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ORIGINAL ARTICLE

Comparisons of efficacy and safety in insulin glargine and lixisenatide plus glulisine combination therapy with multiple daily injection therapy in Japanese patients with type 2 diabetes

Yuji Kawaguchi*, Shoko Miyamoto, Yuriko Hajika, Narumi Ashida, Koji Masumoto, Jun Sawa, Kenji Hamazaki, Yasuro Kumeda

Department of Internal Medicine, Minami Osaka Hospital, Osaka, Japan

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***Correspondence**
Yuji Kawaguchi
Tel.: +81-6-6685-0221
Fax: +81-6-6685-5208
E-mail address:
y.kawaguchi@minamiosaka.com

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ABSTRACT

Aims/Introduction: Multiple daily injection therapy for early glycemic control in patients with type 2 diabetes mellitus is associated with hypoglycemia and weight gain. This study aimed to compare the efficacy (time in range of glucose level 70–180 mg/dL), safety (time below range level 1 of glucose <70 mg/dL), glycemic variability changes, therapeutic indices, body mass index and titration periods between multiple daily injection and insulin glargine U100 and lixisenatide (iGlarLixi) combination (iGlarLixi + insulin glulisine; injected once daily [evenings]) therapies using intermittent continuous glucose monitoring.

Materials and Methods: A total of 40 hospitalized patients with type 2 diabetes were randomly assigned to the iGlarLixi + insulin glulisine group or the multiple daily injection group. An intermittent continuous glucose monitoring system was attached, and each injection was adjusted to achieve the target glucose level according to the respective titration algorithm. Times in and below the range were analyzed using data collected on days 11–13 of the intermittent continuous glucose monitoring.

Results: The time in range did not significantly differ between the groups. However, the time below range level 1 was lower in the iGlarLixi + insulin glulisine group ($P = 0.047$). The changes in glycemic variability, therapeutic indices and body mass index were not significantly different between the groups, although the titration period was significantly shorter in the iGlarLixi + insulin glulisine group ($P = 0.033$).

Conclusions: iGlarLixi + insulin glulisine combination therapy is safe and equally efficacious as multiple daily injection therapy for glycemic control, while avoiding hypoglycemia risk and reducing the number of injections are required.

INTRODUCTION

Uncontrolled type 2 diabetes mellitus is a progressive disease that negatively impacts patients' quality of life because of microvascular complications (such as diabetic retinopathy, nephropathy and neuropathy), complications associated with ischemic heart disease (such as myocardial or cerebral infarction) and issues associated with arteriosclerotic diseases (such as arterial occlusion of the lower limbs)[1]. Early interventions associated with strict glycemic control reduce the 10-year relative risk of total mortality, myocardial infarction and microangiopathy compared with the outcomes of conventional therapies[2].

Glycated hemoglobin (HbA1c) levels <7.0%, corresponding with fasting glucose levels <130 mg/dL and 2-h postprandial

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glucose levels <180 mg/dL, are recommended to prevent microangiopathies[3]. However, just 49.8% of Japanese patients with type 2 diabetes mellitus achieve the recommended HbA1c levels[4]. Furthermore, 35.6% of Japanese patients with type 2 diabetes mellitus treated with basal insulin have HbA1c levels >7.0% due to residual postprandial hyperglycemia despite achieving the target fasting blood glucose level[5]. This rate might be explained by Japanese patients showing lower postprandial additional insulin secretion, compared with their white counterparts[6]. Fasting and postprandial glucose levels rise with HbA1c levels[7]. Therefore, aside from lowering fasting glucose levels with long-acting insulin[8], multiple daily injection (MDI) therapy with bolus insulin before each meal is often required to improve postprandial hyperglycemia. This immediately lowers the glucose levels to reduce severe hyperglycemia, or eliminate glucose toxicity. MDI therapy requires injections at least three times a day, a burdensome regimen for 23.1% of patients globally[9], and might increase hypoglycemia risk and weight gain[10]. In contrast, glucagon-like peptide-1 receptor agonist (GLP-1RA) administered at once-daily injection promotes glucose-dependent insulin secretion and suppresses the postprandial glucagon levels[11]. GLP-1 RA therapy is associated with lower hypoglycemia risk and weight gain than long-acting insulin[12]. Long-acting insulin promotes glucose uptake by the liver and muscles, and suppresses gluconeogenesis in the liver, and it is used when GLP-1 RA therapy is inappropriate or already in use[12]. It is possible to lower fasting and postprandial glucose levels by combining the treatments[13]. However, there is a concern that administration might become complicated as the number of injection devices increases. Interestingly, iGlarLixi (basal insulin glargine U100 [Gla-100] and the short-acting GLP-1 RA lixisenatide [Lixi] at a fixed-dose ratio of 1 U: 1 μg) has been approved for use in Japan. The approved iGlarLixi dose ratio is 3 U:1 μg in the USA, and 3 U:1 μg or 2 U:1 μg in the European Union[14].

