined. To approach this question, we conducted a clinical research study to determine both the effect of SMBG on glycaemic control and the value of a putatively less painful blood sampling technique on SMBG in OHA-treated T2D. In the current study, two puncture sites were compared, fingertip and palm, partly because many nurses answered that palm blood sampling was less painful than fingertip sampling in a preliminary questionnaire (40 of 48 nurses, less painful; eight of 48 nurses, equal) and partly because the blood glucose level by palm blood sampling has been reported to be almost equal to that by fingertip sampling [13]. We show here that SMBG is useful for glycaemic control in OHA-treated patients with T2D and that palm blood sampling, a less painful technique, is beneficial for SMBG.

Materials and methods

Participants

This SMBG-OHA study was a prospective, 24-week, randomized, single centre comparison study to evaluate the efficacy of SMBG on glycaemic control in OHA-treated T2D. Outpatients of Kyoto University hospital were recruited and randomized into three groups: fingertip group, palm group, and no SMBG group using balanced design (age, gender, A_{1C}, and diabetes duration). Seven physicians examined a similar number of patients. Inclusion criteria were as follows: T2D treated with only OHA; aged ≥ 20 years; A_{1C} level $\geq$ 6.2% or fasting blood sugar level ≥ 6.1 mmol/L; no improvement in $A_{1C} \geq 0.5\%$ within 3 months; and no experience of SMBG in 1 year. Exclusion criteria were as follows: type 1 diabetes; secondary diabetes; alcoholism; severe depression or severe psychological condition; malignancy; abnormal haemoglobinemia; participation in other clinical trials or studies; and patients unsuitable for this study judged by physicians. The study protocol was approved by the Institutional Review Board of Kyoto University Hospital (#C-275) and registered on University hospital Medical Information Network in Japan (UMIN000001525). Written informed consent was obtained from all subjects.

Intervention

The study's duration was 24 weeks. Subjects were screened for eligibility and gave informed consent and basic demographic information and medical history, and were provided with the One Touch Ultra Blood Glucose Monitoring System kit®, which allows both fingertip and palm blood sampling. Subjects who performed SMBG were trained once similarly by one of three of the physicians at enrollment; they were requested to perform three SMBG for at least 3 days each week except for seven SMBG on at least 2 days in the week before the next visit. All of the subjects visited the clinic every 6 weeks, and laboratory data including A_{1C}, the number of SMBG, physical findings, and all documented medications were collected. All subjects also received advice on diet and exercise at each visit of their primary physicians with the same education and treatment policies. Subjects and physicians filled in the original questionnaire on SMBG satisfaction at the final visit. In the patient's questionnaire, subjects were questioned on five different subjects: motivation to glycaemic control; willingness to treatment for diabetes; positive response to SMBG; usefulness of SMBG in glycaemic control; and willingness to continue SMBG. The physician's questionnaire raised three issues: patient's motivation to glycaemic control;

willingness of patients to treatment for diabetes; and usefulness of SMBG in glycaemic control.

Measurements

The primary endpoint was change in A_{1C}. Significant difference was determined when average reduction of A_{1C} level was larger than or equal to 0.4% among the SMBG group, fingertip group, and palm group. The value for A_{1C} (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula A_{1C} (%) = A_{1C} (JDS) (%) + 0.4%, considering the relational expression of A_{1C} (JDS) (%) measured by the previous Japanese standard substance and measurement methods and A_{1C} (NGSP) [14]. The secondary endpoints were SMBG compliance (total number of SMBG tests), dropout rate, treatment changes, patient's satisfaction, and physician's satisfaction. When more than 80% of patients or physicians answered the questionnaire affirmatively, they were deemed satisfied with performance of SMBG. SMBG data sheets of the subjects were collected, and the number of SMBG tests was counted at every visit.

Statistical analysis

Sample size was estimated to be 49 in each group to detect a 0.4% change in A_{1C} in 24 weeks with a power 95%, alpha 0.05 two-tailed. To take dropout rate into account, the aim was to include 60 subjects in each group. A_{1C} outcomes and total number of SMBG tests were assessed blindly for statistical analysis. Analysis of variance and Dunnett's multiple comparison tests were used to examine the significance levels for reduction in A_{1C} between the no SMBG group, fingertip group, and palm group. Dependent samples Student's *t*-test was used to compare the means of A_{1C} level between baseline and 24 weeks in the no SMBG, fingertip and palm groups, respectively. Independent samples Student's *t*-test was used to compare the compliance of SMBG between the fingertip and palm groups. Difference in dropout rate between no SMBG, fingertip, and palm groups was analysed by Fisher's exact test. Patient's satisfaction and physician's satisfaction also were evaluated by Fisher's exact test. Person's product–moment correlation test was used to evaluate the relationship between SMBG compliance and change in A_{1C}. p values <0.05 were considered as statistically significant.

Results

Subjects

We screened 445 patients, and 307 patients were provisionally registered. Of these, 137 patients were eligible and were enrolled in the study (Figure 1). They were randomized to three groups: 46, no SMBG group; 46, fingertip group; and 45, palm group. There was no significant difference in the background of the subjects including OHAs (Table 1). No major differences were found in the socioeconomic status or level of education between the studied groups. Five subjects were dropped from the no SMBG group, 12 subjects were dropped from the fingertip group, and 11 subjects were dropped from the palm group (Figure 1). The dropout rate appeared to be higher in the fingertip group (26.1%) and the palm group (24.4%) than that in the no SMBG group (10.9%), but

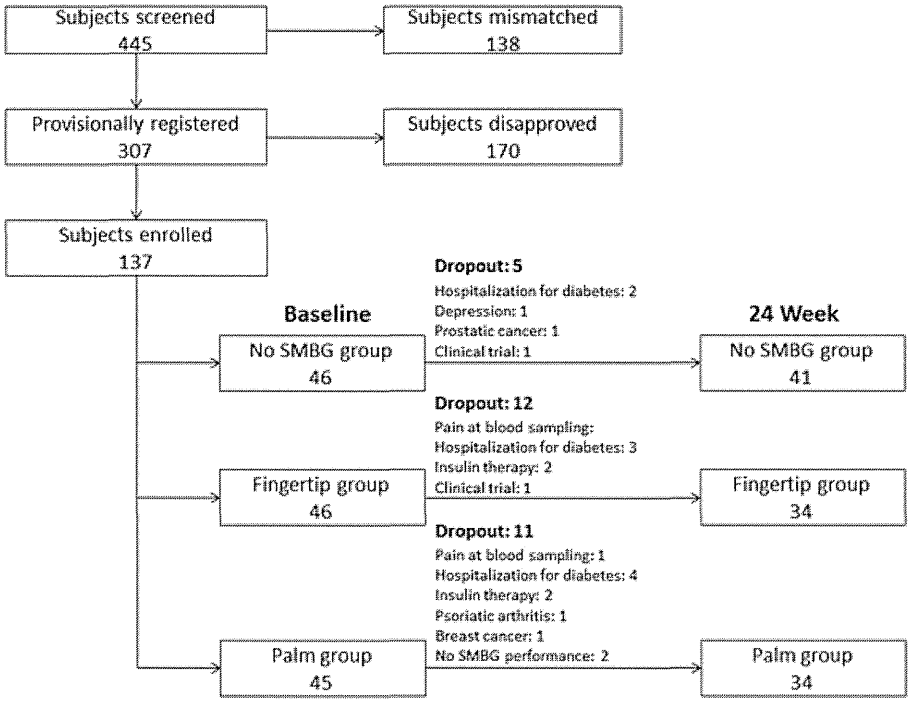

Figure 1. Flow chart of the study. SMBG, self-monitoring of blood glucose

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Table 1. Demographic and clinical features of patients participating in the study

Subjects (137)	Age (years)	Female (%)	Diabetes duration (year)	A_{1C} (%)	Medication				
					SU (%)	Metformin (%)	TZD (%)	αGI (%)	Glinide (%)
No SMBG (46)	63.1 ± 11.9	41.3	8.4 ± 6.7	7.46 ± 0.76	71.7	37.0	8.7	20.0	15.2
Fingertip (46)	64.3 ± 10.0	43.5	8.5 ± 4.7	7.44 ± 0.91	69.6	34.8	10.9	19.6	15.2
Palm (45)	63.9 ± 9.4	42.2	8.2 ± 3.8	7.44 ± 0.85	71.1	33.3	8.8	22.2	13.3

SU, sulfonylurea; TZD, tiazolidine; αGI, alpha glucosidase inhibitor; SMBG, self-monitoring of blood glucose.

the difference was not significant ($p = 0.262$) (Table 2). The dropout rate due to pain at blood sampling appeared to be higher in the fingertip group (50.0%, six of 12 subjects) than that in the palm group (9.1%, one of 11 subjects), but the difference was not significant ($p = 0.116$) (Table 2). The final number of subjects who completed the study was 41 in the no SMBG group, 34 in the fingertip group, and 34 in the palm group (Table 2).

A_{1C} findings

A_{1C} level (mean ± SD) at 24 weeks was decreased from 7.25 ± 0.77% to 7.02 ± 0.59% in the fingertip group ($p < 0.05$) and from 7.35 ± 0.70% to 7.19 ± 0.67% in the palm group ($p < 0.05$) (Figure 2a). On the other hand, A_{1C} level (mean ± SD) was significantly increased from 7.44 ± 0.74% to 7.75 ± 0.85% in the no SMBG group ($p < 0.05$). Accordingly, A_{1C} level (mean ± SE) at 24 weeks was significantly decreased from baseline in the fingertip group (−0.23 ± 0.10%) and the palm group (−0.16 ± 0.06%) compared with that in the no SMBG group (+0.31 ± 0.07%) ($p < 0.05$) (Table 2). Thus, the difference in change in A_{1C} between the fingertip group or the palm group and the no SMBG group was −0.54% (95% CI −0.31 to −0.77, $p < 0.05$) and −0.48% (95% CI −0.29 to −0.67, $p < 0.05$), respectively (Table 2), but there was no statistically significant difference in A_{1C} reduction between the fingertip group and the palm group. There was no difference in hypoglycaemic events between the groups.

