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BRIEF REPORT

Effects of DPP-4 inhibitor linagliptin and GLP-1 receptor agonist liraglutide on physiological response to hypoglycaemia in Japanese subjects with type 2 diabetes: A randomized, open-label, 2-arm parallel comparative, exploratory trial

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Dipeptidyl peptidase-4 (DPP-4) inhibitors reduce the risk of hypoglycaemia, possibly through augmentation of glucose-dependent insulinotropic polypeptide (GIP) action, but not that of glucagon-like peptide-1 (GLP-1) on glucagon secretion. To examine this model in Japanese individuals with type 2 diabetes (T2D), the effects of the DPP-4 inhibitor linagliptin on glucagon and other counter-regulatory hormone responses to hypoglycaemia were evaluated and compared with those of the GLP-1 receptor agonist liraglutide in a multi-centre, randomized, open-label, 2-arm parallel comparative, exploratory trial. Three-step hypoglycaemic clamp glucose tests preceded by meal tolerance tests were performed before and after 2-week treatment with the drugs. Glucagon levels were increased during the hypoglycaemic clamp test at 2.5 mmol/L. This increase was similar in the linagliptin and liraglutide groups, both before and after the 2-week treatment. Changes in other counter-regulatory hormones (ie, growth hormone, cortisol, epinephrine and norepinephrine) were also similar between the groups, but were suppressed substantially after 2-week treatment compared to baseline. In conclusion, we confirmed that the glucagon response to hypoglycaemia was not affected by linagliptin or liraglutide treatment in Japanese individuals with T2D.

KEYWORDS

DPP-4 inhibitor, GLP-1 receptor agonist, glucagon response, hypoglycaemia, sympatho-adrenal response

1 | INTRODUCTION

Dipeptidyl peptidase-4 (DPP-4) inhibitors are widely used in treatment of type 2 diabetes (T2D).[1,2] DPP-4 inhibitors increase biologically intact forms of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), both of which enhance glucose-induced insulin secretion from pancreatic β-cells.[3,4] GLP-1 suppresses glucagon secretion from pancreatic α-cells when glucose levels are high,[5,6] whereas GIP augments glucagon secretion in response to hypoglycaemia.[7–10] Thus, enhancement of GIP and GLP-1 actions by DPP-4 inhibitors should contribute, not only to amelioration of hyperglycaemia, but also to mitigation of severe hypoglycaemia. It was reported that the DPP-4 inhibitor vildagliptin enhances the glucagon response to hypoglycaemia in drug-naïve individuals with T2D,[11] whereas the glucagon response to hypoglycaemia was not affected by GLP-1 receptor (GLP-1R) agonists.[12,13] This might suggest differing effects of DPP-4 inhibitors and GLP-1R agonists on the glucagon response to hypoglycaemia, which needs to be examined in head-to-head studies. In this study, we compared effects of the DPP-4 inhibitor linagliptin with those of the GLP-1R agonist liraglutide on the responses to hypoglycaemia of glucagon and other counter-regulatory hormones in Japanese individuals with T2D.

2 | METHODS

2.1 | Study protocol

This was a multi-centre, randomized, prospective, open-label, 2-arm parallel comparative, exploratory study in Japanese individuals with T2D (UMIN-CTR clinical trial registration number: UMIN000014417). Those eligible were randomized in a 1-to-1 ratio to a linagliptin or liraglutide treatment group by computer-based dynamic allocation, taking gender, age and BMI into consideration for stratified randomization based on screening results at visit 1 (V1, week −4). A meal tolerance test (MTT) and stepped hyperinsulinaemic glucose clamp test (SHGCT) were performed at visit 2 (V2, day 0) and visit 3 (V3, day 14); blood samples were obtained and analysed as described in File S1. Subjects received study medication for 14 days (days 1-14), linagliptin 5 mg once daily or liraglutide 0.3 mg via subcutaneous injection once daily from day 1 to day 6 and 0.6 mg from day 7 to day 14. Liraglutide up to only 0.9 mg once daily is the dosage approved in Japan. Subjects were recruited and screened in 2 medical institutions in Osaka and Fukuoka, Japan after obtaining approval of the respective ethics committees. Randomization was performed at A2 Healthcare Corp in Tokyo, Japan. Data were collected in an inpatient ward of the medical institution in Fukuoka, Japan. Recruitment of subjects began July 5, 2014 and the last follow-up date was April 20, 2015. Written informed consent was obtained from all subjects.