In the Lixilan JP-O1[15] and JP-O2[16] trials (phase III studies carried out in Japan), iGlarLixi was compared with Lixi and Gla-100, respectively. The change in the HbA1c levels from baseline to 26 weeks after administration was significantly lower in the iGlarLixi than in the Lixi and Gla-100 groups. These findings suggested that iGlarLixi treatment might achieve fasting and postprandial glycemic control with a single injection at the start of injection therapy. Nevertheless, MDI therapy is typically selected when glycemic control is inadequate, despite its disadvantages, such as complicated insulin titration and the increased number of injections, which might cause difficulties, particularly in elderly patients with type 2 diabetes mellitus[12]. The use of insulin glargine U300 (Gla-300) in MDI therapy has the advantage of reducing the risk of hypoglycemia compared with Gla-100[17]. If iGlarLixi therapy could achieve the same glucose levels and hypoglycemic risk as MDI therapy, it would be possible to simply introduce insulin to elderly type 2 diabetes mellitus patients. However, according to the results of a 7-point self-monitoring blood glucose (SMBG) in the Lixilan JP-O1 and JP-O2 trials, although the glucose level after breakfast was lowered, the suppression of glucose elevation after supper might be weakened, as Lixi is a short-acting GLP-1 RA[15,16]. Therefore, to suppress the rise of the postprandial glucose level after supper, the addition of insulin glulisine (Glu) pre-supper to the injection of iGlarLixi pre-breakfast can help to achieve stricter glycemic control, and might be a convenient and safe treatment method to replace MDI therapy.

To date, there have been no studies worldwide that have directly compared MDI therapy with twice-daily injections of iGlarLixi pre-breakfast + Glu pre-supper injection. The present study aimed to compare the efficacy, in terms of glycemic variability (GV), and the safety, in terms of hypoglycemia, between iGlarLixi + Glu combination therapy and MDI therapy in type 2 diabetes mellitus patients hospitalized for glycemic control using intermittently scanned continuous glucose monitoring (isCGM).

MATERIALS AND METHODS

Study design and participants

This randomized, open-label, parallel-group, controlled trial of patients with type 2 diabetes mellitus was carried out from August 2020 to May 2021. We explained to the patients the significance, purpose and methodology of the present study, which adhered to the tenets of the Helsinki Declaration (1975, as revised in 2013). Informed consent was obtained from all participants before their participation. The study protocol was approved by the Ethics Committee of Minami Osaka Hospital (No.2020-7), and the trial was registered with the University Hospital Medical Information Network Clinical Trial Registry (UMIN 000041551).

We enrolled 40 participants with type 2 diabetes mellitus (18 men and 22 women), who were admitted to Minami Osaka Hospital for glycemic control. The inclusion and exclusion criteria are presented in Table S1.

Figure 1 shows the study protocol. Patients who consented to participate were assigned using blocked randomization with randomly selected block sizes at a ratio of 1:1 to the iGlarLixi (pre-breakfast administration) + Glu (pre-supper administration) combination therapy group (the iGlarLixi group) or the Gla-300 (pre-breakfast administration) + Glu (pre-meal administration) treatment group (the MDI group). If oral hypoglycemic agents were used for pretreatment, the dosage and administration were not altered and treatment continued (however, participants who were assigned to the iGlarLixi group and were taking a dipeptidyl peptidase-4 inhibitor [DPP-4i] at the time of consent to participate in this study discontinued the DPP-4i treatment). In the iGlarLixi group, the starting regimen of iGlarLixi was five doses before breakfast and 2–20 units of Glu before supper, although this could be adjusted at the discretion of the attending physician. The maximum daily dose of iGlarLixi was 20 doses. In the MDI group, the Gla-300 dose started at 4–20 units before breakfast and the Glu dose started at 2–20 units before each meal, and

the doses could be adjusted at the discretion of the attending physician. In both groups, an isCGM device (Freestyle Libre Pro™; Abbott Diabetes Care, Alameda, CA, USA) was attached for 15 days, beginning the day after the initiation of injections. The doses and units of iGlarLixi, Gla-300 and Glu in each group were titrated 10 days after the initiation of isCGM by following an algorithm based on SMBG (before each meal, 2 h after each meal and before bedtime). The doses of iGlarLixi and the units of Gla-300 were titrated for a target fasting glucose level of 100–130 mg/dL[18], and the units of Glu were titrated for a target 2-h postprandial glucose level of 130–150 mg/dL[19]. The iGlarLixi, Gla-300 and Glu titration algorithms are listed in Table S2. The titration was judged to be completed based on the daily difference of the SMBG level within 10% for 2 days.

The efficacy and safety of each treatment were evaluated using data collected on days 11–13 of the isCGM. Blood sampling was carried out the day after obtaining consent for this study (day 1 of isCGM) and at the end of the testing period (day 15 of isCGM). All participants were weighed before breakfast during this study and ate a hospital-prepared diet of approximately 28 kcal/target bodyweight kg/day. As the participants in the present study had few opportunities to exercise in their daily lives, they were engaged in approximately three metabolic equivalents of exercise, such as gymnastics, exercise bikes and stair climbing for 30 min under the supervision of an exercise therapist.