During the study, treatment with OHA was changed in four patients of the no SMBG group, in two patients of the fingertip group, and in seven patients of the palm group (Table 3). In treatment-unchanged subjects, A_{1C} level (mean ± SD) at 24 weeks was significantly decreased from 7.23 ± 0.77% to 6.98 ± 0.58% in the fingertip group

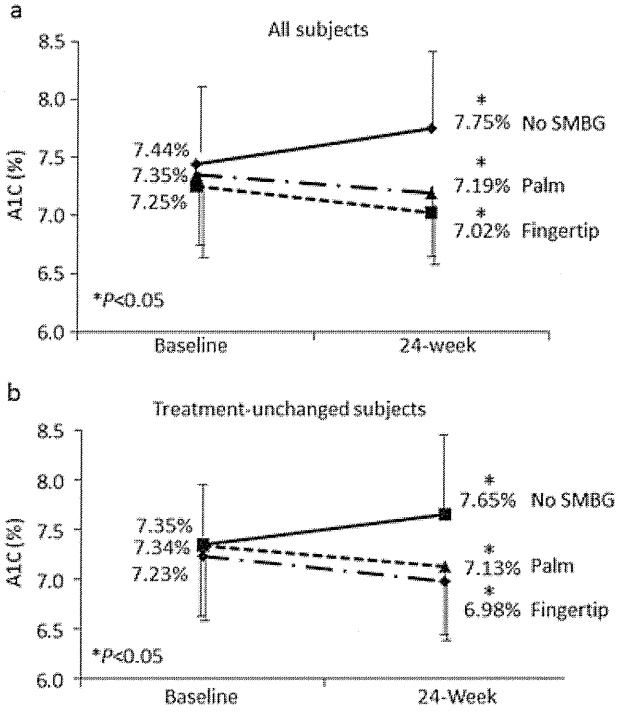

Figure 2. Change in A1C level. (a) A1C level at baseline and 24 weeks in the no SMBG, fingertip, and palm groups. (b) A1C level at baseline and 24 weeks in the no SMBG group, fingertip, and palm groups in treatment-unchanged subjects. SMBG, self-monitoring of blood glucose. *$p < 0.05$

($p < 0.05$) and from 7.34 ± 0.76% to 7.13 ± 0.72% in the palm group ($p < 0.05$), while it was significantly increased from 7.35% ± 0.68% to 7.65 ± 0.80% in the no SMBG group ($p < 0.05$) (Figure 2b). Change in A_{1C} (mean ± SE) at 24 weeks from baseline was +0.30 ± 0.08%, −0.25 ± 0.21%, and −0.21 ± 0.06% in the no SMBG, fingertip, and palm groups, respectively ($p < 0.05$) (Table 3). The difference in change in A_{1C} between the fingertip group or the palm group and the no SMBG group

Table 2. Change in A1C and SMBG compliance (total number of SMBG tests) in all subjects

Subjects number (137)	Final subjects number (129)	Dropout rate	Dropout rate due to pain	Change in A1C (mean ± SE)	ΔA1C from no SMBG (95% CI)	Total number of SMBG tests (mean ± SD)	SMBG frequency (times/day) (mean ± SD)
No SMBG (46)	41	10.9%	—	+0.31 ± 0.07%*	—	—	—
Fingertip (46)	34	26.1%	50.0%	−0.23 ± 0.10%*	−0.54%* (−0.31 to −0.77)	364 ± 160*	2.17 ± 0.92*
Palm (4)	34	24.2%	9.1%	−0.16 ± 0.06%*	−0.48%* (−0.29 to −0.67)	277 ± 154*	1.65 ± 0.92*

SMBG, self-monitoring of blood glucose.
*$p < 0.05$.

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Table 3. Change in A1C and SMBG compliance (total number of SMBG tests) in treatment-unchanged subjects

Final subjects number (129)	Treatment changes	Change in A1C	Treatment-unchanged subjects	Change in A1C (mean ± SE)	ΔA1C from no SMBG (95%CI)	Total number of SMBG tests (mean ± SD)	SMBG frequency (times/day) (mean ± SD)
No SMBG (41)	4 Increased 4	+0.35%	37	+0.30 ± 0.08%*	—		
Fingertip (34)	Increased 2	+0.20%	32	−0.25 ± 0.21%*	−0.55%* (−0.29 to −0.81)	376 ± 160*	2.24 ± 0.95*
Palm (34)	7 Increased 5 Decreased 1 Drug change 1	−0.10%	27	−0.21 ± 0.06%*	−0.51%* (−0.30 to −0.72)	278 ± 148*	1.65 ± 0.88*

SMBG, self-monitoring of blood glucose.
*$p < 0.05$.

was −0.55% (95% CI −0.29 to −0.81, $p < 0.05$) and −0.51% (95% CI −3.0 to −7.5, $p < 0.05$), respectively (Table 3). There was no difference in hypoglycaemic events between the groups.

SMBG compliance

Total number of SMBG was defined as SMBG compliance. SMBG compliance (mean ± SD) by 24 weeks was 364 ± 160 (2.17 ± 0.95 times a day) in the fingertip group and 277 ± 154 (1.65 ± 0.92 times a day) in the palm group; SMBG compliance was significantly increased in the fingertip group compared with that in the palm group ($p < 0.05$) (Table 2). There was a weak, negative correlation between SMBG compliance and change in A_{1C} in the fingertip group (Person's product–moment correlation coefficient = −0.370, $p < 0.05$) (Figure 3a), the palm group (Person's product–moment correlation coefficient = −0.395, $p < 0.05$) (Figure 3b), and all SMBG (fingertip and palm) groups (Person's product–moment correlation coefficient = −0.376, $p < 0.01$) (Figure 3c). In treatment-unchanged

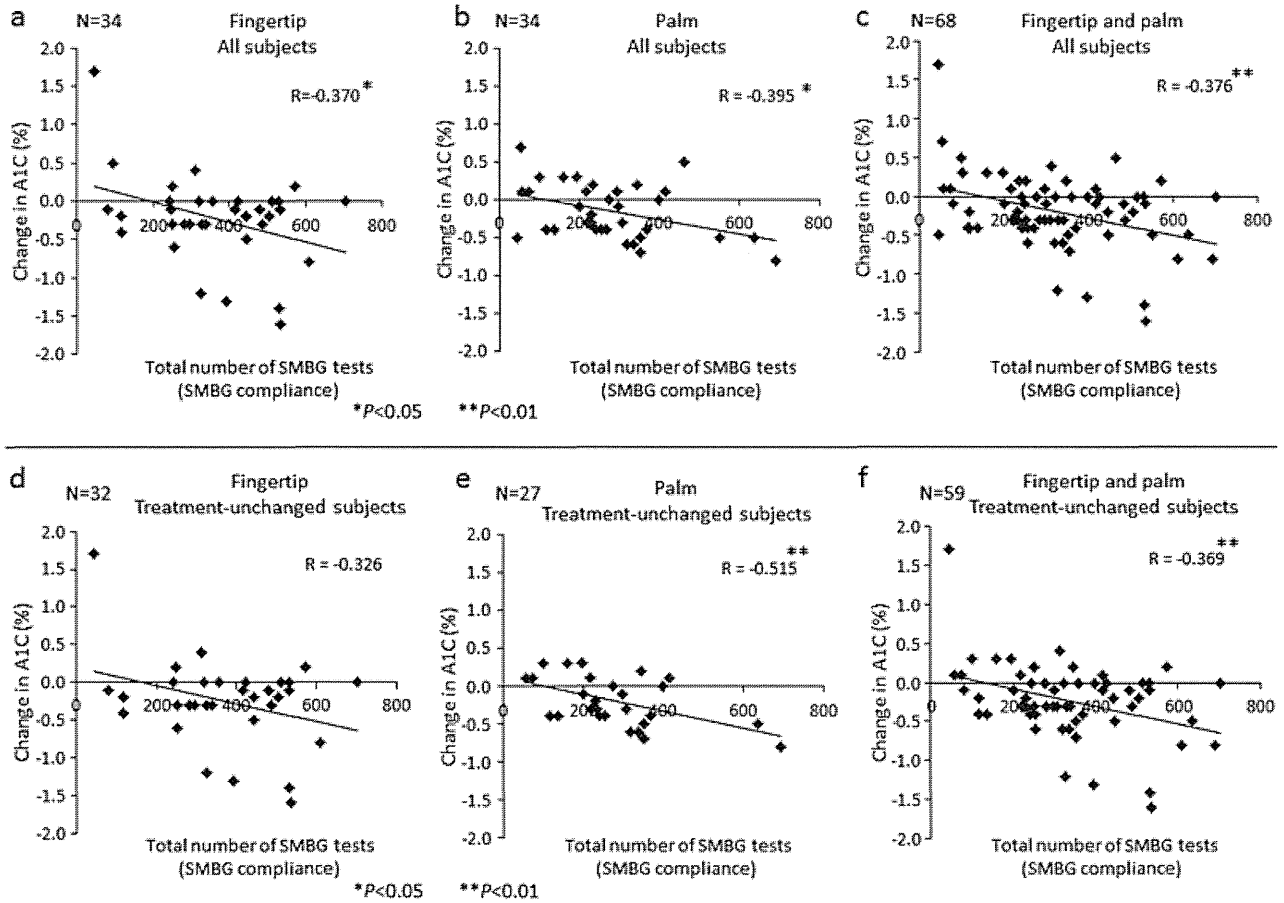

Figure 3. (a–c) Relationship between change in A1C and SMBG compliance (total number of SMBG tests) at 24 weeks in the fingertip group (a), the palm group (b), and all SMBG (fingertip and palm) groups (c) in all subjects. (d–f) Relationship between change in A1C and SMBG compliance (total number of SMBG tests) at 24 weeks in the fingertip group (d), the palm group (e), and all SMBG (fingertip and palm) groups (f) in treatment-unchanged subjects. SMBG, self-monitoring of blood glucose. *$p < 0.05$. **$p < 0.01$