2.2 | Study population

Eligible Japanese individuals with T2D were age 40-70 years with a body mass index (BMI) < 30 kg/m^2, and a treatment history of a single oral hypoglycaemic agent with HbA1c 6.0% to 8.0% (42-64 mmol/mol) or no drug treatment with HbA1c 6.0% to 8.5% (42-69 mmol/mol). Individuals requiring insulin therapy, or those treated with insulin, DPP-4 inhibitors or GLP-1 receptor agonists during the 4 weeks before informed consent, or those treated with pioglitazone during the 12 weeks before informed consent were excluded, as were those with cardiac failure or history of cerebro-cardiovascular disease or pancreatitis. The sample size was not based on a power calculation as this was an exploratory pilot study; however, the sample size would seem to be appropriate in the context of previous, related studies.[9,11]

2.3 | Study assessments

Major outcomes were changes in glucagon and other counter-regulatory hormones (ie, epinephrine, norepinephrine, cortisol and growth hormone [GH]) during SHGCT following 2-week treatment with linagliptin or liraglutide in the per protocol set (PPS) population (ie, subjects who received 14-day administration of study medication with successful completion of SHGCT on both V2 and V3). All adverse events (AE) were collected and AE by system organ class/preferred terms, serious adverse events (SAE) and discontinuation from AE were summarized for the full analysis set (FAS) population (ie, subjects who received at least 1 dose of study medication, with data from at least 1 point of safety available after drug administration, regardless of successful completion of SHGCT on either V2 or V3).

2.4 | Statistical analysis

All analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina). Results are reported as mean ± SE unless otherwise stated. Area-under-the curve (AUC) of each measurement was calculated according to the trapezoidal rule. Repeated measures were analysed with mixed-design analysis of covariance (ANCOVA). The changes of each measurement from 225 to 255 minutes were tested using two sample *t* test. A 2-sided *P* value < .05 was taken to indicate significant difference.

3 | RESULTS

Thirty-five screened subjects were randomized to the linagliptin treatment group or the liraglutide treatment group; they received study assessments at V2 and V3 and were analysed as the FAS population (Table 1 and Figure S1). Six of them did not reach the target blood glucose level in the hypoglycaemic phase at V2 and/or V3 and were excluded from the PPS population.

MTT were performed to increase GIP and GLP-1 prior to SHGCT. Glucose levels were similar between the linagliptin and liraglutide groups at V2, and were decreased similarly at V3, to a greater degree in the liraglutide group (Table S1 and Figure S2). C-peptide and ISR were significantly higher in the liraglutide group at V2 and V3, and were similarly enhanced at V3 compared to V2 in both groups. Glucagon tended to be higher in the liraglutide group at V2 and V3, but was similarly suppressed at V3 compared to V2 in both groups. Intact GIP levels were similar in the two groups at V2, and were increased at V3 only in the linagliptin group; GIP levels were similar between

the two groups at V2 and V3 (Table S1 and Figure S3). Total and intact GLP-1 were not determined in the liraglutide group throughout the study because of the difficulty in differentiating liraglutide from endogenous GLP-1.