Outcome measures

The primary and secondary end-points of this study were calculated from the 3-day isCGM data (days 11–13) for each treatment. The primary efficacy end-point was the time in range (TIR) of a glucose level of 70–180 mg/dL[20], and the primary safety end-point was the time below range (TBR level 1) of a glucose level <70 mg/dL[20].

The secondary end-points were as follows: (i) time above range of a glucose level ≥180 mg/dL, TBR (level 2) of a glucose level <54 mg/dL and nocturnal (00.00–06.00 hours) TBR level 1[20]; (ii) standard deviation (SD) of GV[21] (24-h and from 06.00 to 18.00 hours), coefficient of variation (CV) of GV[22] (24-h and from 06.00 to 18.00 hours), 24-h *M*-value (target glucose level = 100 mg/dL)[23], mean amplitude of glycemic excursion[23] and mean of daily difference for 24 h (average of the difference between the CGM data for days 11–12 and days 12–13)[23]; (iii) mean glucose level (24-h, and from 00.00 to 06.00 hours, 06.00 to 18.00 hours, and 18.00 to 24.00 hours) and 7-point SMBG data (pre- and post- breakfast, lunch and supper, and at bedtime)[24] on day 10 of isCGM (the last day of the titration period); (iv) the area under the glucose curve (AUC) for GV[25], the AUC of the postprandial 2 h after each meal and during the nocturnal time (00.00–06.00) hours; and (v) changes in the body mass index (BMI), HbA1c level, glycated albumin (GA) level, C-peptide immunoreactivity (CPR) and the CPR index (CPI) between baseline and day 15 of isCGM treatment, and the change (delta) between each group and the titration period. Furthermore, in the iGlarLixi group, the primary and secondary end-points by the pretrial DPP-4i (DPP-4i or non-DPP-4i groups) were evaluated.

Statistical analysis

Data are shown as the mean ± SD, unless otherwise noted. Two-tailed Student's *t*-tests were carried out to compare the GV indicators of the iGlarLixi and MDI groups, and χ^2-tests were carried out to compare the differences in frequencies between the two groups. Paired *t*-tests were carried out to compare the indicator measurements at baseline and on day 15 of isCGM in each treatment group. Pearson product-moment correlation analyses were carried out to determine the correlation coefficients between the two variables. Outlier tests were carried out using the Smirnov–Grubbs test. The cut-off for statistical

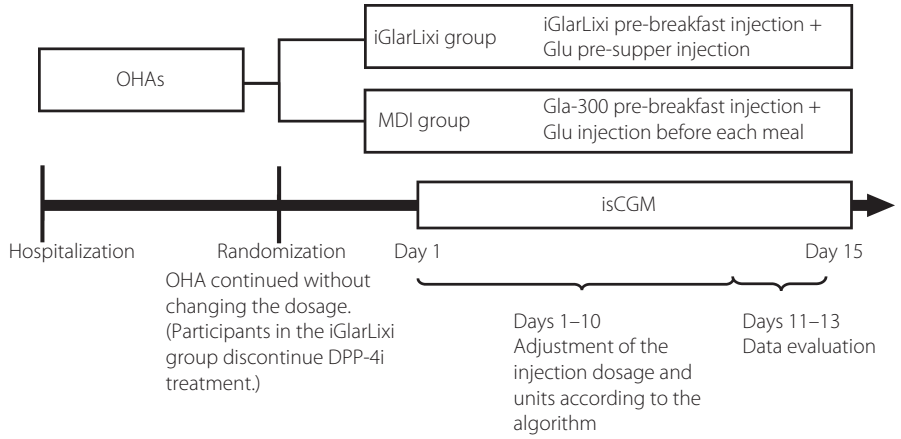

Figure 1 | Study protocol. DPP-4i, dipeptidyl peptidase-4 inhibitor; Gla-300, insulin glargine U300; Glu, insulin glulisine; iGlarLixi, insulin glargine U100 and lixisenatide; isCGM, intermittently scanned continuous glucose monitoring; MDI, multiple daily injections; OHAs, oral hypoglycemic agents.

significance was $P < 0.05$. The sample size, calculated using G power 3.1.9.2 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), with a two-tailed effect = 0.5, an α error = 0.05 and a power = 0.8, showed that a sample size of 34 was required. Therefore, it was judged that a total sample size of 40 patients, with 20 patients in each group would be sufficient. Data were analyzed using EZR version 1.37 software (Saitama Medical Center, Jichi Medical University, Saitama, Japan)[26].