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subjects, SMBG compliance (mean ± SD) by 24 weeks was 376 ± 160 (2.24 ± 0.95 times a day) in the fingertip group and 278 ± 148 (1.65 ± 0.88 times a day) in the palm group (Table 3); SMBG compliance was significantly increased in the fingertip group compared with that in the palm group ($p < 0.05$). Correlation between SMBG compliance and change in A_{1C} was not found in the fingertip group (Person's product–moment correlation coefficient = −0.326), although a tendency seemed possible (Figure 3d). On the other hand, there was a negative correlation between SMBG compliance and change in A_{1C} in the palm group (Person's product–moment correlation coefficient = −0.515, $p < 0.01$) (Figure 3e) and all SMBG (fingertip and palm) groups (Person's product–moment correlation coefficient = −0.369, $p < 0.01$) (Figure 3f).

Patient's and physician's satisfaction with SMBG

Overall, 84.1% of the fingertip group and 90.2% of the palm group answered the questionnaire affirmatively. Thus, it appears that subjects were satisfied with SMBG. Although the proportion of patient's satisfaction seemed somewhat higher in the palm group, there was no statistically significant difference between the fingertip and palm groups ($p = 0.602$) (Table 4).

Physicians thought that patient's motivation to glycaemic control was significantly increased in the palm group by 94.1% compared with that in the fingertip group by 73.5% ($p = 0.045$) (Table 5). Overall, physicians answered the questionnaire affirmatively in 80.0% of the fingertip group and in 94.0% of the palm group. Thus, it appears that physicians also were satisfied with SMBG performed by both groups, but there was significantly greater approval in the palm group than that in the fingertip group ($p = 0.007$) (Table 5).

Discussion

In the present study, we show that SMBG is useful for glycaemic control in OHA-treated T2D. A_{1C} reduction was significantly greater in the fingertip and palm SMBG groups than that in the no SMBG group. SMBG compliance (total number of SMBG tests) was significantly higher in the fingertip group than that in the palm group, but SMBG compliance and change in A_{1C} were more correlated in the palm group than those in the fingertip group.

SMBG is recommended by ADA [2], International Diabetes Federation (IDF) [15], and other local guidelines [16,17], and is recognized as an integral part of self-management of patients using insulin therapy in T2D [18–20]. However, SMBG can improve glycaemic control only if it is carried out three or more times daily in patients using multiple insulin

Table 4. Patient's satisfaction

Questionnaire	Fingertip	Palm	*p* value
Motivation to glycaemic control	Increased 88.2%	Increased 97.1%	0.614
Willingness to treatment for diabetes	Increased 88.2%	Increased 76.5%	0.340
Encourage response to SMBG	Increased 70.6%	Increased 91.2%	0.174
Usefulness of SMBG in glycaemic control	Increased 88.2%	Increased 94.1%	0.673
Willingness to continue SMBG	Increased 85.3%	Increased 91.2%	0.709
Overall	Increased 84.1%	Increased 90.2%	0.602

SMBG, self-monitoring of blood glucose.

Table 5. Physician's satisfaction

Questionnaire	Fingertip	Palm	*p* value
Patient' motivation to glycaemic control	Increased 73.5%	Increased 94.1%	0.045*
Willingness of patients to treatment for diabetes	Increased 79.4%	Increased 93.9%	0.305
Usefulness of SMBG in glycaemic control	Increased 85.3%	Increased 94.1%	0.427
Overall	Increased 80.0%	Increased 94.0%	0.007*

SMBG, self-monitoring of blood glucose.
*$p < 0.05$.

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injections (ADA level of evidence = A) [2]. SMBG is recommended by expert consensus or clinical experience for patients using less frequent insulin injections or OHA or medical nutrition therapy alone (ADA level of evidence = E) [2]. IDF has released a guideline on SMBG in non-insulin-treated T2D that maintains that SMBG is appropriate only when individuals with diabetes and their caregivers and healthcare providers have the knowledge, skills, and willingness to incorporate the necessary SMBG monitoring and therapy adjustments into their diabetes care plan [15].

Recently, Polonsky *et al.* reported that structured SMBG use significantly reduced the A_{1C} level in poorly controlled, non-insulin-treated T2D [21]. A_{1C} reduction from baseline to 12-months was 1.2% in structured testing groups with enhanced usual care, while it was 0.9% in an active control group with enhanced usual care. The difference in A_{1C} reduction between the two groups was 0.3%. They concluded that appropriate use of structured SMBG significantly improves glycaemic control and facilitates more timely/aggressive treatment changes in non-insulin-treated T2D. Bonomo *et al.* showed that relatively simple SMBG policy for T2D not on insulin could be potentially useful when performed with sufficient compliance [22]. Thus, Klonoff *et al.* summarized a consensus report about the current role of SMBG in non-insulin-treated T2D, which described that SMBG is an established practice for patients with non-insulin-treated T2D, and to be most effective, it should be performed in a structured format where information obtained from this measurement is used to guide treatment [23].

In the present study, subjects were encouraged to perform SMBG regularly and intensively, especially in the week before the next visit to the clinic, and to assess their SMBG data with the physician at every visit. Consequently, the average number of SMBG in all subjects was 2.17 times a day in the fingertip group and 1.65 times a day in the palm group, resulting in A_{1C} reductions by 0.54% and by 0.48% in 24 weeks, respectively, compared with that in the no SMBG group. In treatment-unchanged subjects, 2.24 SMBG a day in the fingertip group and 1.65 SMBG a day in the palm group resulted in A_{1C} reduction of 0.55% and 0.51%, respectively, compared with that in the no SMBG group. This evidence indicates that SMBG is useful for glycaemic control when patients perform SMBG regularly and healthcare providers assess SMBG data carefully with patients.

In all subjects, the relationship between SMBG compliance and change in A_{1C} was correlated in the fingertip, palm, and all SMBG (fingertip and palm) groups. In treatment-unchanged subjects, the correlation of SMBG compliance and change in A_{1C} was found in the palm and all SMBG (fingertip and palm) groups, and there was a tendency to correlate in the fingertip group. These findings may suggest that the greater the number of SMBG tests, the more the A_{1C} level might be improved. In addition, approximately once daily SMBG by fingertip or palm blood sampling was minimum for maintenance of A_{1C} level.

The total number of SMBG tests was higher in the fingertip group than that in the palm group. Pain at blood sampling was a cause of complaint regarding SMBG; six patients in fingertip group and one patient in palm group dropped out of the study because of pain. Thus, during the study, it is possible that less motivated patients were dropped and relatively highly motivated patients continued SMBG in the fingertip group. In fact, the number of subjects who performed SMBG less than 200 times in 24 weeks was smaller in the fingertip group compared with that in the palm group. Every patient was able to continue SMBG in the palm group, but sometimes continued to have puncture scars in their palms, in which case the total number of SMBG tests was lower than in the fingertip group. Overall, SMBG compliance in the fingertip group was higher than that in the palm group.

Some patients in the fingertip group who continued SMBG complained of pain at blood sampling; these patients did not perform SMBG frequently and answered the questionnaire that they were not willing to do SMBG in the future. Fear that SMBG is painful also is a barrier to the use of SMBG in patients. In fact, 55 of 170 (32.3%) patients refused to participate because of anticipated painfulness. It has been reported that an SMBG frequency of $\geq$1 per day is significantly related to higher levels of distress, worry, and depressive symptoms in non-insulin-treated patients [24]. The ZODIAC-17 study also showed that OHA-treated T2D patients experienced some worsening of their personal health perception [25]. On the other hand, structured SMBG can significantly improve glycaemic control without decreasing general well-being [21]. Thus, SMBG should be performed with reference to the patient's quality of life, and palm puncture, a less painful method of blood sampling, can be an alternative method for SMBG.

The present study shows that SMBG is helpful for glycaemic control in OHA-treated T2D and that a less painful technique for blood sampling such as palm puncture should be considered to encourage patients to continue SMBG. Although longer term observation is required, on the basis of our results, once daily SMBG can be useful to maintain a stable A_{1C} level in OHA-treated T2D.

Conflict of interest

This study was supported by Johnson & Johnson KK.

No other potential conflicts of interest relevant to this article were reported.

The study conception and protocol were by S.H., Y.S., and N.I. Subject training was by T.F., M.S., and Y.N. Patient examinations were by S.H., S.F., M.O., N.H., A.H., K.N., and N.I. The statistical analysis was by Y.N., D.T., and S.Y. The manuscript development was by S.H. and N.I.

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Basic nutritional investigation

Enteral supplement enriched with glutamine, fiber, and oligosaccharide attenuates experimental colitis in mice

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ABSTRACT

Objective: Ulcerative colitis is a chronic recurrent disease characterized by acute inflammation of the colonic mucosa. In Japan, a dietary supplementation product enriched with glutamine, dietary fiber, and oligosaccharide (GFO) is widely applied for enteral nutrition support. These three components have been suggested to improve intestinal health. In this study, we investigated whether GFO has suppressive effects on mucosal damage in ulcerative colitis in an experimental mouse model.

