Journal of Clinical Diabetes

Nakagawa et al. J clin Diabetes 2021, 5:2

Mini Review Open Access

Anagliptin a DPP4 Inhibitor Slightly Suppresses Glucagon as Measured by LCHRMS

Tomoko Nakagawa*, Yoshio Nagai, and Yasushi Tanaka

Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Japan

Abstract

The etiology and pathology of type 2 diabetes are insufficient insulin action, which involves an increase in insulin resistance due to environmental factors and/or a decrease in insulin secretion mainly due to genetic factors. Glucagon is secreted from alpha cells of the pancreas as a hormone that antagonizes the action of insulin. Abnormal glucagon secretion is also thought to be a factor in raising blood glucose levels. In normal glucose tolerance, when insulin secretion from β cells increases in response to an increase in blood glucose level, the insulin suppresses glucagon secretion from α cells in paracrine manner.

Keywords: Insulin; Glucagon; Glucose tolerance; Beta cells

Introduction

The etiology and pathology of type 2 diabetes are insufficient insulin action, which involves an increase in insulin resistance due to environmental factors and/or a decrease in insulin secretion mainly due to genetic factors. Glucagon is secreted from alpha cells of the pancreas as a hormone that antagonizes the action of insulin. Abnormal glucagon secretion is also thought to be a factor in raising blood glucose levels [1]. In normal glucose tolerance, when insulin secretion from β cells increases in response to an increase in blood glucose level, the insulin suppresses glucagon secretion from α cells in paracrine manner. As another factor controlling glucagon secretion, incretin and amino acids regulate glucagon secretion. In patients with diabetes, alpha cells have insufficient glucagon suppression because beta cells do not secrete enough insulin to raise blood glucose level [2]. Glucagon hypersecretion is thought to be the third leading cause of diabetes. However, details on how glucagon contributes to glycemic control are unknown.

As mentioned above, glucagon could have an important role in glucose homeostasis. Thus, inappropriate or excessive glucagon secretion should have an important influence on glucose homeostasis and the pathophysiology of type 2 diabetes [3,4]. However, it has been difficult to measure the plasma glucagon concentration, because some other peptides derived from proglucagon cross-react with glucagon by using both radioimmunoassays and Enzyme-Linked Immuno-Sorbent Assays (ELISAs) [5,6]. Recently, a new assay was developed for plasma glucagon that employs Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) to achieve higher accuracy and precision. The lower limit of quantification is 1.5 pmol/L for sandwich ELISA and down to 0.5 pmol/L for LC-HRMS. Plasma glucagon responses in healthy volunteers quantified by LC-HRMS method and sandwich ELISA. Blood glucagon levels by LC-HRMS and sandwich ELISA both decreased after OGTT, but gradually increased during the Meal tolerance Test (MMT). The difference between these loads is that the MMT tests included not only glucose but also amino acids and other components that stimulated glucagon secretion [7].

We evaluated the effect of anagliptin on plasma glucagon levels in Japanese patients with type 2 diabetes by using liquid chromatography-high resolution mass spectrometry. Anagliptin, a dipeptidyl peptidase-4 inhibitor, was developed by Sanwa Kagaku Kenkyusho Co, Ltd (Nagoya, Japan) and Kowa Pharmaceutical Co, Ltd (Tokyo, Japan) and has been available in Japan since 2012. It is generally used in Japan. There is increasing evidence that glucagon has a critical role in glucose metabolism, but it has been difficult to measure plasma glucagon precisely due to the existence of multiple peptides derived from pro-glucagon that show cross-reactivity with glucagon [8].