RESULTS

Participant characteristics

Table 1 summarizes the characteristics of the 40 participants included in the present study. The treatments were randomly assigned. Seven male and 13 female patients were assigned to the iGlarLixi group, and 11 male and nine female patients were assigned to the MDI group. All participants were hospitalized throughout the study period and completed the trial without dropping out. During the trial period, three people in the iGlarLixi group ($n = 20$) complained of loose stools and mild nausea, although no serious gastrointestinal symptoms or adverse events (e.g., severe hypoglycemia) requiring the assistance of a third party were reported in both groups. The mean age, BMI, HbA1c levels, GA levels, CPR and CPI were 66.7 years, 27.1 kg/m^2, 8.6%, 21.7%, 2.0 ng/mL and 1.5, respectively. There were no significant differences between the two groups for any of the parameters at baseline ($P > 0.05$), nor was a significant difference in the use of oral hypoglycemic agents other than DPP-4 inhibitors between the groups.

Comparison of efficacy and safety between the iGlarLixi and MDI groups

The TIR, which was the primary end-point of efficacy, did not significantly differ between the iGlarLixi and MDI groups ($P > 0.05$). TBR level 1, which was the primary end-point of safety, was significantly lower in the iGlarLixi than in the MDI group ($P = 0.047$).

In terms of secondary end-points, the nocturnal (00.00–06.00 hours) TBR level 1 was significantly lower in the iGlarLixi than in the MDI group ($P = 0.017$), and the 00.00–06.00 hours mean glucose level was significantly lower in the MDI than in the iGlarLixi group ($P = 0.048$). None of the time above range, TBR level 2, SD of GV (24-h and 06.00–18.00), CV of GV (24-h and 06.00–18.00 hours), 24-h *M*-value, mean amplitude of glycemic excursion, mean of daily difference, mean glucose level (24-h, 00.00–06.00 hours, 06.00–18.00 hours and 18.00–24.00 hours) or 7-point SMBG assessment values differed significantly between the groups, nor did the changes in BMI, HbA1c level, GA level, CPR or CPI values. However, the titration period was significantly shorter in the iGlarLixi than in the MDI group ($P = 0.033$; Table 2).

Figure 2 shows the mean glucose level curves measured via the isCGM over three consecutive days. The AUC of the

Table 1 | Baseline characteristics of the study participants

	Overall ($n = 40$)	iGlarLixi group ($n = 20$)	MDI group ($n = 20$)	*P*-value*
Age (years)	66.7 ± 8.9	66.5 ± 8.6	67.0 ± 9.4	0.875
Duration of diabetes (years)	11.6 ± 8.9	11.7 ± 8.8	11.4 ± 9.1	0.916
Male, *n* (%)	18 (45.0)	7 (35.0)	11 (55.0)	0.340
BMI (kg/m^2)	27.1 ± 4.9	27.4 ± 5.5	26.8 ± 4.4	0.723
HbA1c (%)	8.6 ± 1.1	8.3 ± 1.0	8.8 ± 1.2	0.221
GA (%)	21.7 ± 5.2	20.6 ± 4.7	22.8 ± 5.5	0.174
FPG (mg/dL)	141.2 ± 48.5	133.4 ± 43.2	149.1 ± 53.3	0.312
CPR (ng/mL)	2.0 ± 1.2	2.1 ± 1.2	1.9 ± 1.3	0.605
CPI	1.5 ± 1.0	1.6 ± 0.8	1.4 ± 1.2	0.506
eGFR (mL/min/1.73 m^2)	64.8 ± 23.2	64.9 ± 24.4	64.8 ± 22.7	0.985
TG level (mg/dL)	168.1 ± 86.2	156.6 ± 66.7	179.7 ± 102.5	0.405
LDL-C level (mg/dL)	96.6 ± 38.5	95.8 ± 36.3	97.5 ± 41.4	0.894
HDL-C level (mg/dL)	51.0 ± 13.2	50.6 ± 14.2	51.5 ± 12.5	0.832
S-albumin level (g/dL)	3.9 ± 0.4	3.8 ± 0.4	3.9 ± 0.3	0.573
DPP-4 inhibitor, pretrial (*n*)	18	10	8	0.751
Antihyperglycemic drugs				
Metformin (*n*)	24	13	11	0.747
DPP-4 inhibitor (*n*)	8	–	8	–
SGLT-2 inhibitor (*n*)	17	9	8	1.000
α-Glucosidase inhibitor (*n*)	1	1	0	1.000

Data are presented as the means ± standard deviation. BMI, body mass index; CPI, C-peptide index; CPR, C-peptide immunoreactivity; DPP-4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GA, glycated albumin; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; iGlarLixi, insulin glargine U100 and lixisenatide; LDL-C, low-density lipoprotein cholesterol; MDI, multiple daily injections; SGLT-2, sodium–glucose cotransporter 2; TG, triglyceride. *Student's *t*-test or the χ^2-test is used to compare data between the two groups. Antidiabetic drug dosages did not change throughout the study period.