Methods: C57BL/6 mice received 2.5% dextran sulfate sodium in drinking water for 5 d to induce colitis. Then, they were given 0.25 mL of GFO or a 20% glucose solution twice daily for 10 d. Another set of mice receiving unaltered drinking water was used as the normal control group.

Results: The body weight loss and disease activity index were significantly lower in the GFO-treated mice compared with the glucose-treated mice ($P < 0.05$). The decrease in colon length induced by dextran sulfate sodium was significantly alleviated in GFO-treated mice compared with glucose-treated mice ($P < 0.01$). In addition, the histologic findings showed that intestinal inflammation was significantly attenuated in mice treated with GFO. Furthermore, treatment with GFO significantly inhibited the dextran sulfate sodium–induced increase in the mRNA expression of interleukin-1β.

Conclusion: These results suggest that GFO has potential therapeutic value as an adjunct therapy for ulcerative colitis.

Introduction

Ulcerative colitis (UC) is a major form of inflammatory bowel disease characterized by an acute inflammation of the colonic mucosa leading to rectal bleeding and diarrhea. Patients with UC have repeated, chronic, relapsing–remitting inflammatory conditions, and chronic inflammation of colon has been reported to promote the risk of colonic cancer [1]. Aminosalicylates and corticosteroids are applied as standard medications to decrease such intestinal mucosal inflammation [2]. However, in some cases, these medications have side effects and are not effective enough to achieve the complete remission of UC [3]. A more effective intervention with fewer side effects is required for better UC treatment.

GFO is a commercial enteral supplement enriched with glutamine, dietary fibers, and oligosaccharide. This medicinal supplementation product is widely applied as enteral nutrition support and perioperative nutrition management. The dietary fibers in GFO are polydextrose and hydrolyzed guar gum, which are water soluble. The oligosaccharide in GFO is lacto-sucrose, a kind of galacto-oligosaccharide. The fiber and the oligosaccharide are prebiotics that stimulate the growth

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and activity of gastrointestinal microflora [4,5]. Previous studies have suggested that an abnormal regulation of the mucosal immune response toward the commensal bacterial flora plays an important role in the pathogenesis of UC [6]. Some studies have reported that the administration of prebiotics such as inulin [7], resistant starch [8], lactulose [9], oligofructose [10], and goat's milk oligosaccharides [11] effectively decrease severe clinical symptoms and prolong the remission of intestinal mucosal inflammation by improving the intestinal microbial balance in rats with experimental colitis. In a human trial involving 10 patients with Crohn's disease, the administration of a fructo-oligosaccharide significantly decreased the activity indices of Crohn's disease [12]. These data suggest that modification of the intestinal microflora by GFO might be a useful adjunctive treatment for UC. Glutamine, the other of the three constituents of GFO, is a major oxidative fuel for the intestine that supports intestinal mucosal functions [13]. Previous studies have shown that glutamine has anti-inflammatory effects on intestinal epithelial cells of rodent models of inflammatory bowel disease [14–16] and cell lines [17] and biopsy samples [18] of the human gut. The aim of the present study was to investigate the hypothesis that GFO has multiple therapeutic effects on UC in the case of mice with colitis induced by dextran sulfate sodium (DSS).

Materials and methods

Animals

C57BL/6 male 11-wk-old mice weighing 25 to 30 g (Shimizu Laboratory Supplies Co. Ltd., Kyoto, Japan) were used in this study. The mice were housed in a specific pathogen-free environment at an ambient temperature of 23°C with a 12-h light–dark cycle. They were given a standard chow and tap water ad libitum. All procedures were approved by the animal care committee of Kyoto University Graduate School of Medicine.

Induction of colitis and GFO treatment

Experimental colitis was induced by an oral administration of DSS (M 5000; Wako Pure Chemicals, Osaka, Japan) [19–21]. The mice received 2.5% (w/v) DSS in drinking water for 5 d, from day 0 to day 4, as previously described [19]. A pack of GFO (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was dissolved in 50 mL of distilled water. The energy and nutrient contents of the GFO and glucose solution are listed in Table 1. The mice received 0.25 mL of GFO or a 20% glucose solution twice daily for 10 d, from day 0 to day 9. Another set of animals receiving unaltered drinking water was used for the normal control group. On the morning after 10 full days, all mice were euthanized by cervical dislocation and colon samples from each animal group were obtained for histologic evaluation and RNA extraction.

Determination of disease activity index

The clinical activity of colitis was evaluated by stool consistency, hemoccult testing (ColoScreen Occult Blood Test, Helena Laboratories, Beaumont, TX, USA), and changes in body weight during the experimental period to determine the disease activity index (DAI), as described previously [20,22]. DAI scoring was as follows: weight loss: 0, no change or positive change; 1, 1% to 5%; 2, 6% to 10%; 3, 11% to 20%; 4, >21%; stool consistency: 0, well-formed pellets; 2, loose stools; 4, diarrhea; fecal blood: 0, negative hemoccult test; 2, positive hemoccult test; 4, gross bleeding. The DAI, ranging from 0 to 4, was the sum of the scores for these parameters divided by 3.

Table 1
Components of GFO

	kcal/50 mL	Glutamine (g)	Fiber (g)	Oligosaccharide (g)	Sodium (mg)
GFO (1 pack)	36	3	5	1.5	1.2
Glucose 20%	40	0	0	0	0

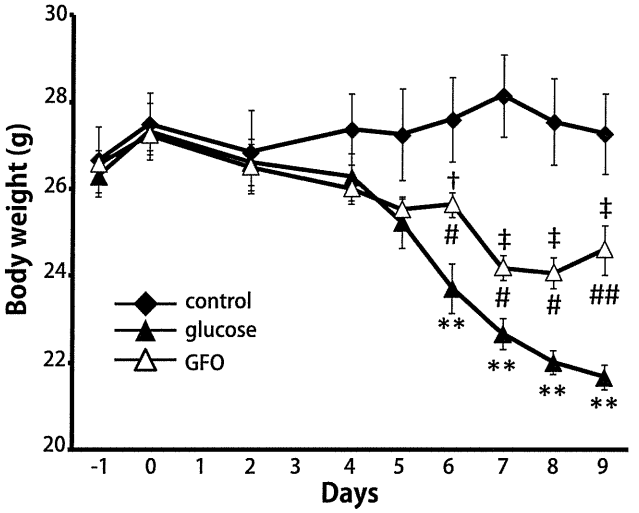

Fig. 1. Effects of GFO on body weight change in mice with colitis induced by dextran sulfate sodium. Serial changes in body weight were observed throughout the experiment in normal control mice ($n = 4$; closed diamonds), glucose-treated mice with colitis induced by dextran sulfate sodium ($n = 5$; closed triangles), and GFO-treated mice with colitis induced by dextran sulfate sodium ($n = 6$; open triangles). Each value represents mean ± SE. $^{**}P < 0.01$ between control mice and glucose-treated mice with colitis induced by dextran sulfate sodium. $^{\dagger}P < 0.05$ and $^{\ddagger}P < 0.01$ between control mice and GFO-treated mice with colitis induced by dextran sulfate sodium. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ between glucose-treated and GFO-treated mice with colitis induced by dextran sulfate sodium.

Tissue sample preparation

After measuring the length of the large intestine as an indirect marker of inflammation, it was washed in physiologic saline. Segments were taken from 0.5 cm of the distal colon for the analysis of mRNA expression levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). Colon samples were cooled

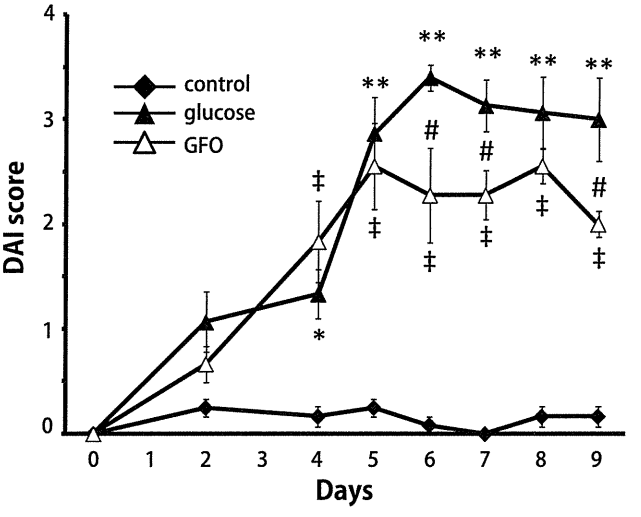

Fig. 2. DAI of normal control mice and mice with colitis induced by dextran sulfate sodium treated with or without GFO. The DAI, a combined score for bleeding, weight loss, and stool consistency, is divided by 3. Normal control mice ($n = 4$; closed diamonds), glucose-treated mice with colitis induced by dextran sulfate sodium ($n = 5$; closed triangles), and GFO-treated mice with colitis induced by dextran sulfate sodium ($n = 6$; open triangles). Each value represents mean ± SE. $^{*}P < 0.05$ and $^{**}P < 0.01$ between control mice and glucose-treated mice with colitis induced by dextran sulfate sodium. $^{\ddagger}P < 0.01$ between control mice and GFO-treated mice with colitis induced by dextran sulfate sodium. $^{\#}P < 0.05$ between glucose-treated and GFO-treated mice with colitis induced by dextran sulfate sodium. DAI, disease activity index.