Twenty-four patients with type 2 diabetes were enrolled in a prospective, single-center, randomized, open-label study and were randomly allocated to 4 weeks of treatment with metformin (1000 mg/day) or anagliptin (200 mg/day). A liquid test meal labeled with sodium (13C) acetate was ingested before and after the treatment period. We selected metformin as an active comparator to assess the influence on the plasma glucose level, since metformin is recommended as a first-line oral hypoglycemic agent in many countries [9]. We also compared the effects of anagliptin and metformin on gastric emptying by performing the [13C] acetate breath test. Samples of blood and expired air were collected over 3 hours. Plasma levels of glucose, glucagon, C-peptide, Glucagon-Like Peptide-1 (GLP-1), and Glucose-dependent Insulinotropic Polypeptide (GIP) were measured, and gastric emptying was also evaluated. The primary outcome of this study was the change from baseline of the incremental plasma glucagon AUC (iAUC) following ingestion of the liquid test meal after the 4-week treatment period. Secondary outcomes included the changes from baseline of the plasma glucose, C-peptide, GLP-1, and GIP iAUC after 4 weeks, as well as the changes of T1/2 and Tlag of gastric emptying. Furthermore, changes of HbA1c and fasting plasma glucose from baseline after 4 weeks were determined. A recent single-arm study comparing the plasma glucagon level measured by the same LC-HRMS method that we used or by a commercial ELISA kit showed significant reduction of plasma glucagon by the same anagliptin regimen as ours [10]. It is important to improve early secretion of insulin for improvement of blood glucose level [11].

***Corresponding author:** Tomoko Nakagawa, Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Japan, Tel: +81 44-977-8111; E-mail: tomonaka@marianna-u.ac.jp

Received: March 03, 2021; **Accepted:** March 17, 2021; **Published:** March 24, 2021

Citation: Nakagawa T, Nagai Y, Tanaka Y (2021) Anagliptin a DPP4 Inhibitor Slightly Suppresses Glucagon as Measured by LCHRMS. J Clin Diabetes 5: 117.

Literature Review

Twenty-two patients completed the study (metformin group: n=10; anagliptin group: n=12). Glycemic control showed similar improvement in both groups. At screening, 5 subjects (3 in the anagliptin group and 2 in the metformin group) were on treatment with metformin (500 mg/day). Demographic variables were well balanced between the two groups with regard to age, gender, duration of diabetes, BMI, glycemic control, blood pressure, and lipid profile.

The plasma glucagon profile and changes of the iAUC for plasma glucagon after ingestion of the liquid test meal are shown in Figure 1 and Table 1.

After 4 weeks of treatment, the glucagon iAUC was decreased by 4.2 pmol•h/L (95% CI-6.1, -2.3) in the anagliptin group and increased by 0.8 pmol•h/L (95% CI-3.9, -5.6) in the metformin group, with a slight difference between the two groups (-5.0 pmol•h/L; 95% CI-10.0, -0.1, p=0.048).

In addition, active GLP-1 was increased at 0.5 h in the anagliptin group compared with the corresponding time point before treatment (data not showed). Also, the plasma level of GIP was increased, and plasma C-peptide was also increased versus baseline. Neither anagliptin nor metformin delayed gastric emptying.

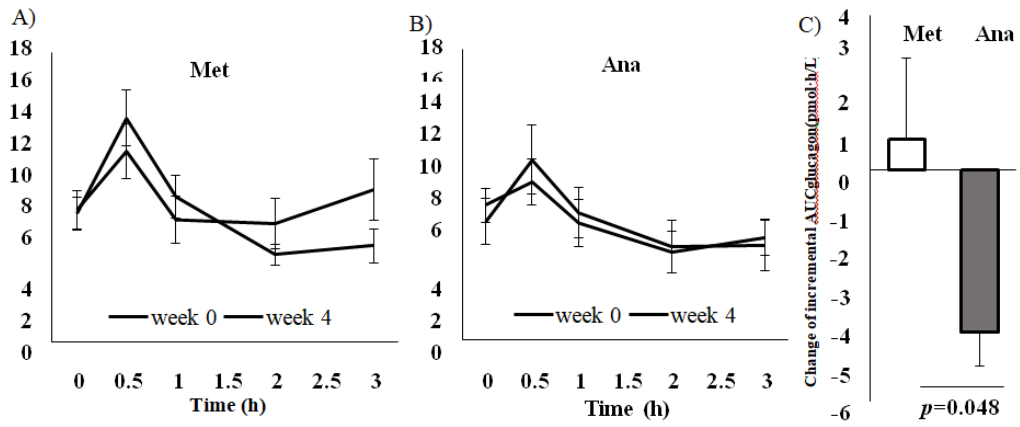

Figure 1: Plasma glucagon profile following ingestion of a liquid test meal before (dotted line) and after (solid line) 4 weeks of treatment with