Table 2 | Self-monitoring blood glucose and intermittently scanned continuous glucose parameters of glucose variability and diabetes-related factors in patients treated with insulin glargine U100 and lixisenatide combination therapy or multiple daily injections therapy

	iGlarLixi group	MDI group	*P*-value
Percentage of time in target glucose range (70–180 mg/dL)	93.1 ± 8.8	90.4 ± 9.1	0.100
Patients with time in target glucose range (70–180 mg/dL) >70%, *n* (%)	19 (95.0)	20 (100)	0.311
Percentage of time below target glucose range (<70 mg/dL)	1.5 ± 2.5	2.9 ± 4.7	0.047*
Patients with time below target glucose range (<70 mg/dL) <4%, *n* (%)	18 (90.0)	14 (70.0)	0.114
Percentage of time above target glucose range (>180 mg/dL)	5.4 ± 8.2	6.7 ± 8.8	0.390
Percentage of time below target glucose range (<54 mg/dL)	0.0 ± 0.1	0.2 ± 0.7	0.127
Percentage of nocturnal time below target glucose range (<70 mg/dL)	0.3 ± 1.1	1.6 ± 3.4	0.017*
24-h SD of glycemic variability (mg/dL)	28.6 ± 8.4	30.5 ± 10.0	0.266
06:00–18:00 h SD of glycemic variability (mg/dL)	29.0 ± 11.0	27.1 ± 11.9	0.369
24-h CV of glycemic variability (%)	24.7 ± 5.8	26.4 ± 6.5	0.144
06:00–18:00 h CV of glycemic variability (%)	23.0 ± 7.3	21.1 ± 7.4	0.332
24-h *M*-value (target glucose level: 100 mg/dL)	3.4 ± 3.2	4.0 ± 2.7	0.590
MAGE (mg/dL)	73.6 ± 26.8	81.4 ± 37.0	0.190
MODD in glucose level (mg/dL)	19.8 ± 6.6	22.6 ± 11.0	0.335
24-h mean glucose level (mg/dL)	115.0 ± 17.2	113.7 ± 18.0	0.680
00.00–06.00 hours mean glucose level (mg/dL)	97.4 ± 19.7	90.7 ± 17.4	0.048*
06.00–18.00 hours mean glucose level (mg/dL)	124.5 ± 19.7	121.7 ± 22.8	0.476
18.00–24.00 hours mean glucose level (mg/dL)	113.8 ± 20.9	120.8 ± 24.6	0.097
Preprandial glucose level at breakfast (mg/dL)	118.4 ± 15.8	110.5 ± 16.8	0.134
Preprandial glucose level at lunch (mg/dL)	115.1 ± 29.4	128.1 ± 18.4	0.103
Preprandial glucose level at supper (mg/dL)	140.9 ± 30.8	125.4 ± 35.4	0.149
Postprandial glucose level 2 h after breakfast (mg/dL)	120.7 ± 30.5	124.7 ± 38.6	0.718
Postprandial glucose level 2 h after lunch (mg/dL)	149.2 ± 35.4	133.5 ± 43.4	0.219
Postprandial glucose level 2 h after supper (mg/dL)	137.1 ± 22.4	141.3 ± 32.7	0.638
Bedtime glucose level (mg/dL)	112.0 ± 25.7	110.3 ± 33.8	0.859
AUC of the postprandial 2 h after breakfast (mg/dL h)	265.0 ± 58.7	270.3 ± 64.3	0.642
AUC of the postprandial 2 h after lunch (mg/dL h)	325.4 ± 98.3	316.2 ± 124.9	0.654
AUC of the postprandial 2 h after supper (mg/dL h)	257.1 ± 66.6	275.9 ± 65.0	0.120
AUC of the nocturnal time (mg/dL h)	585.2 ± 117.8	544.8 ± 104.2	0.049*
Delta BMI (kg/m^2)	0.8 ± 0.6	0.5 ± 0.7	0.198
Patients without weight gain, *n* (%)	18 (90.0)	18 (90.0)	1.000
Delta HbA1c (%)	0.6 ± 0.4	0.8 ± 0.5	0.390
Delta GA (%)	3.1 ± 2.3	4.7 ± 2.7	0.065
Delta CPR (ng/mL)	0.6 ± 1.2	0.4 ± 1.2	0.584
Delta CPI	0.1 ± 0.8	0.0 ± 0.9	0.631
Titration period (days)	6.0 ± 2.5	7.8 ± 2.5	0.033*
iGlarLixi (doses/day)	10.3 ± 3.6	–	–
Glu (U/day)	6.1 ± 3.0	20.0 ± 10.9	<0.001*
Gla-300 (U/day)	–	12.0 ± 5.8	–
Total daily dose of insulin (U/day)	16.3 ± 5.3	31.9 ± 14.2	<0.001*

Data are presented as the mean ± standard deviation. Data between the groups are compared using Student's *t*-test or the χ^2-test. AUC, area under the curve; BMI, body mass index; CPI, C-peptide index. Glu, insulin glulisine; CPR, C-peptide immunoreactivity; CV, coefficient of variation; GA, glycated albumin; Gla-300, insulin glargine U300; HbA1c, glycated hemoglobin; iGlarLixi, insulin glargine U100 and lixisenatide; isCGM, intermittently scanned continuous glucose monitoring; MAGE, mean amplitude of glycemic excursion; MDI, multiple daily injections; MODD, mean of daily difference; SD, standard deviation; SMPG, self-monitoring plasma glucose. *Indicates a statistically significant difference between groups.

postprandial 2 h after each meal was not significantly different between the two groups, but the AUC of the nocturnal time was significantly smaller in the MDI than in the iGlarLixi group ($P = 0.049$). In the iGlarlixi group, there was no significant difference in the primary and secondary end-points by pretrial DPP-4i (Table S3).