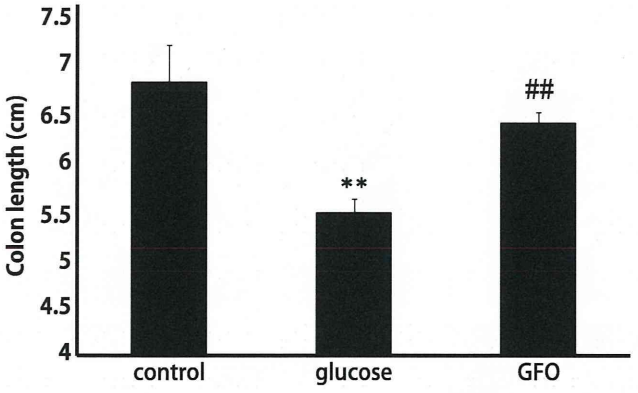

Fig. 3. Colon length of normal control mice and mice with colitis induced by dextran sulfate sodium treated with or without GFO. Normal control mice (n = 4), glucose-treated mice with colitis induced by dextran sulfate sodium (n = 5), and GFO-treated mice with colitis induced by dextran sulfate sodium (n = 6). Each value represents mean ± SE. $^{**}P < 0.01$ between control mice and glucose-treated mice with colitis induced by dextran sulfate sodium. $^{\#\#}P < 0.01$ between glucose-treated and GFO-treated mice with colitis induced by dextran sulfate sodium.

with liquid nitrogen and stored at −80°C until use. The rest of the central colon was fixed in Bouin fluid for histologic analysis.

mRNA assessment by semiquantitative reverse transcription–polymerase chain reaction

Total RNA from the distal colon and proximal colon was extracted using the TRIzol solution (Invitrogen, Grand Island, NY, USA) according to the manufacturer's instructions. First-strand cDNA was synthesized by the SuperScript II Reverse Transcriptase System (Invitrogen) according to the manufacturer's instructions. SYBER Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was prepared for real-time quantitative polymerase chain reaction (PCR) using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The PCR was performed for 10 min at 90°C, followed by 50 cycles at 95°C for 15 s and at 60°C for 1 min. The signal of each product was standardized against the β-actin signal for each sample. The primer pairs for TNF-α, IL-1β, and β-actin were: TNF-α (300 bp): 5′-GGC AGG TCT ACT TTG GAG TCA TTG C-3′ and 5′-CCG AAT TCA CTG GAG CCT CGA ATG T-3′; IL-1β (381 bp): 5′-GCA ACT GTT CCT GAA CTC A-3′ and 5′-CTG CAC TAC AGG CTC CGA G-3′; β-actin (517 bp): 5′-ATA TCG CTG CGC TGG TCG TC-3′ and 5′-GCT CTC CCT CAC GCC ATC CT-3′.

Immunohistochemistry

After fixing, the colon samples were embedded in paraffin, and 3-μm sections stained with hematoxylin and eosin and periodic acid-Schiff reaction (PAS) were prepared. PAS stains the acidic mucin of colonic goblet cells a turquoise blue color. In active UC, the amount of mucin is decreased, particularly in more severely ulcerated areas [23].

Single oral administration of GFO

C57BL/6 mice were fasted for 16 h and orally administered GO 12.5 g/kg of body weight or glucose 1.5 g/kg of body weight (same carbohydrate calories as GFO). Blood samples were collected at 0, 30, and 60 min after glucose loading or GFO ingestion and were centrifuged at 1800 × g at 4°C for 10 min. After collecting the supernatant of the samples, plasma and serum were stocked at −80°C. The plasma glucose levels were measured by the glucose oxidase method. Serum insulin levels were measured by enzyme-linked immunosorbent assay. Total gastric inhibitory polypeptide (GIP) and total glucagon-like peptide-1 (GLP-1) levels were measured using a mouse GIP enzyme-linked immunosorbent assay kit (Linco Research, St Charles, MO, USA) and a mouse GLP-1 enzyme-linked immunosorbent assay kit (Meso Scale Discovery, Gaithersburg, MD, USA), respectively.

Statistical analysis

All data were expressed as mean ± standard error. Statistical analysis was performed using Student's t test or one-way analysis of variance with the Fisher protected least signicicant difference post hoc test, where appropriate, using SPSS 11.0 (SPSS, Inc., Chicago IL, USA). A significant statistical difference was considered to be present at $P < 0.05$.

Results

Symptomatic and macroscopic changes

Changes in body weight

Throughout the experiment, the control mice did not show significant changes in body weight. Glucose-treated mice administered DSS showed a gradual decrease in body weight, one of the

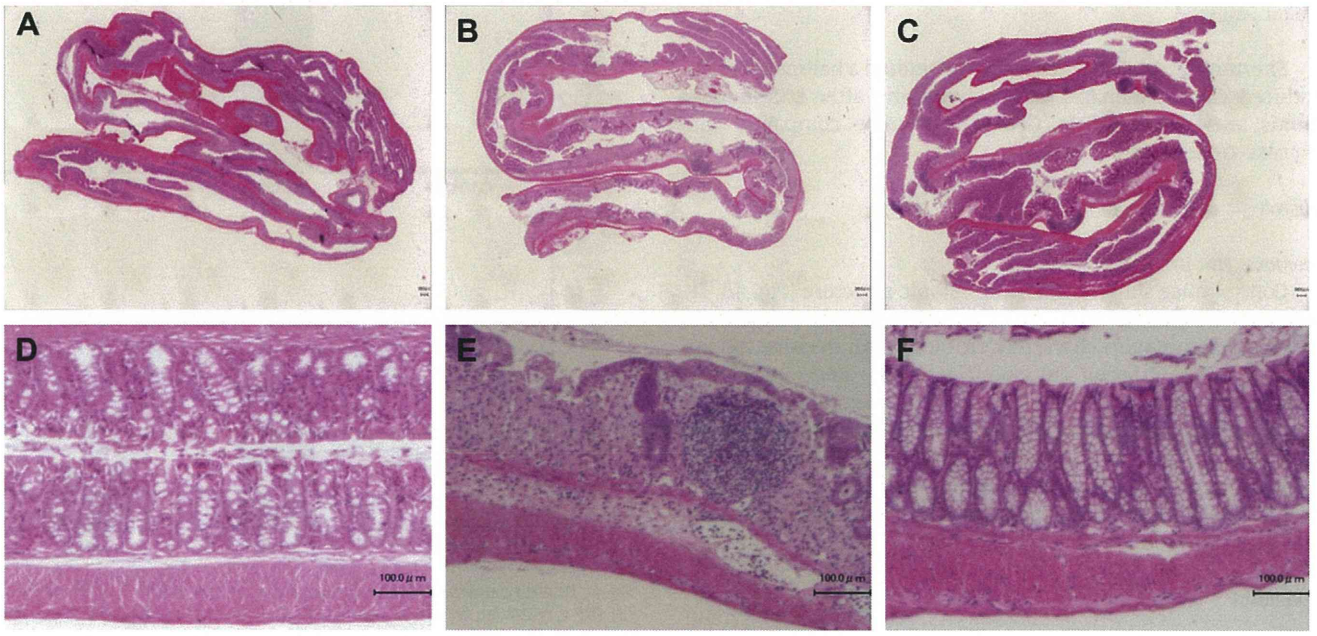

Fig. 4. Histologic findings of colon samples stained with hematoxylin and eosin. (A, D) Normal control mice, (B, E) glucose-treated mice with colitis induced by dextran sulfate sodium, and (C, F) GFO-treated mice with colitis induced by dextran sulfate sodium. Original magnifications 8× in A–C and 48× in D–F.

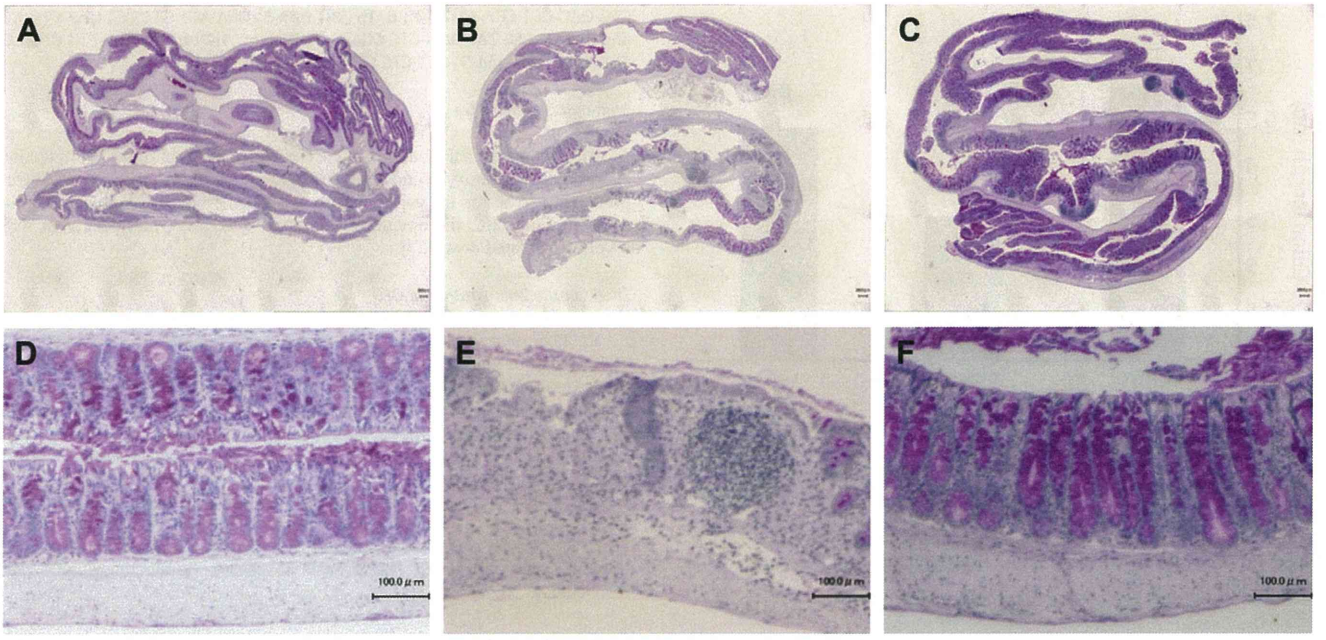

Fig. 5. Histologic findings of colon samples stained with periodic acid-Schiff. (A, D) Normal control mice, (B, E) glucose-treated mice with colitis induced by dextran sulfate sodium, and (C, F) GFO-treated mice with colitis induced by dextran sulfate sodium. Original magnifications 8× in A–C and 48× in D–F.

major symptoms of colitis [22]. The decrease in body weight in the GFO-treated mice was significantly prevented compared with that in glucose-treated mice (Fig. 1).