SHGCT were performed immediately after MTT. Glucose levels at V2 and V3 in both groups were similarly decreased during euglycaemic and hypoglycaemic phases (Figure S4). After discontinuation of insulin infusion at 255 minutes, blood glucose recovered similarly at V2 and V3 in both groups. The recovery from hypoglycaemia exhibited no significant difference between groups and glucose infusion rates (IIR) from 255 to 300 minutes were similar between groups and between visits. Glucose infusion rates were also similar between groups and between visits (Figure S7). ISR at V3 was higher than that at V2 during the hyperglycaemic and euglycaemic phases, but there was no difference in ISR during the hypoglycaemic phase between the two groups or between visits (Table 2 and Figure S4). During the hyperglycaemic and euglycaemic phases, glucagon at V2 and V3 were nearly unchanged and were similar in both groups (Table 2 and Figure S5). Glucagon increased rapidly during the hypoglycaemic phase and there was no difference in glucagon response between groups or between visits. Total GIP levels were similar between groups at V2 and V3. Intact GIP levels were similar between groups at V2, and were increased only in the linagliptin group at V3. GH, cortisol, epinephrine and norepinephrine were increased during the latter half of the hypoglycaemic phase at V2 and V3 (Table 2 and Figure S6). The changes in GH, cortisol, epinephrine and norepinephrine at V3 were less than those at V2 in both groups. The overall results in the FAS population were fully consistent with those of the PPS population (data not shown).

3.1 | Safety

AE and SAE were analysed in the FAS population. No AE was reported in the linagliptin group. One subject in the liraglutide group experienced nausea, which was assessed as non-serious and treatment-related. No SAE was experienced by any individual in either group.

4 | DISCUSSION

A main finding of this study comparing linagliptin with liraglutide in Japanese individuals with T2D confirms our general understanding that incretin therapies do not prevent hypoglycaemia-induced glucagon secretion, as was previously reported for the DPP-4 inhibitor vildagliptin and the GLP-1R agonists albiglutide and lixisenatide in individuals with T2D.[11–14] This supports the notion that these therapies are not associated with increased risk of hypoglycaemia. Importantly, the present study shows that linagliptin did not enhance hypoglycaemia-induced glucagon secretion even though biologically intact GIP was significantly elevated. It has been shown previously that vildagliptin enhanced hypoglycaemia-induced glucagon secretion in drug-naive individuals with T2.[11] Discrepancies between that study and ours could be explained by the following possibilities. First, different DPP-4 inhibitors were used for different treatment periods (ie, vildagliptin, 4 weeks; linagliptin, 2 weeks). Second, different comparisons were made (ie, vildagliptin vs placebo; linagliptin vs liraglutide; before vs after linagliptin treatment). Third, different ethnic groups were studied (ie, Caucasian vs Japanese); T2D in East Asians, including Japanese, is characterized primarily by β-cell dysfunction with less adiposity and it differs phenotypically from T2D in Caucasians,[15] showing greater HbA1c reduction in response to DPP-4 inhibitors and GLP-1R agonists.[16,17] Finally, subjects with different baseline HbA1c levels were enrolled (ie, mean HbA1c 6.3% vs 7.3%). It could therefore be possible that glucagonotropic effects of GIP might be impaired by chronic hyperglycaemia, similarly to the insulinotropic effects of GIP.[18] Interestingly, enhancement of the hypoglycaemia-

TABLE 1 Demographic and baseline characteristics of full analysis set and per protocol set populations

	Full analysis set			Per protocol set		
	Linagliptin	Liraglutide	*P* value	Linagliptin	Liraglutide	*P* value
n (male/female)	18 (13/5)	17 (12/5)		15 (11/4)	14 (10/4)	
Age (years)	58.56 ± 7.81	57.76 ± 7.63	.764	59.53 ± 7.12	56.79 ± 7.66	.326
BMI (kg / m^2)	23.82 ± 2.29	23.98 ± 3.15	.871	23.47 ± 1.93	23.88 ± 3.49	.702
Waist circumference (cm)	86.79 ± 8.86	87.46 ± 10.85	.842	86.05 ± 9.2	85.53 ± 10.8	.889
Duration (years)	4.00 ± 3.66	3.59 ± 5.73	.801	4.67 ± 3.66	3.50 ± 6.02	.530
FPG (mg/dL)	143.89 ± 31.19	139.65 ± 27.36	.672	146.73 ± 33.54	137.21 ± 28.34	.418
HbA1c (%)	7.38 ± 0.71	7.21 ± 0.59	.441	7.37 ± 0.75	7.14 ± 0.57	.375
OAD use (%)	0	0		0	0	
Systolic BP (mm Hg)	130.44 ± 10.99	128.24 ± 9.26	.526	131.40 ± 11.56	127.93 ± 8.91	.376
Diastolic BP (mm Hg)	78.89 ± 6.91	78.47 ± 8.24	.871	78.67 ± 7.47	78.43 ± 9.10	.939
Total-cholesterol (mg/dL)	217.28 ± 38.44	224.76 ± 45.15	.600	219.13 ± 36.38	217.29 ± 44.89	.904
HDL-cholesterol (mg/dL)	59.17 ± 16.19	61.41 ± 15.74	.680	61.53 ± 16.08	63.21 ± 16.78	.785
Triglyceride (mg/dL)	157.39 ± 96.03	120.65 ± 34.94	.143	148.20 ± 99.38	112.79 ± 32.47	.208
DPP-4 activity (nmol/mL/min)	8.73 ± 1.66	9.02 ± 1.95	.639	8.76 ± 1.72	9.19 ± 2.09	.542