Changes in the BMI, HbA1c level, GA level and endogenous insulin secretory capacity between baseline and day 15 of isCGM treatment in the iGlarLixi and MDI groups

The BMI, HbA1c level and GA level significantly decreased between the pre- and post-trial time points in both treatment groups. There was no significant difference in CPI between the

two groups before and after the study period. However, the CPR significantly decreased in the iGlarLixi group ($P = 0.018$; Table 3).

Correlation between iGlarLixi doses, Glu and Gla-300 units, and the baseline CPR in each treatment group

In the present study, we analyzed the correlations between the baseline CPR and iGlarLixi doses and Glu units administered in the iGlarLixi group, and between the baseline CPR and Gla-300 and Glu units administered in the MDI group. One participant with a high CPR level, which was judged to be an outlier by the Smirnov–Grubbs test, was excluded from the analysis. A significant negative correlation was observed between the iGlarLixi doses and CPR values in the iGlarLixi group (Figure 3a). No correlation was found between any other measure and the CPR values (Figure 3b–d).

DISCUSSION

In the present study, we showed that a single injection of iGlarLixi and Glu was non-inferior to MDI therapy regarding efficacy, based on the TIR. Furthermore, the treatment improved safety, as evidenced by the significantly decreased TBR level 1, especially the decreased nocturnal TBR level 1, based on the isCGM. Patients with diabetes can show a TIR percentage of 70% and an HbA1c level of approximately 7.0%[27]; a target TIR using isCGM of ≥70% is recommended[3]. Both the iGlarLixi and MDI groups achieved a TIR of at least 70%, and an increased TIR correlates with a reduced complications risk[28–30].

Regarding safety, a TBR <4% is a common target for type 1 and type 2 diabetes[3], and this target level was achieved in both treatment groups, although the TBR in the iGlarLixi group was significantly lower than that in the MDI group. A TBR level 2 was rarely observed in either group. However, nocturnal hypoglycemia unawareness has been shown to reduce physical activity, quality of life and sleep quality on the subsequent day[31]. The MDI treatment had a smaller nocturnal AUC and increased the risk of nocturnal hypoglycemia. Therefore, attention should be paid to hypoglycemia unawareness at night.

There were no significant differences between the two groups regarding the GV indices. Among the GV indices, CV and SD were the most popular metrics, as they are simple, familiar and clearly defined[32]. In the present study, the SD in the isCGM of the iGlarLixi group of 28.6 mg/dL was lower than that reported in the LixiLan-L study carried out with iGlarLixi[33]. The SD of the 7-point SMBG was 32.4 mg/dL[33], possibly because the postprandial glucose elevation after supper was further suppressed by the Glu administered before supper. Although there was no significant difference between the groups in the present study, the SD was lower in the iGlarLixi than in the MDI group, and hypoglycemia was significantly suppressed. This might be a result of the lowering of the fasting glucose level through the effect of the long-acting insulin and the lowering of the postprandial glucose elevation through the effect of the GLP-1 RA. GLP-1 RAs promote insulin secretion in a blood glucose-dependent manner and suppress postprandial glucagon levels[11], while simultaneously maintaining the reverse regulatory process of hypoglycemia[34]. Therefore, they possibly correct any hypoglycemia that might be caused by the long-acting insulin.

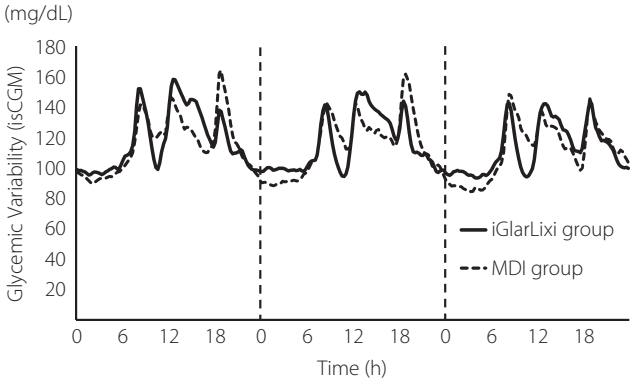

Figure 2 | Three-day mean glycemic variability curve of the 20 participants in each of the insulin glargine U100 and lixisenatide (iGlarLixi) and multiple daily injections (MDI) insulin groups based on the intermittently scanned continuous glucose monitoring (isCGM) data. The solid and dotted lines show the glycemic variability curves of participants in the iGlarLixi and MDI groups, respectively. Patients in the iGlarLixi group received iGlarLixi (pre-breakfast injection) + insulin glulisine (pre-supper injection) treatment. Patients in the MDI insulin group received insulin glargine U300 (Gla-300; pre-breakfast injection) + insulin glulisine (pre-meal injection) treatment.