Disease activity index

Mice exposed to DSS for 5 d developed colitis characterized by a decreased body weight (Fig. 1), loose stools, and fecal blood. The DAI scores throughout the experimental period are shown in Figure 2. The DAI scores were significantly lower in GFO-treated mice than in glucose- treated mice on days 6, 7, and 9.

Colon length

Shortening of colon is generally considered a hallmark of DSS-induced colonic damage. Colon shortening after DSS-induced colitis was decreased in GFO-treated mice compared with glucose-treated mice (Fig. 3).

Histologic appearance

Hematoxylin and eosin stain

Control mice showed a normal colonic structure (Fig. 4A, D), whereas the glucose-treated mice administered DSS developed colonic inflammation characterized by edema, massive inflammatory cell infiltration (mononuclear cells, neutrophils, and eosinophils), mucosal ulceration, and crypt destruction (Fig. 4B, E). In contrast, although there was mild thickening of the colon wall, the GFO-treated mice administered DSS showed much less infiltration of inflammatory cells and exhibited a relatively normal mucosal structure, indicating that colonic inflammation was attenuated in the GFO-treated mice (Fig. 4C, F).

PAS stain

The control mice exhibited a high intensity of PAS staining of the goblet cells and epithelial surface mucin through the length of the colon samples (Fig. 5A, D). In contrast, the glucose-treated DSS mice showed strongly decreased PAS-positive cells (Fig. 5B, E). However, the intensity of PAS staining was substantially similar in the control and GFO-and DSS-treated mice, indicating

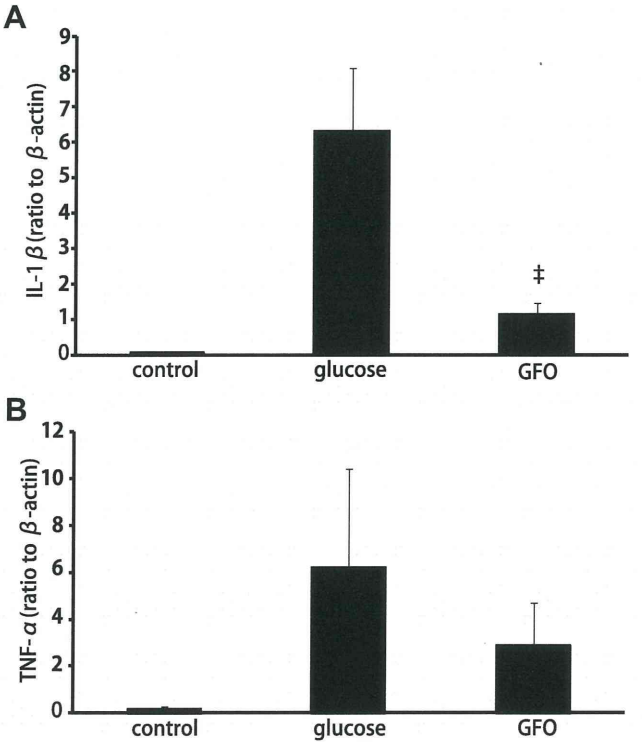

Fig. 6. Real-time quantitative polymerase chain reaction analysis of (A) IL-1β and (B) TNF-α mRNA expressions in the distal colon. Each value represents mean ± SE (n = 3–6). $^{‡}P < 0.01$ between glucose-treated and GFO-treated mice with colitis induced by dextran sulfate sodium. IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α.

that GFO attenuated the mucosal damage and maintained the architecture of normal colonic mucosa (Fig. 5C, F).

Expression of proinflammatory cytokines

The mRNA expressions of the proinflammatory cytokines IL-1β and TNF-α in colonic mucosa were evaluated by real-time quantitative PCR (Fig. 6). The IL-1β mRNA levels in the GFO-treated mice were significantly decreased compared with those in glucose-treated mice ($P < 0.01$). The mRNA expression levels of TNF-α tended to be lower in GFO-treated mice compared with glucose-treated mice, although the difference was not significant.

Effect of GFO on GIP and GLP-1 secretion

Fasting concentrations of glucose, insulin, GIP, and GLP-1 were similar in the glucose-administered and GFO-administered mice. After the administration of glucose and GFO, the levels of glucose, insulin, and GIP were significantly higher in the glucose-administered mice than in the GFO-administered mice (Fig. 7A–C). In contrast, GLP-1 levels were significantly higher in the GFO-administered mice than in the glucose-administered mice (Fig. 7D).

Discussion

In the present study, we observed the therapeutic effects of GFO, a dietary supplementation product enriched with glutamine, dietary fiber, and oligosaccharide, on intestinal damage in mice with DSS-induced colitis. DSS has been used to experimentally induce acute and chronic models of colitis. After administration of the DSS solution, the mice showed signs of diarrhea, gross rectal bleeding, weight loss, and distinctive histologic phenotypes similar to those of UC in human subjects [21]. In this study, body weight loss, a shorter colon, an increased DAI, and histologic abnormalities in the DSS-treated mice were significantly suppressed in GFO-treated mice compared with vehicle-treated mice, demonstrating that GFO prevents the development of experimental UC.

GFO is composed of glutamine, dietary fiber, and oligosaccharide. Each component has been reported to have beneficial effects on the intestinal condition [4,5,13,24]. It is well known that fiber and oligosaccharide are prebiotics that produce short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate in the colon by anaerobic bacterial fermentation [25]. One of the most abundant SCFAs, butyrate, has been considered a major fuel source for the colonocytes [26] and has been shown to increase mucosal growth and epithelial proliferation in the small and large intestines [27,28]. Several studies have reported that inflammatory bowel disease is associated with an impairment of SCFA production [29], and that the butyrate enema is highly effective in treating active UC [30]. Therefore, GFO may have a therapeutic effect by decreasing mucosal damage through an incremental increase of SCFA production. In addition, glutamine is a major oxidative fuel for the intestine that supports intestinal mucosal functions [13]. Baskerville et al. [31] reported that animals receiving an intravenous infusion of

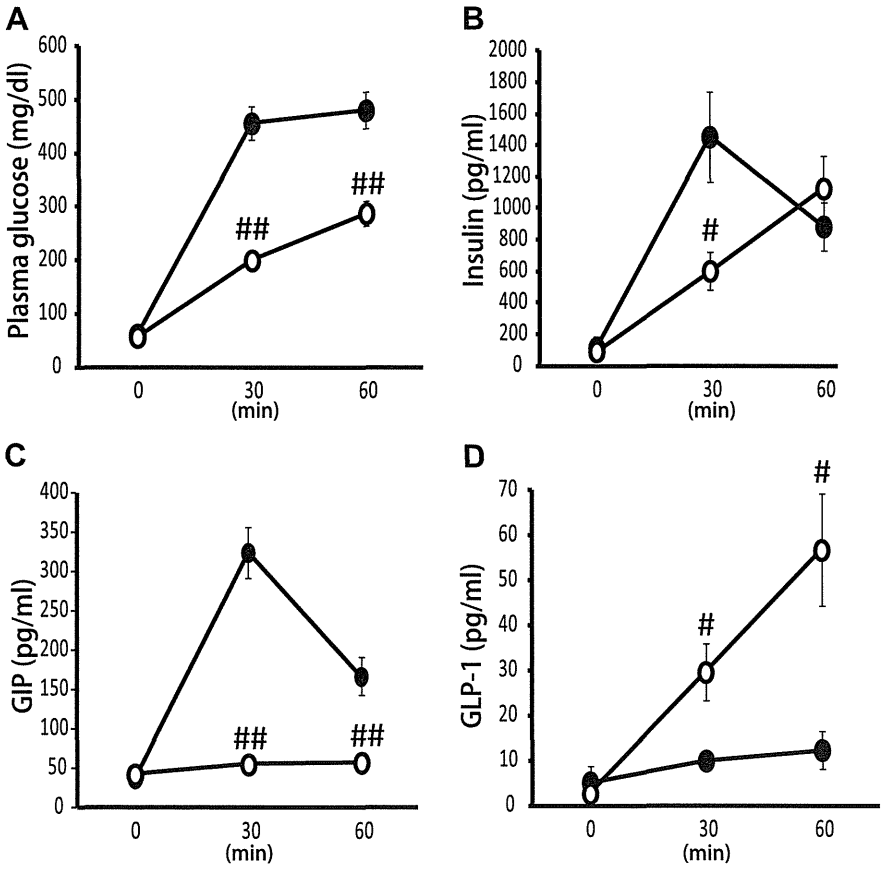

Fig. 7. Effects of GFO on GIP and GLP-1 secretion. Levels of plasma glucose (A), serum insulin (B), plasma total GIP (C), and plasma total GLP-1 (D) after an oral ingestion of glucose ($n = 7$; closed circle) or GFO ($n = 7$; open circle). Each value represents mean ± SE. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ between glucose-administered and GFO-administered mice. GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1.

glutaminase developed diarrhea, mild villous atrophy, mucosal ulcerations, and intestinal necrosis. In addition, supplementation of glutamine to a parenteral nutrition solution significantly maintained the height of intestinal villi, the thicknesses of the mucosa, and the integrity of the intestinal wall in endotoxemic rats [32].