Each value represents mean ± standard deviation.

Abbreviations: BMI, body mass index; BP, blood pressure; DPP-4, dipeptidyl peptidase-4; FPG, fasting plasma glucose; HDL, high density lipoprotein; OAD, oral anti-diabetic drugs.

TABLE 2 Response to hypoglycaemia in the per protocol population

		Linagliptin					Liraglutide					Δ(V3 - V2)				
		V2		V3			V2		V3			Linagliptin		Liraglutide		
		Mean	S.E.	Mean	S.E.	*P* value	Mean	S.E.	Mean	S.E.	*P* value	Mean	S.E.	Mean	S.E.	*P* value
ISR	Δ{225 - 255} (pmol / m^2)	− 84.85	24.21	− 105.90	17.37	.493	− 105.70	23.42	− 95.82	19.84	.733	− 21.05	29.89	9.89	28.36	.461
	AUC{225 - 255} (pmol / m^2 × min)	2057.94	317.03	2010.04	250.84	.907	1824.22	283.26	2135.27	286.97	.463	− 47.90	403.86	311.05	411.03	.539
Glucagon	Δ{225 - 255} (pg / mL)	70.87	16.59	55.73	10.75	.393	56.43	12.24	62.21	13.25	.284	− 15.13	17.16	5.79	5.18	.260
	AUC{225 - 300} (pg / mL × min)	9151.00	930.08	8653.50	700.72	.358	9051.43	787.85	8776.61	786.45	.226	− 497.50	523.15	− 274.82	216.39	.699
Growth hormone	Δ{225 - 255} (ng / mL)	5.07	1.04	2.48	0.87	.023	2.58	0.80	0.94	0.39	.081	− 2.60	1.01	− 1.65	0.87	.486
	AUC{225 - 300} (ng / mL × min)	329.10	45.03	256.65	48.81	.222	275.08	49.58	219.30	47.28	.264	− 72.44	56.62	− 55.78	47.72	.825
Cortisol	Δ{225 - 255} (μg / dL)	7.09	1.49	3.05	1.12	.035	3.34	1.47	1.51	0.96	.315	− 4.05	1.74	− 1.83	1.75	.377
	AUC{225 - 300} (μg / dL × min)	1302.25	71.33	970.80	65.19	.000	1225.45	103.09	907.61	64.00	.001	− 331.45	68.13	− 317.84	77.99	.896
Epinephrine	Δ{225 - 255} (ng / mL)	0.98	0.22	0.53	0.14	.103	0.72	0.20	0.33	0.09	.091	− 0.45	0.16	− 0.39	0.12	.769
	AUC{225 - 300} (ng / mL × min)	36.25	7.56	20.25	3.60	.006	28.23	6.19	13.23	2.25	.004	− 16.00	4.92	− 15.00	4.35	.881
Norepinephrine	Δ{225 - 255} (ng / mL)	0.29	0.07	0.14	0.04	.041	0.23	0.05	0.15	0.04	.079	− 0.15	0.06	− 0.08	0.04	.422
	AUC{225 - 300} (ng / mL × min)	30.09	3.37	27.90	2.02	.277	29.76	2.19	27.96	1.78	.313	− 2.19	1.93	− 1.80	1.72	.883
Total GIP	Δ{225 - 255} (pmol / L)	67.07	35.14	60.08	23.76	.871	− 66.22	58.52	− 29.28	29.28	.579	− 6.98	34.20	36.94	48.61	.452
	AUC{225 - 300} (pmol / L × min)	14196.60	2810.26	13006.23	1441.86	.620	9828.73	766.24	14330.31	1533.50	.014	− 1190.37	2349.66	4501.58	1581.68	.058
Intact GIP	Δ{225 - 255} (pmol / L)	9.17	5.50	11.71	6.34	.765	− 8.04	8.08	0.13	5.57	.413	2.54	7.37	8.17	7.06	.587
	AUC{225 - 300} (pmol / L × min)	1434.30	296.39	3386.85	436.02	<.0001	1096.42	150.22	1528.59	173.40	.065	1952.55	335.06	432.17	214.47	.001
Total GLP-1	Δ{225 - 255} (pmol / L)	2.19	0.71	2.50	1.00	.804	ND		ND			0.31	0.92	ND		
	AUC{225 - 300} (pmol / L × min)	433.04	45.82	430.15	41.01	.923						− 2.89	29.34	ND		
Intact GLP-1	Δ{225 - 255} (pmol / L)	− 0.71	0.26	0.61	0.69	.091	ND		ND			1.32	0.70	ND		
	AUC{225 - 300} (pmol / L × min)	95.23	10.54	225.71	20.42	<.0001	ND		ND			130.48	18.94	ND		