Table 3 | Changes in the body mass index, glycated hemoglobin level, glycated albumin level and endogenous insulin secretory capacity between pre- and post-treatment timepoints in the insulin glargine U100 and lixisenatide and multiple daily injections groups

iGlarLixi group ($n = 20$)	Pre-treatment	Post-treatment	*P*-value
BMI (kg/m^2)	27.4 ± 5.5	26.6 ± 5.3	<0.001*
HbA1c (%)	8.3 ± 1.0	7.7 ± 0.8	<0.001*
GA (%)	20.6 ± 4.7	17.4 ± 3.5	<0.001*
CPR (ng/mL)	2.1 ± 1.2	1.5 ± 0.5	0.036*
CPI	1.6 ± 0.8	1.5 ± 0.5	0.616
MDI group ($n = 20$)	Pre-treatment	Post-treatment	*P*-value
BMI (kg/m^2)	26.8 ± 4.4	26.3 ± 4.3	0.004*
HbA1c (%)	8.8 ± 1.2	8.0 ± 1.1	<0.001*
GA (%)	22.8 ± 5.5	18.1 ± 4.2	<0.001*
CPR (ng/mL)	1.9 ± 1.3	1.5 ± 1.7	0.167
CPI	1.4 ± 1.2	1.4 ± 1.4	0.854

Data are presented as means ± SDs. Pre-and post-treatment measurements are compared using paired *t*-tests. BMI, body mass index; CPI, C-peptide index; CPR, C-peptide immunoreactivity; GA, glycated albumin; HbA1c, glycated hemoglobin; iGlarLixi, insulin glargine and lixisenatide; MDI, multiple daily injections. *Indicates a statistically significant difference between time points.

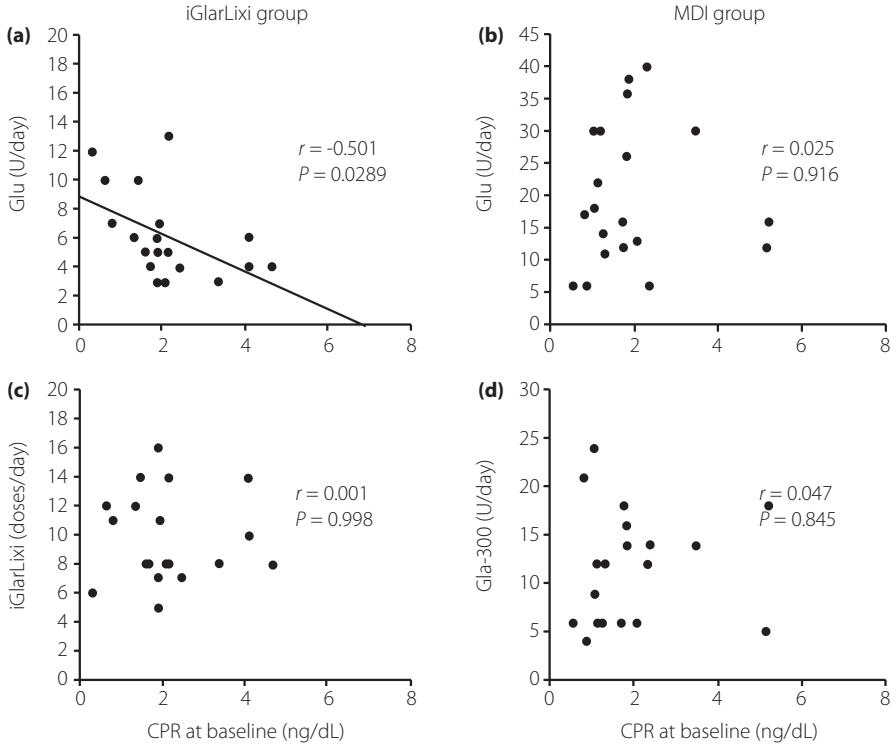

Figure 3 | Correlations between insulin glargine U100 and lixisenatide (iGlarLixi) doses and the number of insulin glulisine (Glu) and insulin glargine U300 (Gla-300) units per day, and baseline C-peptide immunoreactivity (CPR) measurements in each treatment group. A Pearson product-moment correlation test was used to determine the correlation coefficients between the two variables shown in each graph. Patients in the iGlarLixi group received iGlarLixi (pre-breakfast injection) + Glu (pre-supper injection) treatment. (a) Relationship between the Glu units and CPR at baseline. (c) Relationship between iGlarLixi doses and CPR at baseline. Patients in the multiple daily injections (MDI) insulin group received insulin Gla-300 (pre-breakfast injection) + Glu (pre-meal injection) treatment. (b) Relationship between Glu and CPR at baseline. (d) Relationship between Gla-300 units and CPR at baseline.

The CV of the GV is associated with hypoglycemic risk, and when the CV is <25%, the risk of hypoglycemia is extremely low[32]. In the present study, the mean CVs of the iGlarLixi and MDI groups were 24.7% and 26.4%, respectively, and this difference might have contributed to the significant differences in the TBR and nocturnal TBR between the two groups.