A) Metformin (Met, n=10) or

B) Anagliptin (Ana, n=12). Changes between before and the end of treatment with metformin (a closed bar:■) or

C) Anagliptin (open bar:□) were calculated for incremental AUC of glucagon (pmol•h/L). Data are presented as mean values ± SEM.

	Duration (No. of weeks)	Metformin group (n=10)		Anagliptin group (n=12)		Difference of AUC change between the groups	Confidence interval	
		Mean	SEM	Mean	SEM		Lower	Upper
Glucose (mg•h/dL)	0w	152.9	(12.9)	168.4	(15.1)	-	-	-
	4w	118.7	(11.6)	119.3	(14.3)	-	-	-
	Change of AUC	-34.2	(10.8)	-49.1	(12.4)	-14.9	-49.2	19.4
Glucagon (pmol•h/L)	0w	0.3	(2.1)	0.1	(1.6)	-	-	-
	4w	1.1	(2.2)	-4.1	(1.9)	-	-	-
	Change of AUC	0.8	(2.1)	-4.2	(0.9)	-5.0	-10.0	-0.1
C-peptide (ng•h/mL)	0w	6.5	(0.7)	6.5	(0.7)	-	-	-
	4w	6.8	(0.8)	7.2	(0.8)	-	-	-
	Change of AUC	0.3	(0.4)	0.7	(0.4)	0.4	-0.8	1.5
Total GLP-1 (pmol•h/L)	0w	8.3	(3.7)	12.4	(4.1)	-	-	-
	4w	15.6	(4.4)	7.0	(3.2)	-	-	-
	Change of AUC	7.4	(3.2)	-5.4	(2.4)	-12.8	-20.9	-4.7
Active GLP-1 (pmol•h/L)	0w	4.7	(1.9)	11.0	(4.9)	-	-	-
	4w	11.0	(3.8)	17.5	(8.3)	-	-	-
	Change of AUC	6.3	(2.9)	6.5	(10.4)	0.2	-23.2	23.6
Active GIP (pmol•h/L)	0w	77.0	(16.4)	58.0	(5.6)	-	-	-
	4w	58.0	(7.1)	129.6	(14.8)	-	-	-
	Change of AUC	-19.0	(11.9)	71.6	(17.1)	90.6	45.2	136.0

Table 1: iAUC0-3h after intake of the liquid test meal before and after 4 weeks of treatment and between group differences of iAUC changes.