Abbreviations: AUC, area under the curve; CPR, C-peptide reactivity; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; ISR, insulin secretion rate; S.E., standard error; V2, visit 2; V3, visit3.

induced glucagon response by GIP or vildagliptin was observed in T2D individuals with mean baseline HbA1c values between 6.3% and 6.5%,[7,11] but there was little enhancement by vildagliptin in individuals with T2D with a mean baseline HbA1c value of 7.7%.[14] Further investigations are required to clarify the discrepancies between these two studies.

In the current study, the hypoglycaemia-induced responses of the counter-regulatory hormones GH, cortisol, epinephrine and norepinephrine were attenuated at V3 compared to those at V2, which is similar to the effect of DPP-4 inhibitor linagliptin and the GLP-1 receptor agonist liraglutide (Table 2 and Figure S6). The lack of placebo or euglycaemic control groups in our study does not permit exclusion of the possibility that the subjects' experiences at V2 might affect the counter-responses of GH, cortisol, epinephrine and norepinephrine seen at V3. Furthermore, the variable IIR could result in different insulin levels between tests that might affect responses of the counter-regulatory hormones. However, a recent study in T2D individuals reported suppressive effects on hypoglycaemia-induced sympatho-adrenal response after treatment with the GLP-1 receptor agonist lixisenatide,[13] which are similar to our present findings. Co-administration of some incretin therapies in insulin and/or sulfonylurea-treated individuals with T2D would seem to have potential for preventing hypoglycaemia-induced release of cortisol and catecholamine, and thus possibly reduce cardiovascular event risks. However, further investigations are needed to clarify the underlying mechanisms and clinical relevance of the current findings, as most previous studies on incretin therapies did not show similar suppressive effects in individuals with T2D or type 1 diabetes.[11,19,20] In conclusion, neither the DPP-4 inhibitor linagliptin nor the GLP-1 receptor agonist liraglutide impaired the alpha-cell responsiveness to hypoglycaemia in Japanese individuals with T2D.

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Conflict of interest

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Author contributions

D. Y. and Y. S. contributed to the conception and design of the research and writing of the manuscript. T. E., S. I. and M. S. contributed to the design of the research, collection of data, and critical revision of the manuscript for important intellectual content. H. K., K. T., Y. S., S. S. and B. A. contributed to the analysis and interpretation of data and critical revision of the manuscript for important intellectual content. K. M. contributed to the design of the research, statistical analysis and critical revision of the manuscript for important intellectual content. All authors approved the version to be published. D. Y. and Y. S. are the guarantors of this work.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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