Ulcerative colitis is closely associated with an increased activation of intestinal immune cells, and most medications are focused on decreasing the inflammation [2]. Proinflammatory cytokines, such as TNF-α and IL-1β, were increased in the colonic mucosa of patients with UC [33,34]. Some studies have reported that the administration of prebiotics, including lactulose, oligofructose, and goat's milk oligosaccharides, produce intestinal anti-inflammatory effects in rats with experimental colitis [9–11]. Glutamine also has been shown to have anti-inflammatory effects on intestinal epithelial cells of rodents in vivo [14–16], on a human intestinal cell line [17], and on primary human intestinal epithelial cells [18]. In the present study, GFO treatment significantly inhibited the DSS-induced increase in mRNA expression of IL-1β. Moreover, the histologic findings indicated that the infiltration of lymphocytes into the mucosal tissue was significantly attenuated in the GFO-treated mice. These findings suggest that GFO attenuates the mucosal damage of UC partly through its anti-inflammatory effects.

When nutrients pass through the intestine, enteroendocrine cells secrete many kinds of gastrointestinal hormone, including GIP, GLP-1, and GLP-2 [35–37]. GLP-1 and GLP-2 are pro-glucagon–derived peptides and are secreted simultaneously in response to the ingestion of a meal from intestinal L cells, which are located mainly in the distal ileum and colon [37]. GLP-1 binds to its receptor on the surface of pancreatic β cells to stimulate insulin secretion. In contrast, GLP-2 has various biological effects on the intestine, such as stimulation of intestinal mucosal growth [38], intestinal nutrient transport [39], intestinal blood flow [40], and inhibition of crypt cell apoptosis [41]. In addition, human GLP-2 has been reported to decrease the severity of colonic injury in a murine model of DSS-induced colitis [42]. In vivo animal studies have shown that some kinds of dietary fiber and SCFAs are potent stimulators of GLP-2 secretion [28,43]. It is also accepted that glutamine promotes GLP-1 secretion [35,36]. In this context, we evaluated the effect of a single oral administration of GFO on plasma GLP-1 levels and plasma GIP levels in mice. GIP levels were significantly higher in glucose-administered mice than in GFO-administered mice, probably because the absorption of oligosaccharide contained in GFO is slower than that of glucose. In contrast, GLP-1 levels were extremely higher in GFO-administered mice than in glucose-administered mice at 30 and 60 min after the administration, indicating that the glutamine in GFO stimulates GLP-1 secretion. Therefore, we speculate that GFO stimulates GLP-2 secretion concomitantly with GLP-1 secretion and attenuates the development of the mucosal damage of UC by an increase of GLP-2 secretion, although the direct measurement of plasma GLP-2 levels is remains to be studied.

Conclusion

We observed that the GFO supplement (combination of glutamine, dietary fiber, and oligosaccharide) has therapeutic effects on DSS-induced experimental colitis in mice. Although further studies are needed to clarify the mechanism of action of GFO, GFO may be a useful adjunct therapy for UC.

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Efficacy and Safety of Switching from Basal Insulin to Sitagliptin in Japanese Type 2 Diabetes Patients

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Key words
- sitagliptin
- basal insulin
- insulin secretion capacity

Abstract

Basal-supported oral therapy (BOT) is often used to treat poorly controlled type 2 diabetes. However, patients sometimes experience nocturnal and early morning hypoglycemia. Thus, maintaining targeted glycemic control by BOT is limited in some patients. We assessed the efficacy and safety of replacing basal insulin by sitagliptin therapy in Japanese type 2 diabetes patients on BOT. Forty-nine subjects were sequentially recruited for the 52-week, prospective, single arm study. Patients on BOT therapy were switched from basal insulin to sitagliptin. The primary endpoint was change in HbA1c in 52 weeks. The secondary endpoints were dropout rate, changes in body weight, frequency of hypoglycemia, and relationship between change in HbA1c and insulin secretion capacity evaluated by glucagon loading test. The average dose of basal insulin was 15.0±8.4 units. Sixteen subjects (31.3%) were dropped because replacement by sitagliptin was less effective for glycemic control. In these subjects, diabetes duration was longer, FPG and HbA1c at baseline were higher, and insulin secretion capacity was lower. Change in HbA1c in 52 weeks was −4 mmol/mol (95% CI −5 to −4 mmol/mol) ($p<0.05$). Change in body weight was −0.71 kg (95% CI −1.42 to −0.004 kg) ($p<0.05$). Frequency of hypoglycemia was decreased from 1.21±1.05 to 0.06±0.24 times/month. HbA1c level was improved if C-peptide index (CPI) was over 1.19. In conclusion, basal insulin in BOT can be replaced by sitagliptin with a decrease in HbA1c level and frequency of hypoglycemia in cases where insulin secretion capacity was sufficiently preserved.

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Introduction

Basal insulin preparation is recommended by the American Diabetes Association (ADA)/European Association for the Study of Diabetes (EASD) consensus algorithm when lifestyle interventions and oral glucose-lowering agents no longer achieve the glycemic goal of hemoglobin A1c (HbA1c) level less than 53 mmol/mol [1,2]. Recently, 2 long-acting insulin analogues, insulin glargine and insulin detemir, are available that attain glycemic targets more effectively and safely [3,4]. There are no significant differences reported in glycemic control and overall hypoglycemia between the 2 analogues [5]. The combination of basal insulin and oral hypoglycemic agents (OHAs), known as basal-supported oral therapy (BOT), is often used to treat poorly controlled type 2 diabetes [6,7]. Better glycemic control, fewer hypoglycemic episodes, and less weight gain are obtained by BOT than by biphasic insulin [8]. In addition, BOT is relatively cost effective with the same glycemic control level as biphasic insulin regimen [9]. BOT is also helpful in Japanese type 2 diabetes patients. In the ALOHA (Add-on to Lantus® to OHA) study, in which 5223 Japanese type 2 diabetes patents participated, mean HbA1c was reduced from 75±13 to 60±13 mmol/mol in 24 weeks [10]. Although BOT is well-tolerated and effective for glycemic control, patients sometimes experience nocturnal and early morning hypoglycemia. In the ALOHA study, 0.97% of the patients experienced frequent hypoglycemia. In the 4 T-study, 1.3% of BOT-treated patients experienced hypoglycemia with loss of consciousness [8]. Another problem of BOT is that postprandial glucose is high, although morning fasting blood glucose level is within normal range. An increase in dosage of basal insulin or sulfonyl ureas (SUs); which are most commonly administrated in BOT-treated Japanese patients, is not always effective, and can result in increased hypoglycemia. In Japanese interview forms, frequency of hypoglyc-

emia induced by SUs is reported to be 1.3–2.8%. Thus, maintaining targeted glycemic control by BOT is limited in some patients.

Dipeptidyl peptidase-4 (DDP-4) inhibitor is a newly developed OHA that prevents degradation of the incretin hormones, glucagon-like peptide-1, and gastric inhibitory polypeptide [11]. This compound stimulates glucose-dependent insulin secretion and suppresses glucagon release, and can improve both fasting and postprandial glucose levels. Four different DPP-4 inhibitors are available in Japan: sitagliptin, vildagliptin, alogliptin, and linagliptin. Of these, sitagliptin is most widely used, partly because it was the first approved DPP-4 inhibitor and the safety and efficacy are acceptable in Japanese clinical practice. Generally, sitagliptin is more effective for glycemic control in Japanese patients compared to Caucasian patients [12,13]. Sitaglipitn is usually combined with low dosage of SUs in Japan, less than or equal to 2 mg/day of glimepiride and 40 mg/day of gliclazide, which is enough for glycemic control when combined with sitagliptin [14]. Patients also show improved glycemic control even if insulin secretion capacity is insufficient for oral therapy [14,15]. The main pathophysiology of Japanese type 2 diabetes is impairment of insulin secretion [16,17]. Insulin secretion capacity in Japanese populations is only about half of that in Caucasians [18]. Both decreased basal and early phase insulin secretion contribute more to Japanese type 2 diabetes [16], and insulin therapy is usually required in those with C-peptide index (CPI) lower than 0.8 [19]. However, basal insulin therapy is not always ideal in some patients because postprandial glucose is still high and preprandial glucose is low, which results in large fluctuations in blood glucose. On the other hand, DPP-4 inhibitor might nevertheless ameliorate decreased early phase insulin secretion. This encouraged us to consider whether basal insulin can be replaced with sitagliptin in type 2 diabetes patients treated with SUs and basal insulin in at least some BOT cases.

We show here that sitagliptin can be switched from basal insulin in patients with C-peptide index (CPI) and/or secretory unit of islet in transplantation (SUIT) equal to or larger than 1.19 and/or 36.4, respectively, with beneficial effects on glycemic control.