Discussion

In the present randomized study, we evaluated the effect of anagliptin on plasma glucagon in patients with type 2 diabetes by using a new LC-HRMS assay and we obtained three main findings. First, after 4 weeks of treatment with metformin (1000 mg/day) or anagliptin (200 mg/day), improvement of glycemic control was comparable, including fasting plasma glucose, HbA1c, and the glucose profile during after ingestion of a 200 kcal liquid test meal. Second, anagliptin slightly decreased the iAUC of plasma glucagon during MMT and increased early plasma levels of active GLP-1, active GIP, and C-peptide levels. Third, neither anagliptin nor metformin caused any delay of gastric emptying. A recent single-arm study comparing the plasma glucagon level measured by the same LC-HRMS method that we used or by a commercial ELISA kit showed significant reduction of plasma glucagon by the same anagliptin regimen as ours. That study recruited patients on insulin and the baseline plasma C-peptide AUC was lower than in our subjects, while the plasma glucagon AUC was higher. These differences of baseline plasma glucagon and C-peptide levels between the two study populations can be explained because glucagon secretion from pancreatic β cells is inhibited by endogenous insulin secretion in a paracrine manner. In that study, the baseline plasma glucagon level was higher and the reduction by anagliptin treatment was greater than in the present study. Therefore, it seems difficult to detect a decline of glucagon secretion when baseline secretion is not high in patients with preserved endogenous insulin secretion. And also, the change in the area under the curve for plasma glucagon during the MMT before and after anagliptin treatment measured using LC-HRMS and ELISA (a commercially available sandwich ELISA kit (10-1271-01, Mercodia, Uppsala, Sweden)) was similar. Therefore, the performance of a commercially available ELISA kit was equivalent to LC-HRMS for the analysis of postprandial glucagon kinetics before and after anagliptin treatment. 75 g OGTT was performed on 2,121 Japanese with suspected glucose intolerance. The patients with mild diabetes diagnosed by 75 g OGTT have decreased insulin secretion 30 and 60 minutes after loading. Compare with normal glucose tolerance, total insulin secretion after loading was maintained despite the decrement of early secretion. It is important to improve early secretion of insulin for improvement of blood glucose level. Anagliptin improved blood glucose control by improving the early secretion of insulin.

Conclusion

In conclusion, in patients with type 2 diabetes maintained endogenous insulin secretion, anagliptin increased the plasma level of active GLP-1 and GIP in association with a slight stimulation of insulin secretion and slight inhibition of glucagon secretion but did not delay gastric emptying.

References

1. Ichikawa R, Takano K, Fujimoto K, Motomiya T, Kobayashi M, et al. (2019) Basal glucagon hypersecretion and response to oral glucose load in prediabetes and mild type 2 diabetes. Endocr J 66: 663-675.
2. Kawamori D, Akiyama M, Hu J, Hambro B, Kulkarni RN (2011) Growth factor signaling in the regulation of α-cell fate. Diabetes Obes Metab 13 (Suppl 1): 21-30.
3. Unger RH (1976) The banting memorial lecture 1975 diabetes and the alpha cell. Diabetes. 25: 136-51.
4. Unger RH, Cherrington AD (2012) Glucagonocentric restructuring of diabetes: A pathophysiologic and therapeutic makeover. J Clin Invest 122: 4-12.
5. Albrechtsen NJW, Hartmann B, Veedfald S, Windeløv JA, Plamboeck A, et al. (2014) Hyperglucagonaemia analysed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? Diabetologia 57: 1919-1926.
6. Matsuo T, Miyagawa JI, Kusunoki Y, Miuchi M, Ikawa T, et al. (2016) Postabsorptive hyperglucagonemia in patients with type 2 diabetes mellitus analyzed with a novel enzyme-linked immunosorbent assay. J Diabetes Investig 7: 324-31.
7. Miyachi A, Kobayashi M, Mieno E, Goto M, Furusawa K, et al. (2017) Accurate analytical method for human plasma glucagon levels using liquid chromatography-high resolution mass spectrometry: Comparison with commercially available immunoassays. Anal Bioanal Chem 409: 5911-5918.
8. Marshall SM (2017) 60 years of metformin use: a glance at the past and a look to the future. Diabetologia 60: 1561-1565.
9. Ahrén B (2015) Glucagon--Early breakthroughs and recent discoveries. Peptides 67: 74-81.
10. Katahira T, Kanazawa A, Shinohara M, Koshibu M, Kaga H, et al. (2018) Postprandial plasma glucagon kinetics in type 2 diabetes mellitus: comparison of immunoassay and mass spectrometry. J Endocr Soc 3: 42-51.
11. Tanaka Y, Atsumi Y, Asahina T, Hosokawa K, Matsuoka K, et al.(1998) Usefulness of revised fasting plasma glucose criterion and characteristics of the insulin response to an oral glucose load in newly diagnosed Japanese diabetic subjects. Diabetes Care 21: 1133-1137.