In the present study, there was no significant difference in the 7-point SMBG on day 10 of isCGM treatment between the two groups. However, in a study comparing IDegLira (fixed combination of insulin degludec and liraglutide) treatment with MDI treatment, the glucose levels in the MDI treatment were significantly lower than in the IDegLira group after lunch, before supper, after supper and at bedtime based on 9-point SMBG[35]. Lixi is a short-acting GLP-1 RA with a half-life of 2–4 h, and its strong binding affinity to the GLP-1 receptor induces a daytime hypoglycemic effect through once-daily administration[36]. However, the suppression of the postprandial glucose levels after supper might be weakened as a result[15,16]. In such cases, administration of fast-acting insulin before supper, as was done in the present study, might enable a glycemic control like that of MDI treatment.

Regarding the titration period, in a study, in which inpatients were treated with MDI using Gla-100, the mean titration period to achieve the target glucose level by titrating according to the algorithm was 8.0 days[37], which was similar to the average of 7.8 days for the MDI group in the present study. In contrast, the mean titration period of the iGlarLixi group was 6.0 days, which was significantly shorter than that of the MDI group. There are three possible reasons for this difference in the titration period. First, the MDI group was titrated four times a day, whereas the iGlarLixi group was titrated twice a day according to the algorithm. Furthermore, the number of times was related to the complexity. Second, the iGlarLixi group reached the target glucose level quickly, with almost no occurrence of hypoglycemia. Third, as Lixi continued to have residual effects even after supper, the required number of units of Glu was low, and it was easy to titrate.

The BMI, HbA1c and GA levels significantly decreased after treatment in both groups, although the CPR was

significantly reduced only in the iGlarLixi group. A possible reason for this finding is that the CPR of the iGlarLixi group at baseline was higher, although not significantly, than that of the MDI group. Thus, the equivalent glycemic control reduced the CPR to similar levels in both groups, thus resulting in a significantly greater reduction of the CPR in the iGlarLixi group.

In the iGlarLixi group, the Glu dose (U/day) and CPR values at baseline were significantly negatively correlated. There is a correlation between the relative contribution rate of the incretin effect on insulin and fasting glucose levels. Interestingly, it has been suggested that the incretin effect is enhanced by correcting the fasting glucose level by administering basal insulin[38]. In addition, the HbA1c lowering effect of GLP-1 RAs correlates with the CPI, which is an index of residual pancreatic β-cell function[39]. Thus, it might be possible to lower the fasting glucose level and enhance the incretin effect by administering a sufficient amount of iGlarLixi to type 2 diabetes mellitus patients with a stable CPR. In such patients, Glu injection before supper becomes unnecessary, and once-daily iGlarLixi injection might be as effective as MDI treatment.

The present study had several limitations. This was a randomized, controlled trial carried out in a single hospital, with a small sample size of 20 patients in each group. To obtain more real-world results, it will be necessary to carry out multicenter joint studies using a common protocol and to increase the sample size of each group to ≥100 individuals. Furthermore, as the present study was carried out over a short period during which the patients were hospitalized, we analyzed the results of isCGM over just 2 weeks. For this reason, we could analyze the indicators related to GV obtained through isCGM, although at least 1 year would be required to investigate the long-term impact of changes in bodyweight and HbA1c levels. Furthermore, we could analyze safety issues according to the presence of adverse events, such as hypoglycemia and gastrointestinal symptoms.

In conclusion, the data obtained from the isCGM showed that in patients with type 2 diabetes mellitus, iGlarLixi once-daily + Glu once-daily treatment is as effective in achieving glycemic control as MDI therapy. Furthermore, avoiding the risk of hypoglycemia and offering the convenience of requiring fewer injections might increase the quality of life in patients with type 2 diabetes mellitus.

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DISCLOSURE

Conflict of interest: Y Kawaguchi received lecture honoraria or speaker fees from Sanofi K.K., Novo Nordisk Pharma, Takeda Pharmaceutical Co., Boehringer Ingelheim, Sumitomo Dainippon Pharma Co., Ltd., Astellas Pharma Inc. and Kowa Company, Ltd. The other authors declare no conflict of interest.

Approval of the research protocol: The study protocol was approved by the Ethics Committee of Minami Osaka Hospital. The study was carried out according to the tenets of the Declaration of Helsinki.

Informed consent: Informed consent was obtained from all participants before their participation.

Approval date of registry and the registration no. of the study/-trial: Approved on August 4, 2020 (Approval No. 2020-7).

Animal studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Selection and exclusion criteria.

Table S2 | The insulin glargine U100 and lixisenatide + insulin glargine U300 and insulin glulisine titration algorithms.

Table S3 | Intermittently scanned continuous glucose monitoring parameters of glucose variability and diabetes-related parameters in patients treated with insulin glargine U100 and lixisenatide by pretrial dipeptidyl peptidase-4 inhibitor.