Materials and Methods

Study design and participants

This was a prospective, 52-week, single center, and single arm intervention study to evaluate the effects on glycemic control of replacement of basal insulin to sitagliptin in type 2 diabetes patients inadequately controlled with BOT. Outpatients of Takashima General Hospital were recruited consecutively for a sample size of 45 subjects. Inclusion criteria were: type 2 diabetes treated with basal insulin (insulin glargine or detemir) and SUs (glimepiride or gliclazide) ± metformin ± thiazolidinedione ± α-glucosidase inhibitors for more than 1 year; aged ≥ 20 years; HbA1c level ≥ 52 mmol/mol; no improvement in HbA1c ≥ 5 mmol/mol within 3 months in BOT; and a fasting C-peptide reactin (CPR) of > 0.5 ng/ml. Exclusion criteria were: type 1 diabetes; secondary diabetes; alcoholism; severe depression, or severe psychological condition; malignancy; and abnormal hemoglobinemia. The study protocol was approved by the Institutional Review Board of Takashima General Hospital, and registered at the University hospital Medical Information Network in Japan (UMIN000005499). Written informed consent was obtained from all subjects.

Procedures and intervention

The duration of the study was 52 weeks. Subjects were screened for eligibility and gave basic demographic information, medical history, and frequency of hypoglycemia. Within a month before changing therapy from basal insulin to sitagliptin, glucagon loading test was performed without any OHAs or basal insulin for more than 24 h to evaluate insulin secretion capacity. When basal insulin was replaced by sitagliptin, the dosage of glimepiride or gliclazide was decreased to equal to or less than 2.0 mg/day or 40 mg/day, respectively, to prevent increased hypoglycemia if the subjects had been treated with more than 2.0 mg/day glimepiride or 40 mg/day gliclazide. If the subjects had been treated with equal to or less than 2.0 mg/day of glimepride or 40 mg/day of gliclazide, that dosage of SUs was maintained. Metformin (Met) and thiazolidinedione (TZD) were continued without any changes during the study. α-Glucosidase inhibitors were discontinued. The dosage of SUs was changed depending on the frequency of hypoglycemic episodes and glycemic control level. Sitagliptin was started at 50 mg/day, the usual initial dosage in Japan, which was increased to 100 mg/day if the HbA1c level did not reach 52 mmol/mol, since titration to 100 mg/day is acceptable.

Measurements

The primary endpoint was the change in HbA1c in 52 weeks. The secondary endpoints were dropout rate due to lesser efficacy of replacement by sitagliptin of basal insulin on glycemic control, change in body weight in 52 weeks, change in body mass index (BMI) in 52 weeks, change in frequency of hypoglycemia in 52 weeks, adverse events, and the correlation between change in HbA1c at the 8th week and insulin secretion capacity or CPI or SUIT at baseline. HbA1c are expressed in mmol/mol according to the recommendation of IFCC. CPI was calculated by the formula: [100 × fasting CPR (ng/ml)]/[18 × FPG (mM)] [19]. SUIT index was calculated by the formula: [250 × fasting CPR (nM)]/[(FPG − 3.43) (mM)] [20]. Blood glucose and C-peptide level were measured before (0 min) and 6 min after intravenous administration of 1 mg glucagon.

Statistical analysis

Sample size was estimated to be 34 to detect a 4 mmol/mol change in HbA1c in 52 weeks with a power of 95%, alpha 0.05 2-tailed, beta 0.20, standardized effect size 0.7. To take the dropout rate of 30% into account, the aim was to include 45 subjects. IBM SPSS Statistics was used for analysis. Dependent samples Student's *t*-test was used to compare the means of HbA1c level, insulin secretion capacity, BMI, body weight, age, and diabetes duration of the subjects between baseline and 52th week. Person's product-moment correlation test was used to evaluate the relationship between change in HbA1c and insulin secretion capacity or CPI or SUIT. To evaluate cutoff values of diabetes duration, FPG, HbA1c, 0-min CPR, 6-min CPR, delta-CPR, CPI, SUIT, and receiver operating characteristics curve (ROC) analysis were used. Independent sample Student's *t*-test was used to compare the mean of change in HbA1c in 52 weeks between subjects treated with sitagliptin + glimepiride and sitagliptin + gliclazide. Dunnett analysis was used to compare change in HbA1c in 52 weeks among subjects treated with sitagliptin + SUs and sitagliptin + SUs + MET and sitagliptin + SUs + TZD. A p-value of < 0.05 was considered as statistically significant.

Results

Participants

Forty-nine patients were eligible and were consecutively enrolled in the study (Table 1). Average age of subjects was 70.0±10.2 years; ratio of male was 60.8%; duration of diabetes was 14.3±8.2 years; average body weight was 62.3±10.4 kg; average BMI was 24.3±3.8 kg/m^2; and HbA1c was 64±9 mmol/mol. All subjects were treated with SUs; 17 subjects (34.7%) were treated with glimepiride (average dose 1.67±1.47 mg) and 32 (65.3%) were treated with gliclazide (average dose 33.8±12.0 mg). Average dosage of basal insulin analogues was 15.0±8.4 units. Glucagon loading test showed that 0-min CPR, 6-min CPR, CPI, and SUIT were 1.65±1.02 ng/ml, 3.37±1.98 ng/ml, 1.19±0.64, and 36.5±22.1, respectively. Sixteen subjects (32.6%) were dropped due to an increase in HbA1c in 8th week; 6 (29.4%) and 11 (34.4%) were dropped in glimepiride- and gliclazide-treated subjects, respectively (Table 2). No subjects were dropped for other reasons. Thirty-three subjects completed the study.

HbA1c findings and dosage of SUs and sitagliptin

Therapy adherence was confirmed by certified diabetes educators (nurses) in the study. Adherence of BOT therapy and the switching therapy were almost 100% for both therapies (data not shown).

HbA1c level in 52 weeks in final subjects was significantly decreased from 61±8 to 57±8 mmol/mol ($p<0.01$) (Table 2). Change in HbA1c in 52 weeks was −4 mmol/mol (95% CI; −5 to −4 mmol/mol) ($p<0.05$). HbA1c levels in 52 weeks in glimepiride-treated subjects (n=12) were significantly decreased from 63±9 mmol/mol to 55±9 mmol/mol ($p<0.01$). Change in HbA1c in 52 weeks was −8 mmol/mol (95% CI; −11 to −5 mmol/mol) ($p<0.05$). HbA1c levels in 52 weeks in gliclazide-treated subjects (n=21) were significantly decreased from 54±6 to 58±7 mmol/mol ($p<0.05$). Change in HbA1c in 52 weeks was −2 mmol/mol (95% CI; −4 to −0 mmol/mol) ($p<0.05$). There was a significant difference in change in HbA1c in 52 weeks between glimepiride-treated and gliclazide-treated subjects ($p<0.01$). The original dosages of glimepiride and gliclazide before the study were 1.58±0.93 mg/day and 38.2±14.0 mg/day, respectively; the initial dosages at the beginning of the study were significantly decreased to 0.96±0.40 mg/day and 24.8±8.7 mg/day, respectively ($p<0.05$); and the final dosages were significantly increased to 1.42±0.57 mg/day and 31.4±12.0 mg/day, respectively, compared to the initial dosages ($p<0.05$), and were almost equal to the original dosages (Table 2). Final dosage of sitagliptin was 74.2±25.4 mg/day in all subjects; 70.8±25.7 mg/day

Subjects (n)	49	**Basal insulin**	15.0±8.4 Units
Age (years)	70.0±10.2	**Medications**	SU 100%
			Glimepiride 34.7%
			1.67±1.47 mg
			Gliclazide 65.3%
			33.8±12.0 mg
Male	60.8%		
Diabetes duration (years)	14.3±8.2		Metformin 22.4%
			636±131 mg
Complications	Nephropathy		Thiazolidinedione 16.3%
	61.2%		10.3±3.9 mg
	Retinopathy		α-Glucosidase inhibitors
	69.4%		8.1%
	Neuropathy	**Glucagon test**	1.65±1.02
	42.8%	0-min CPR (ng/ml)	
	Cardiovascular diseases 34.7%	6-min CPR (ng/ml)	3.37±1.98
Weight (kg)	62.3±10.4	Delta CPR (ng/ml)	1.72±1.23
BMI (kg/m^2)	24.3±3.8	**CPI**	1.19±0.64
HbA1c (mmol/mol)	64±8	**SUIT**	36.5±22.1

Table 1 Demographic and clinical features of subjects participating in the study.

Table 2 Changes in HbA1c, and dosages of SUs and sitagliptin in final subjects.

Subjects (n)	Dropout rate (%) (n)	HbA1c level baseline (mmol/mol)	HbA1c level 52nd week (%)	Change in HbA1c (mmol/mol) (95% CI)	Original dosage of SUs (mg)	Initial dosage of SUs (mg)	Final dosage of SUs (mg)	Final dosage of sitagliptin (mg)
Final 33	32.6% 16	61±7	57±7**	−4* (−5 to −4)	–	–	–	74.2±25.4
Glimepiride 12	29.4% 5	63±9	55±9**	−8* (−11 to −5)	1.58±0.93	0.96±0.40*	1.42±0.57*	70.8±25.7
Gliclazide 21	34.4% 11	60±6	58±7*	−2* (−4 to −0)	38.2±14.0	24.8±8.7*	31.4±12.0*	77.3±25.5
SUs 23	30.3% 10	60±7	56±7*	−4* (−6 to −2)	–	–	–	67.4±24.3
SUs+Met 7	36.4% 4	64±9	58±8	−6* (−10 to −2)	–	–	–	87.5±23.1
SUs+TZD 3	62.5% 5	65±6	63±5	−2* (−5 to −0)	–	–	–	100±0.0

*$p<0.05$, **$p<0.01$